

Krista Varady
Emily N. C. Manoogian
Valter D. Longo *Editors*

Intermittent and Periodic Fasting, Aging and Disease

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Editors

Krista Varady
Department of Kinesiology and Nutrition
University of Illinois at Chicago
Chicago, IL, USA

Emily N. C. Manoogian
Regulatory Biology Laboratory
Salk Institute for Biological Studies
La Jolla, CA, USA

Valter D. Longo
Director of the Longevity Institute, Leonard
Davis School of Gerontology
University of Southern California
Los Angeles, CA, USA

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Preface

Various forms of fasting, which have been part of the common and in some cases required practices by many religious groups for thousands of years, have recently become some of the most adopted interventions to promote health. However, fasting, like eating, can have positive, negative, and neutral effects on health and longevity depending on its length, type, and frequency but also on a wide range of characteristics of the person who is fasting. For example, 12–13 h of daily fasting is associated with health and sleep benefits but when the fasting period surpasses 14–16 h it is associated with an increased risk of hospitalization with gallstone disease (Sichieri et al. 1991) and even higher cardiovascular and overall mortality if the prolonged fasting involves skipping breakfast (Rong et al. 2019).

Thus, to allow fasting to become part of the toolkit of healthcare professionals for disease prevention and treatment it will be important to standardize and test in randomized clinical trials the fasting method utilized while explaining the exact method by which it was carried out. Whereas only some fasting-based interventions may seek FDA approval, a standard similar to the one leading to drug approval should be used to determine whether a particular type of fasting is, in fact, able to prevent or treat a disease or if it may affect biological age.

This book will first focus on calorie and protein restriction research, which has generated an enormous number of valuable publications in the past 100 years, most pointing to their anti-aging properties, but many also pointing to side effects or their efficacy only in certain genetic backgrounds or in specific age ranges. Following the chapters on these everyday restrictions, it will focus on time-restricted eating (TRE) and therefore on the length of the daily periods in which food is consumed or not. These TRE chapters will address its benefits on markers for aging and diseases but also on the quality and length of sleep, as well as its origins in circadian biology. Several chapters will also focus on alternate day fasting (ADF) and therefore the alternation of days of normal or high calorie consumption with days of no or low calorie intake and its effect on aging and disease markers, with one chapter emphasizing fasting responses in the brain. From these forms of intermittent fasting (IF), the subsequent chapters will shift to periodic fasting (PF) or the studies of longer fasting periods usually lasting 3 days or longer but applied once a month or less, in

most cases. These chapters will cover water only or similar very low calorie consumption fasting, or the use of fasting mimicking diets (FMDs) developed to allow a much higher calorie consumption while achieving the fasting response. First the focus will be on periodic fasting and FMDs lasting 3–7 days and in many cases applied once a month and then on periods of water only or similar fasting lasting up to several weeks or longer but in most cases carried out only once a year or less frequently.

In general, there is no doubt that the various fasting methods can have remarkable effects on aging, disease risk factors, but can also reverse or at least ameliorate a number of diseases. However, the near future will require many additional randomized clinical trials, to allow healthcare professionals to adopt only the fasting methods demonstrated to be effective against aging and/or disease prevention or treatment. It will be important to assess their overall effect not only on the disease being treated but also on both the short- and long-term effects, so that lifespan and health span are both optimized. For example, achieving weight loss or diabetes reversal by fasting methods may not only be beneficial but it may also be detrimental if the patient regains the weight or if the insulin resistance and diabetes return.

Taking into account multiple pillars including clinical, basic, and epidemiological studies but especially those examining health span will be of central importance for these dietary interventions to be considered to complement or replace pharmacological interventions.

Los Angeles, CA, USA

Valter Longo

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About the Editors

Krista Varady, PhD, is a Professor of Nutrition at the University of Illinois, Chicago. Her research focuses on the efficacy of intermittent fasting for weight management and metabolic disease risk reduction in adults with obesity. She has been studying fasting for almost 20 years and is one of the top researchers in this field. Her work is funded by the NIH, American Heart Association, and the University of Illinois. She has published over 100 publications on this topic and is also the author of two books for the general public, entitled the “Every Other Day Diet” and “The Fastest Diet.”

Emily N. C. Manoogian, PhD, is a staff scientist and the head of clinical research in Dr. Satchin Panda’s lab at the Salk Institute for Biological Studies. She has studied circadian rhythms for the past 15 years at the mechanistic and clinical levels. Currently, her research focuses on how lifestyle interventions that optimize circadian health, such as time-restricted eating, can improve a variety of age-related diseases including pre-diabetes, diabetes, cardiovascular disease, cancer, mental health, and cognitive decline.

Valter Longo, PhD, is the Edna Jones Professor in Gerontology, the Director of the USC Longevity Institute and group leader at the IFOM Cancer Research Institute in Milan, Italy. His laboratories study the fundamental mechanisms of aging in yeast, rodents, and humans by using genetics and biochemistry techniques. The focus is on the nutrient-response signal transduction pathways that regulate disease and longevity. This work led to the discovery of the effects of periodic fasting and fasting mimicking diets on multi-system stem cell activation and regeneration in mice and to clinical trials on a range of age-related diseases.

Part I

Calorie Restriction

Chapter 1

Caloric Restriction and Biomarkers of Aging



Susan B. Racette and Sai Krupa Das

Abstract Calorie restriction (CR) is characterized by a reduction in calorie intake without malnutrition. In the context of geroscience, CR is a promising nutritional strategy that targets the biology of aging and therefore has the potential to delay the onset or slow the progression of age-related diseases. Life span extension by CR has been demonstrated in a variety of species, including yeast, drosophila, worms, rodents, and dogs, providing compelling evidence for the geroprotective potential of CR. In humans, optimizing health span is arguably a more meaningful goal of CR interventions and CR lifestyles. Numerous physiological effects of CR on biomarkers of human aging and health span have been explored, both in observational studies and in well-controlled intervention trials, providing an abundance of rich data that overwhelmingly support CR as a promising strategy for attenuating biological aging. The development of novel biomarkers of aging has advanced the field of geroscience and provided new opportunities for exploring the impact of CR on biological aging. In this chapter, we discuss the influence of CR on various biomarkers of aging and health span, with the biomarkers organized in three broad categories: cellular aging, phenotypic aging, and functional aging. We present results of CR studies in humans that demonstrate typical improvements in cardiometabolic indices, as well as effects on novel epigenetic biomarkers of cellular aging and the pace of biological aging that are based on DNA methylation. Finally, the chapter closes with highlights of ongoing CR initiatives funded by the National Institute on Aging and future directions for exploring numerous unanswered but important questions about the role of CR in enhancing health span.

S. B. Racette (✉)

College of Health Solutions, Arizona State University, Phoenix, AZ, USA

e-mail: susan.racette@asu.edu

S. K. Das

Jean Mayer USDA Human Nutrition Research Center on Aging, Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA

1.1 Introduction

Biological aging is the gradual deterioration in the integrity of bodily systems over time, during which the loss of molecular fidelity in tissues exceeds repair capacity (Hayflick 2007). Chronological aging increases at the same pace for everyone, whereas biological aging can be accelerated or decelerated as a consequence of lifestyle behaviors (Hayflick 2007; Ferrucci et al. 2018). Biological aging can be described in terms of primary aging and secondary aging (Holloszy 2000). Primary aging is the progressive deterioration in tissue structure and function that occurs with advancing age and is proposed to occur independently of disease, lifestyle behaviors, and environmental factors. Secondary aging, in contrast, reflects the deterioration in tissue structure and function that occurs due to disease processes, adverse lifestyle behaviors, and harmful environmental exposures. Various biomarkers have been identified to reflect biological aging, which is important for evaluating the merits of interventions that are designed to be geroprotective, such as calorie restriction (CR).

1.2 Biological Aging Is Associated with Functional Decline and Chronic Disease Risk

The physiological process of aging is characterized by molecular, cellular, metabolic, hormonal, immune, and neurocognitive changes that independently and collectively contribute to decrements in physical function and increases in chronic disease risk (Hayflick 2007; Lopez-Otin et al. 2013). Recent scientific advances have led to the transformative hypothesis that interventions targeting the fundamental biology of human aging have the potential to delay, if not prevent, the onset of aging-associated conditions, thereby extending the length of time a person is healthy—referred to as health span. The unprecedented growth of the aging population and increasing prevalence of chronic diseases have created an urgent need for interventions that promote the maintenance of health into old age.

1.3 Calorie Restriction as a Geroprotective Strategy

CR is characterized by a reduction in calorie intake without malnutrition. In the context of geroscience, CR is a strategy that is intended to impact primary aging favorably; this is distinct from obesity treatment approaches that are designed to promote weight loss and ameliorate the metabolic consequences associated with obesity. CR has the distinction of being one of the few non-pharmacological, non-genetic interventions that directly target the biology of aging. Other promising dietary approaches include intermittent fasting regimens (de Cabo and Mattson

2019) and methionine restriction (Kitada et al. 2021; Zhang et al. 2022). By targeting biological aging, CR has been shown to delay the onset or slow the progression of age-related diseases in various organisms (Fontana et al. 2010a; Colman et al. 2009, 2014; Mattison et al. 2017). In rodents, CR has potent anticancer effects, reduces adiposity, and protects against atherosclerosis, cardiomyopathy, and neurodegeneration as well as autoimmune, renal, and respiratory diseases (Lane et al. 1996, 1997; Fontana and Partridge 2015; Ingram and de Cabo 2017). In rhesus monkeys, which are genetically and physiologically similar to humans, long-term CR conferred profound benefits in protecting against conditions and diseases that are common in human geriatric populations, such as sarcopenia, osteoporosis, arthritis, cancer, type 2 diabetes, and cardiovascular disease, which lowered the risk of age-related morbidity more than twofold (Colman et al. 2009, 2014; Mattison et al. 2017; Lane et al. 1999). Collectively, the geroprotective effects of CR interventions in yeast, worms, flies, rodents, and non-human primates raised the possibility that CR acts through mechanisms that are conserved across species, providing strong rationale for studying CR in humans.

Effects of CR on Longevity Equally impressive are the effects of CR on increasing longevity, which may be considered the most definitive evidence of geroprotection. Longer life span with CR has been demonstrated convincingly in yeast, drosophila (Mair et al. 2003), worms, rodents (McCay et al. 1935; Holloszy and Schechtman 1991; Holloszy 1997), and dogs (Kealy et al. 2002; Lawler et al. 2008). The earliest experimental evidence for CR's benefits date back to the 1930s, when Dr. Clive McCay et al. discovered that retarding the growth rate of young rats extended their life span (McCay et al. 1935; McCay and Crowell 1934). Elegant CR intervention studies in rats conducted several decades later in Dr. John Holloszy's lab revealed that CR, whether implemented as the sole intervention or combined with exercise, increased both mean and maximal life span (Holloszy 1997). This benefit is distinct from that of exercise alone, which promoted a smaller increase in mean life span compared to CR and did not extend maximal life span (Holloszy 1988). A very intriguing study that demonstrated the life-extending effects of CR in drosophila addressed the important question of whether CR must be initiated in early life to exert longevity benefits (Mair et al. 2003). The results were both encouraging and sobering: CR initiated in midlife had robust, rapid, and comparable benefits on longevity as CR initiated in early life; however, switching from a CR diet in early life to a fully fed condition in midlife quickly reversed the benefits of CR and caused even higher mortality compared to flies that were fully fed throughout life.

The effects of long-term, controlled CR interventions on life span in rhesus monkeys were variable, showing benefits in one of the two colonies (Colman et al. 2009; Mattison et al. 2017), but not in the other (Mattison et al. 2012). This discrepancy is believed to be attributable to diet quality and other differences in experimental designs between the two studies (Maxmen 2012). In humans, there is very intriguing observational evidence from Okinawa, Japan, that a dietary pattern characterized by approximately 11% CR initiated during youth and continued into mid-adulthood was associated with greater mean and maximal life span and likely

contributed to the Okinawans’ distinction as the longest-lived human population (Willcox et al. 2006).

CR Studies in Humans While longer life span is a strong indicator of the gero-protective effects of CR, greater health span is perhaps a more meaningful goal of CR interventions and CR lifestyles. Numerous physiological effects of CR on biomarkers of human aging have been explored both in observational studies and in well-controlled intervention trials. Figure 1.1 provides an outline (for reference throughout this chapter) of human studies in which CR was implemented for at least 6 months’ duration in adults without obesity and in which aging biomarkers were quantified.

Observational Studies of CR in Humans Although randomized controlled trials are considered the gold standard for many research studies, natural experiments and observational studies provide unique and unmatched opportunities to examine the influence of specific dietary patterns followed for long durations. Such lengthy interventions and follow-up periods are beyond the time frame that is feasible in most human intervention studies. A noteworthy example was Biosphere 2, an ecological mini-world located near Tucson, Arizona, in which Dr. Roy Walford, one of the pioneers of CR, together with seven other healthy adults without obesity, lived for a 2-year period (1991–1993). An unexpected problem growing crops resulted in a calorically restricted diet (~29% CR) during most of the 2-year experiment. The silver lining for geroscience was the rich biomarker data that were obtained from the eight inhabitants (Walford et al. 1992, 2002). Another unique opportunity to evaluate the effects of long-term CR was provided by “CRON” individuals who voluntarily and independently had been following a CR

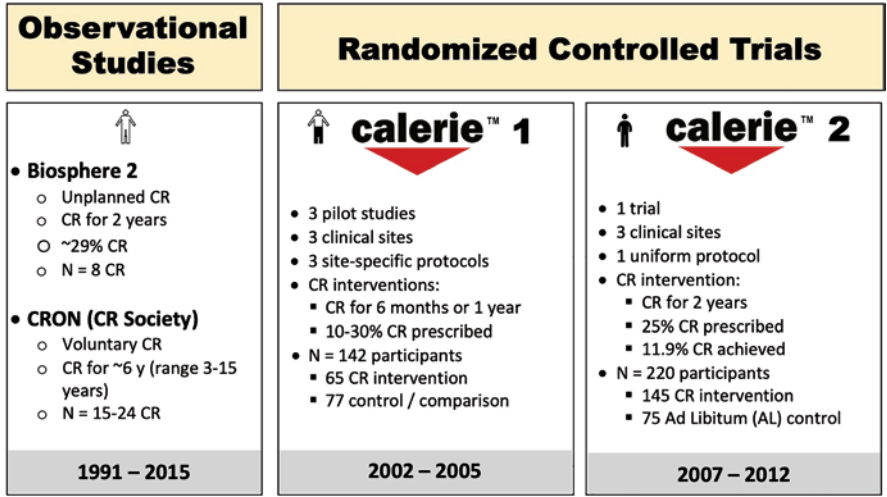


Fig. 1.1 Studies of calorie restriction in humans

diet for an average of 6 years (range 3–15 years) when Dr. Luigi Fontana began assessing their cardiometabolic health (Fontana et al. 2004). The goal of their dietary approach was to achieve caloric restriction with optimal nutrition (CRON); this is the lifestyle adopted by the CR Society. The physiological and metabolic profiles of the CRON individuals were impressive and supported the geroprotective effects of CR observed in other species.

Randomized Controlled Trials of CR in Humans The first randomized controlled trial of CR in humans without obesity was the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) trial. Funded by the National Institute on Aging (NIA), CALERIE™ was conducted in two phases at three clinical centers: Pennington Biomedical Research Center in Baton Rouge, Louisiana; the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston, Massachusetts; and Washington University School of Medicine in St. Louis, Missouri. CALERIE™ Phase 1 was conducted between 2002 and 2005 and involved site-specific pilot studies to test approaches to achieve and maintain CR (Das et al. 2007; Heilbronn et al. 2006; Racette et al. 2006). The CALERIE™ Phase 2 trial, conducted from 2007 to 2012, was a multi-site, single-protocol study designed to test the hypothesis that 2 years of 25% CR would improve biomarkers of aging and biomarkers of age-related chronic disease (Rochon et al. 2011; Ravussin et al. 2015). CALERIE™ 2 participants were 220 healthy adults aged 21–50 years at baseline (upper age limit was 47 years for women to avoid the metabolic effects of menopause) who were randomized in a 2:1 allocation ratio to a CR intervention (“CR” group) or an ad libitum intake control condition (“AL” group). All participants were healthy and not taking medications other than oral contraceptives and had no evidence of disease or disease risk factors based on extensive blood, urine, physical, and psychological testing for eligibility.

The CR prescription was 25% for 2 years, reflecting a daily energy intake level that was 25% below baseline, weight-maintenance energy needs. Each participant in the CR group received an individualized daily energy intake prescription (kcal/day) that was 25% lower than their baseline daily energy expenditure; baseline energy expenditure was measured for 4 consecutive weeks using doubly labeled water. This method provides the most accurate estimate of free-living energy expenditure and energy intake while people lead their usual lives. The CALERIE™ 2 trial was characterized by careful design, rigor, and high commitment of the research team personnel and study participants. An important consideration when reviewing the results of the CALERIE™ 2 trial is that the average level of CR achieved throughout the 2-year intervention was $11.9 \pm 0.7\%$ (mean $\pm$ SE), with a range of 0.9%–31.2% among CR participants. These results highlight the difficulty of sustaining a CR diet for 2 years and the inter-individual variability in adherence. Despite achieving a more moderate level of CR, on average, than the prescribed level of 25%, it is encouraging that this modest level of CR yielded improvements in several biomarkers of aging, with relatively few effects that were deemed potentially adverse.

1.4 Biomarkers of Aging Relevant to Calorie Restriction

Biomarkers are intended to reflect important biological indices of health. Biomarkers of aging can be categorized as cellular, phenotypic, and functional to reflect the physiological mechanisms involved and the clinical manifestations that are associated with aging. Several novel and traditional biomarkers have been proposed to reflect human aging and health span. The focus of this chapter is the various biomarkers that reflect health span and the potential influence of CR on biological aging indices in humans and other species.

Table 1.1 provides an overview of the **favorable**, **neutral**, and **negative** effects of CR on cellular, phenotypic, and functional biomarkers that have been observed in rodent intervention studies 🐭, rhesus monkey intervention studies 🐒, human observational studies 👤, human intervention studies of 6 months to 1 year in duration 🕒, and human intervention studies of 2 years in duration 🕒. We intentionally included only studies that assessed age-associated biomarkers among rodents (mice and rats), monkeys (rhesus macaques), or humans without obesity. Human studies were restricted to those in which the duration of CR was at least 6 months. We acknowledge that this does not represent an all-inclusive compilation of CR studies.

1.4.1 Calorie Restriction and Cellular Aging Biomarkers

TAME Biomarkers Novel surrogates for cellular aging include blood-based biomarkers proposed by the Targeting Aging with METformin (TAME) Biomarkers Workgroup (Justice et al. 2018). TAME encompasses the following multi-system metabolic markers that reflect the health status of various tissues, organs, and metabolic processes: interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), and tumor necrosis factor α receptor II (TNFR2) as markers of inflammation and intercellular signaling; growth differentiation factor 15 (GDF15) as a marker of a stress response and mitochondrial dysfunction; insulin-like growth factor 1 (IGF-1) and insulin as markers of nutrient signaling; cystatin C as a marker of renal aging; N-terminal B-type natriuretic peptide (NT-proBNP) as a marker of heart failure and cardiovascular health; glycated hemoglobin (HbA1c) as a marker of glucose regulation and metabolic aging; and molecular signatures that reflect epigenetic, transcriptional, and proteomic processes that reflect aging.

Inflammation Reduced inflammation is considered an important mechanism that mediates the beneficial effects of CR on aging (Heilbronn and Ravussin 2003). In the CRON population, long-term CR was associated with lower concentrations of the inflammatory protein hsCRP (Fontana et al. 2004), which may be mediated, in part, by an increase in serum cortisol (Yang et al. 2016). In the CALERIE™ 2 trial, 2 years of CR resulted in 40% to 50% reductions in circulating concentrations of the pro-inflammatory cytokines TNF α and hsCRP (Ravussin et al. 2015), as well as

Table 1.1 Effects of calorie restriction on biomarkers of aging and health span

	Favorable Effects	Neutral Effects	Negative Effects	References
Biomarkers of cellular aging				
Inflammatory markers: IL-6, hsCRP, TNFα				Lane et al. (1997), Meyer et al. (2006), Weiss et al. (2006), Fontana et al. (2007), Yang et al. (2016), Meydani et al. (2016), Dorling et al. (2021), Spadaro et al. (2022)
Nutrient signaling: IGF-1, insulin				Fontana et al. (2004), Das et al. (2007), Heilbronn et al. (2006), Weiss et al. (2006), Dorling et al. (2021), Mercken et al. (2013a), Ramsey et al. (2000)
Dehydroepiandrosterone sulfate (DHEAS)				Willcox et al. (2006), Heilbronn et al. (2006)
Oxidative stress or oxidative damage				Ning et al. (2013), Zainal et al. (2000), Meydani et al. (2011), Il'yasova et al. (2018), Redman et al. (2018)
Cellular senescence				Ning et al. (2013), Cohen et al. (2004), Fontana et al. (2018a)
Autophagy				Yang et al. (2016), Ning et al. (2013)
Mitochondrial function or mitochondrial damage				Mercken et al. (2013a), Ning et al. (2013), Civitarese et al. (2007), Sparks et al. (2017)
DNA methylation, gene expression, RNA processing, transcription				Spadaro et al. (2022), Maegawa et al. (2017), Rhoads et al. (2018), Mercken et al. (2013b)
Biological age—Epigenetic clocks (DNA methylation-based): GrimAge, DNAm PhenoAge				Waziry et al. (2023)
Pace of biological aging (DNA methylation-based): DunedinPoAm, DunedinPACE				Waziry et al. (2023)
Biomarkers of phenotypic aging				
Blood pressure				Walford et al. (1992), Meyer et al. (2006)

(continued)

Table 1.1 (continued)

	Favorable Effects	Neutral Effects	Negative Effects	References
Cardiovascular health: diastolic function, carotid intima-media thickness, heart rate variability	⬆️⬆️			Meyer et al. (2006), Riordan et al. (2008), Stein et al. (2012)
Metabolic syndrome, insulin resistance, glucose regulation	⬇️⬆️⬆️⬆️			Ravussin et al. (2015), Weiss et al. (2006), Dorling et al. (2021), Ramsey et al. (2000), Kalant et al. (1988), Kennitz et al. (1994), Gresl et al. (2001), Fontana et al. (2010b), Larson-Meyer et al. (2006), Fontana and Klein (2007), Huffman et al. (2022)
Anthropometrics: weight, waist and hip circumferences	⬆️⬆️⬆️⬆️	⬆️		Fontana et al. (2004), Das et al. (2007), Racette et al. (2006), Colman et al. (1998), Redman et al. (2007), Das et al. (2017)
Body composition: whole-body and abdominal adiposity	⬇️⬆️⬆️⬆️	⬆️		Fontana et al. (2004), Das et al. (2007), Racette et al. (2006), Colman et al. (1998), Redman et al. (2007), Das et al. (2017), Phillips et al. (2022)
Bone mineral density, bone mineral content, bone architecture, bone strength, bone turnover	⬇️⬆️	⬇️⬆️	⬇️⬆️	Ingram and de Cabo (2017), Colman et al. (2007, 2008), Redman et al. (2008), Villareal et al. (2006, 2016)
Core body temperature	⬇️⬆️⬆️	⬆️		Lane et al. (1996), Heilbronn et al. (2006), Ravussin et al. (2015), Soare et al. (2011)
Energy expenditure or metabolic rate (basal, resting, sleeping, daytime, or 24-h metabolic rate)	⬆️⬆️⬆️	⬆️⬆️		Lane et al. (1996), Das et al. (2007), Heilbronn et al. (2006), Raman et al. (2007), Weyer et al. (2000), Ravussin et al. (2015), Redman et al. (2018)
Biological age (physiology-based): Klemmra-Doual method (KDM), homeostatic dysregulation index (HDI), PhenoAge	⬆️			Belsky et al. (2017)

	Favorable Effects	Neutral Effects	Negative Effects	References
Biomarkers of functional aging				
Cardiorespiratory fitness: $\text{VO}_{2\text{max}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	↑	↑		Racette et al. (2017), Weiss et al. (2007)
Muscle strength	↑	↑	↑	Racette et al. (2017), Weiss et al. (2007)
Grip strength	↔	↔		Villareal et al. (2016), Peters et al. (2022)
Cognition	↑	↑		Leclerc et al. (2020), Silver et al. (2023)
Sleep	↑	↑		Martin et al. (2016)
Sexual health	↑	↑		Martin et al. (2016)

Key: 🐭 rodents, 🐒 rhesus monkeys, 👤 human observational studies, 👤 human interventions of 6 months to 1 year, 👤 human intervention of 2 years

their downstream target intercellular adhesion molecule-1 (Meydani et al. 2016). The anti-inflammatory effects of CR were further supported by reductions in total white blood cell, lymphocyte, and monocyte counts (Meydani et al. 2016). Serum cortisol, however, was increased to a small but statistically significant extent only after the first year of CR in the CALERIETM 2 trial; the increase was not sustained at the 2-year time point (Fontana et al. 2016).

Oxidative Stress Oxidative stress and the accumulation of oxidative damage are implicated strongly in the pathogenesis of multiple age-associated chronic diseases, including heart disease, type 2 diabetes, cancer, and neurodegeneration (Valko et al. 2016). A reduction in oxidative stress is one mechanism by which CR is hypothesized to slow aging (Fontana et al. 2010a; de Cabo et al. 2014). Urinary F2-isoprostanes are considered reliable markers of non-enzymatic lipid peroxidation and serve as indicators of tissue oxidative damage and systemic oxidative stress (Il'yasova et al. 2010). In the CALERIETM 2 trial, reductions of 13% and 27% in 2,3-dinor-iPF(2 α)-III and iPF(2 α)-II, respectively, were observed after 2 years of CR (Il'yasova et al. 2018). Furthermore, a sub-study of participants at the Pennington site found that change in 2,3-dinor-iPF(2 α)-III was associated with metabolic adaptation in 24-h energy expenditure, suggesting that CR in humans may slow aging via a biological link between reductions in energy expenditure and oxidative stress (Redman et al. 2018).

SASP, Sirtuins, DNA Repair, and Autophagy Other important biomarkers of cellular aging include senescence-associated secretory phenotype (SASP) proteins, which are cellular senescence biomarkers (Fontana et al. 2018b); silent information regulators (sirtuins) (Cohen et al. 2004; Cantó and Auwerx 2009), which are proteins that regulate important biological pathways, such as ribosomal DNA recombination, gene silencing, and DNA repair; mitochondrial function; and autophagy, which is a favorable process through which damaged or dysfunctional proteins are degraded and recycled (Chung and Chung 2019). Long-term, voluntary CR among CRON practitioners in the CR Society contributed to favorable effects on cellular quality control processes in vastus lateralis muscle (Yang et al. 2016). Specifically, molecular chaperones and mediators of autophagy were higher among 15 men and women who had been following a CRON diet for 3–15 years compared to 10 age-matched control participants who were not practicing CR. Six months of ~25% CR in the CALERIETM 1 pilot study was shown to increase mitochondrial DNA content, increase the expression of genes that encode mitochondrial proteins, and reduce DNA damage, but did not alter the activity of several key mitochondrial enzymes (Civitarese et al. 2007). These predominantly promising findings were not replicated in the CALERIETM 2 trial, in which an investigation of potential mitochondrial effects after the first year of CR revealed no significant alterations in pathways involved in mitochondrial biogenesis, maximal muscle ATP synthesis rate, or other indices of mitochondrial function (Sparks et al. 2017). The effect of CR on autophagy in mice was dependent on the age at which CR was initiated (Sheng et al. 2017). Interestingly, favorable

increases in autophagy occurred in middle-aged and older mice, whereas suppression of autophagy occurred in young mice.

Biological Aging Biomarkers Novel epigenetic biomarkers of cellular aging that were developed to reflect biological age and the pace of biological aging are based on DNA methylation (DNAm) as an indicator of epigenetic modifications. Epigenetic clocks (Horvath and Raj 2018) provide estimates of biological age and age advancement, relative to chronological age. Horvath's epigenetic clock (Horvath 2013) predicts the DNA methylation age of various human tissues and cells based on the methylation state of 353 CpG dinucleotides. Hannum's clock (Hannum et al. 2013) represents a quantitative model of the rate at which a person's methylome ages and was developed using more than 450,000 CpG sites from 656 adults aged 19–101 years. Levine's DNAm PhenoAge biomarker (Levine et al. 2018) was developed using 513 CpG sites selected from 20,169 CpGs tested, 9 blood biomarkers (selected from 42 clinical markers), chronological age, biomarker and mortality data from 9926 adults aged 18 years and older in the National Health and Nutrition Examination Survey (NHANES) III, and DNA methylation and blood chemistry data obtained at two time points from 456 adults aged 21–100 years in the Invecchiare in Chianti (InCHIANTI) Study. Lu et al.'s DNAm GrimAge biomarker (Lu et al. 2019) was designed to predict life span and health span using DNAm-based surrogate biomarkers for seven plasma proteins (adrenomedullin, beta-2 microglobulin, cystatin C, GDF-15, leptin, plasminogen activation inhibitor 1, and tissue inhibitor metalloproteinase 1), a DNAm-based biomarker for smoking pack years, as well as chronological age and biological sex of 2356 adults in the Framingham Heart Study Offspring Cohort. Another novel metric, AgeAccelGrim, was developed from DNAm GrimAge to estimate epigenetic age acceleration (Lu et al. 2019). An enhanced version of DNAm GrimAge, named GrimAge2 (Lu et al. 2022), is proposed to be a stronger epigenetic biomarker of mortality and morbidity risk by incorporating two additional DNAm-based surrogates of the plasma proteins hsCRP and HbA1c.

In the CALERIE™ 2 trial, 2 years of CR did not significantly alter either the GrimAge clock or the DNAm PhenoAge clock relative to the AL control condition (Waziry et al. 2023). A subsequent exploration of a dose-response relationship was conducted to determine whether achieving greater than 10% CR during the 2-year intervention was associated with favorable changes in biological clock ages. Neither the GrimAge clock nor the PhenoAge clock differed based on this criterion of achieving greater than 10% CR (vs. achieving 10% CR or less).

DNA Methylation Pace of Aging Whereas the biological aging clocks are regarded as static metrics that reflect mortality risk at a single point in time, the pace of biological aging indices represents the rate at which an individual is aging relative to each calendar year increase in chronological age. DunedinPoAm, which stands for Dunedin Study Pace of Aging from methylation, was developed by Dr. Daniel Belsky et al. (Belsky et al. 2020) to quantify the rate of biological aging based on changes in several age-related blood-based biomarkers that reflect the integrity of

the cardiovascular, metabolic, renal, hepatic, immune, periodontal, and pulmonary systems. An enhanced pace of aging metric, DunedinPACE (Belsky et al. 2022), was later developed by the same investigators; PACE stands for *Pace of Aging Calculated from the Epigenome*. Unique and important aspects of DunedinPACE are that it was developed using data from the Dunedin study cohort, which consisted of 1037 individuals who were born in the same city (Dunedin, New Zealand) as part of a single birth cohort (born 1972–1973) and assessed at the same four time points (baseline and 6, 12, and 20 years thereafter) at the same ages (26, 32, 38, and 45 years of age). DunedinPACE is comprised of the following 19 biomarkers: body mass index (BMI), waist-to-hip ratio, glycated hemoglobin, leptin, mean arterial blood pressure, $\text{VO}_{2\text{max}}$ as a measure of cardiorespiratory fitness, forced vital capacity ratio (FEV1/FVC), forced expiratory volume in one second (FEV1), total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, lipoprotein(a), apolipoprotein B100/A1 ratio, estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), high-sensitivity C-reactive protein (hs-CRP), white blood cell count, mean periodontal attachment loss, and the number of tooth surfaces affected by dental caries (as a reflection of tooth decay). The advantages of DunedinPACE over DunedinPoAm (as stated by the investigators who developed these metrics) are that it includes four time points (versus three time points) over a 20-year follow-up period (versus 12 years of follow-up) and includes only highly reliable DNA methylation probes that were demonstrated to have low variability across replicate measurements (vs. including all CpG sites without restriction).

In the CALERIE™ 2 trial, the pace of aging based on DunedinPACE was slower in the CR group than in the AL group (Waziry et al. 2023; Rubin 2023). Reductions in DunedinPACE after 1 year of CR (Cohen's $d = -0.29$; 95% confidence interval -0.45 to -0.13) were maintained at the 2-year time point (Cohen's $d = -0.25$; 95% confidence interval -0.41 to -0.09 ; $P < 0.003$ for both time points). These results correspond to a favorable average reduction of 2–3% in the pace of aging among CR participants (Waziry et al. 2023), with larger than 3% improvements among some participants, but worsening observed in other participants. Interestingly, a dose-response relationship was observed: the CR treatment effect of achieving >10% CR was $d = -0.33$ at 1 year and $d = -0.33$ at 2 years, whereas the effects were less in the group that achieved <10% CR ($d = -0.19$ at 1 year; $d = -0.14$ at 2 years). An instrumental variable analysis that modeled the effect of 20% CR revealed that the treatment effect would be $d = -0.43$ (95% confidence interval -0.67 to -0.19) at 1 year and $d = -0.40$ (95% confidence interval -0.67 to -0.12) at 2 years ($P < 0.005$ for both).

1.4.2 Calorie Restriction and Phenotypic Aging Biomarkers

Cardiovascular and Cardiometabolic Disease Risk Phenotypic aging biomarkers are broad and reflect numerous risk factors for cardiovascular and cardiometabolic diseases, such as heart disease, stroke, atherosclerosis, and type 2 diabetes.

Decreasing risks for these diseases reduces lifetime coronary events and morbidity (Hwang et al. 2018). Cardiovascular and cardiometabolic benefits of long-term CR in the CRON population included lower blood pressure, lower glucose and insulin concentrations, and a more favorable blood lipid profile (i.e., lower total and LDL-cholesterol, higher HDL-cholesterol, lower triglycerides, and lower ratio of total/HDL cholesterol). Importantly, several CRON participants provided medical records that preceded their initiation of a CR diet, providing evidence that their baseline values for the various health metrics were comparable to the age-matched referent (non-CR) group and that statistically significant and clinically meaningful improvements in these parameters occurred after following a CR diet (Holloszy and Fontana 2007).

In the CALERIE™ 1 CR intervention studies, 6 months and 1 year of CR led to significant improvements in blood pressure, insulin sensitivity, serum lipid and lipoprotein concentrations, and left ventricular diastolic function (Riordan et al. 2008). In the CALERIE™ 2 trial, 2 years of CR profoundly improved glucose regulation and insulin sensitivity, based on the homeostasis model assessment of insulin resistance (HOMA-IR), and improved multiple risk factors to levels well below the conventional risk thresholds used in clinical practice (Ravussin et al. 2015; Il'yasova et al. 2018; Kraus et al. 2019). Importantly, the baseline values already were in the normal range and the majority of vascular and metabolic indices improved significantly in the CR vs. AL group at 1 year, with maintenance of the improvements at 2 years.

Anthropometrics and Body Composition Midlife adiposity has been associated with earlier onset of Alzheimer's dementia, neuropathology, pre-symptomatic cerebral amyloid accumulation (Chuang et al. 2016), and frailty in old age (Stenholm et al. 2014). Anthropometric and body composition measures reflect whole-body fat mass as an index of adipose tissue-related health risk, regional adiposity as a reflection of metabolic risks associated with visceral and subcutaneous abdominal adiposity, lean body mass as a metric of sarcopenia risk, and bone mineral density as a metric of osteoporosis risk. CR has consistently been shown to improve adiposity-related anthropometric measures (i.e., reductions in body weight, body mass index [BMI], and waist circumference) and reduce adiposity (whole-body and regional) in animal models (Mattison et al. 2003) and in humans (Fontana et al. 2004). In the CALERIE™ 1 pilot trials of 6 months or 1 year of CR, significant reductions were observed for weight, BMI, waist circumference, whole-body fat mass (Das et al. 2007; Racette et al. 2006; Redman et al. 2007), and visceral and subcutaneous abdominal adipose tissue by computed tomography and magnetic resonance imaging (Racette et al. 2006). Results of the CALERIE™ 2 trial were consistent; CR induced significant reductions in whole-body fat mass (−5.4 kg) and central adiposity (6.1 cm decrease in waist circumference and 2.8 kg decrease in trunk fat mass determined by dual-energy x-ray absorptiometry) (Das et al. 2017). Additionally, 1 year of CR attenuated the increase in extramyocellular lipid content of the tibialis muscles of CR participants relative to AL controls (Sparks et al. 2017).

In contrast to the predominantly favorable changes in adiposity that occur with CR, the reduction in fat-free mass was not trivial (−2.0 kg) (Das et al. 2017). This

result is consistent with the composition of weight loss observed in many other dietary intervention studies that did not include an exercise component. A portion of the fat-free mass loss is attributable to bone loss, a potentially adverse consequence of long-term CR diets (Liu and Rosen 2023). In fact, change in bone mineral density was used as a safety metric during the CALERIE™ 2 intervention (Rochon et al. 2011). Three CR participants had decreases in bone mineral density of 5% or more from baseline, necessitating temporary discontinuation of CR for two participants and permanent discontinuation for one participant (Ravussin et al. 2015). In the overall CALERIE™ 2 sample, bone mineral density decreased significantly in the lumbar spine, total hip, femoral neck, and other regions of the hip at both 1 year and 2 years in the CR group relative to the control group (Villareal et al. 2016). Bone turnover, another metric of bone health, also showed adverse changes in response to CR, with bone resorption exceeding bone formation (Villareal et al. 2016). The influence of CR on bone architecture and quality has yet to be explored.

Core Body Temperature and Energy Metabolism Lower core body temperature and reductions in core body temperature in response to CR are considered favorable adaptations that may contribute to slower aging. This has been demonstrated in rodents, monkeys (Lane et al. 1996), and humans after 6 months of CR (Heilbronn et al. 2006), but not after 2 years of modest CR in the CALERIE™ 2 trial (Ravussin et al. 2015). Energy metabolism metrics that are used as indicators of energy conservation and potentially slowed aging include resting metabolic rate (RMR), RMR adjusted for changes in body composition (RMR residual), sleeping metabolic rate, and 24-hour sedentary energy expenditure. While acute weight loss is known to lower resting metabolism, the effects of longer-term CR have been variable in rhesus monkeys. In the CALERIE™ 1 pilot studies, 6 months of CR led to reductions in RMR (Das et al. 2007), sleeping energy expenditure (Heilbronn et al. 2006), and 24-hour sedentary energy expenditure in a metabolic chamber (Heilbronn et al. 2006). In the CALERIE™ 2 trial, the effect of the CR intervention on RMR residual was significant only at 1 year (Ravussin et al. 2015); the lack of difference between the CR and AL groups at 2 years may be attributable to the lower level of CR achieved during year 2 (~8.3% CR) than during year 1 (~15.2% CR) (Dorling et al. 2020).

Biological Age Biological aging algorithms based on phenotypic biomarkers (Hastings et al. 2019; Kwon and Belsky 2021) incorporate various indices, such as cholesterol, hemoglobin A1c, systolic blood pressure, white blood cell count, uric acid, and high-sensitivity C-reactive protein (Belsky et al. 2015). Phenotypic aging algorithms include the Kleméra–Doubal Method (KDM) Bioage (Kleméra and Doubal 2006), Homeostatic Dysregulation Index (HDI) (Cohen et al. 2013), and Levine phenotypic age (Levine 2013). KDM and HDI were assessed in the CALERIE™ 2 trial to address the question of whether long-term modest CR influences biological age. Favorable effects were observed: the CR group had a younger KDM biological age than the AL control group at both the 1-year and 2-year time points (treatment by time interaction: $\beta = 0.60$; 95% confidence interval -0.99 to 0.21 ; $P = 0.003$) (Belsky et al. 2017). In addition, KDM biological age advanced more

slowly during the 2-year CR intervention (0.11 years per calendar year, 95% confidence interval: -0.13 to 0.36) than during 2 years of the AL control condition (0.71 years per calendar year, 95% confidence interval: 0.41 to 1.01). Interestingly and not surprisingly, there was a trend for an inverse dose-response relationship between %CR and the rate of biological aging: CR participants who achieved a higher %CR exhibited a slower rate of biological aging compared to participants who were less adherent to the CR diet (Belsky et al. 2017). Consistent with the KDM Bioage result, HDI was suppressed significantly in CR but not AL ($P < 0.001$). An important observation, based on sensitivity analyses, was that the beneficial effects of CR on biological age were independent of weight loss, challenging the view that weight loss is a prerequisite for CR-related improvements in biomarkers of aging and longevity.

1.4.3 Calorie Restriction and Functional Aging Biomarkers

Cardiorespiratory Fitness Arguably, the most relevant biomarker of functional aging is cardiorespiratory fitness, which has been deemed a vital sign due to its very strong association with longevity in numerous large-scale epidemiologic studies in the United States and worldwide (Hanscombe et al. 2021; Davidson et al. 2018). The Henry Ford Exercise Testing Project of more than 57,000 adults revealed that cardiorespiratory fitness was a strong predictor of survival during more than 10 years of follow-up (Blaha et al. 2016). Similarly, the Cooper Center Longitudinal Study of 16,533 adults indicated that lower cardiorespiratory fitness was associated with a higher risk of cardiovascular death during 28 years of follow-up (Wickramasinghe et al. 2014). The largest study, which included 498,135 participants in the UK Biobank, demonstrated that lower cardiorespiratory fitness was associated with higher mortality during 4.9 years of follow-up (Celis-Morales et al. 2017).

The gold standard measure of cardiorespiratory fitness is maximal oxygen consumption ($\text{VO}_{2\text{max}}$), determined during a graded exercise test with respiratory gas exchange analysis. In the CALERIE™ 2 trial of 2 years of CR, $\text{VO}_{2\text{max}}$ was quantified using the Cornell treadmill protocol (Racette et al. 2017). $\text{VO}_{2\text{max}}$ increased when expressed relative to body mass ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), which is the most common metric used when classifying an individual's fitness level based on sex- and age-specific reference tables (Kaminsky et al. 2015) or assessing changes in fitness over time. In CALERIE™ 2, relative $\text{VO}_{2\text{max}}$ increased 5% from baseline to 2 years, whereas a decrease of 3% was observed in the AL control group (Racette et al. 2017). Exercise time during the $\text{VO}_{2\text{max}}$ treadmill test also increased to a greater extent in the CR group (+2.9 min) than in the AL group (+1.8 min), suggesting that aerobic capacity and endurance were enhanced by CR. In contrast, absolute $\text{VO}_{2\text{max}}$, expressed as L/min, decreased in both the CALERIE™ 1 trial (Weiss et al. 2007) and the CALERIE™ 2 trial (Racette et al. 2017), likely due to smaller body size and smaller muscle mass after the CR interventions.

Functional Aging Biomarkers Other biomarkers of functional aging include gait speed, energy efficiency, muscle strength, grip strength, frailty, fatigue, cognition, and

sleep quality. Consistent with the reductions in lean body mass that accompany long-term CR, muscle strength of the knee extensors and knee flexors decreased in absolute terms in CALERIE™ 1 (Weiss et al. 2007) and CALERIE™ 2 (Racette et al. 2017). However, when expressed relative to body mass, muscle strength was either unchanged (Weiss et al. 2007) or increased (Racette et al. 2017) after 1 or 2 years of CR, respectively. The clinical implications of these findings with aging are uncertain. Hand grip strength did not change significantly in CALERIE™ 2 (Villareal et al. 2016). Male C57BL/6NCrl mice that followed a 15% CR regimen initiated at 4 months of age and continued throughout life demonstrated greater limb grip strength at ages 10 and 18 months, but not at ages 26 or 28 months, relative to controls (Peters et al. 2022).

Cognition, Sleep, and Hunger CR is proposed to have benefits on cognitive health for individuals with or at risk for various neurodegenerative diseases (Dias et al. 2020). In the CALERIE™ 2 trial, spatial working memory improved on the Cambridge Neuropsychological Test Automated Battery (CANTAB), whereas other indices of cognitive function remained unchanged (Leclerc et al. 2020). The change in spatial working memory was not associated with diet quality, assessed using either the Dietary Inflammatory Index or the Healthy Eating Index (Silver et al. 2023). While there is intriguing data that CR may improve cognition in animal models, depending on the level of CR achieved and the time of life that it is initiated (Dias et al. 2020), human trials of adults with normal cognition at baseline have not shown impressive effects of medium-term or long-term CR interventions thus far. It is likely that much longer-term CR and follow-up periods or older adult populations are required to determine the impact of CR on cognitive health. Sleep quality often deteriorates with aging, and there is an extensive array of adverse health consequences associated with sleep deprivation and poor sleep quality. Sleep metrics did not change after 2 years of CR in the CALERIE™ 2 trial; the only benefit observed was on sleep duration at the 1-year time point (Martin et al. 2016). Perceived hunger assessed by the Eating Inventory did not change in the CR group at 1 year or 2 years in the CALERIE™ 2 trial (Ravussin et al. 2015), whereas hunger assessed using a visual analog scale increased to a small but statistically significant extent (~3 mm on a 100 mm scale) in the CR group during the 2-year intervention (Dorling et al. 2020).

1.5 Other Considerations of Calorie Restriction on Biomarkers of Aging

As is evident from studies of CR in humans, non-human primates, and other organisms, the influence of CR on biomarkers of aging is variable and dependent on numerous factors. The level of CR, duration of CR, quality of the CR diet, and age at which CR is initiated appear to be of major importance for many biomarkers. Additional factors that would be expected to impact one or more aging biomarkers include specific dietary components, physical activity and exercise patterns, cardiorespiratory

fitness, sleep quality, stress, tobacco use, alcohol consumption, environmental exposures, sociodemographic characteristics, disease risk factors and comorbidities that are already present, fetal imprinting, genetic traits, and epigenetic modifications.

1.6 On the Horizon

The CALERIETM trial demonstrated numerous impressive benefits on biological aging and markers of health span in healthy adults, but it remains unknown whether rates of biological aging and risk of age-related diseases can be attenuated with exposure to a CR intervention in early to mid-adulthood in humans. To address this important question, the CALERIETM Legacy study (R01 AG071717, NCT05651620, Das SK—Contact PI), also funded by the National Institute on Aging, currently is exploring whether participation in the CALERIETM 2 trial several years ago has had long-term impacts on aging or aging biomarkers. Results from the CALERIETM Legacy study will provide novel and valuable information on the relationship between CR during early to mid-adulthood and cellular, phenotypic, and functional aging biomarkers 12–19 years later. Lifestyle behaviors during the intervening 12 to 19 years are essential to consider; therefore, a comprehensive dietary and physical activity survey is capturing these lifestyle aspects.

The National Institute on Aging is funding other cooperative agreement research trials of CR in humans. These prospective, randomized controlled intervention studies are being conducted in two distinct age groups of adults. The trial of adults aged 25–45 years is titled “A planning project to pilot test and optimize dietary approaches to slow aging and design a long-term trial” (U01AG073204, NCT05549362, Martin CK—Contact PI). Also referred to as the “DiAL Health Research Study” (for Dietary Approaches to Longevity and Health), this trial involves a 6-month intervention of 25% CR or time-restricted eating (TRE), with a target enrollment of 90 adults without obesity who will be randomized to one of five groups: traditional CR, adaptive CR, traditional TRE, adaptive TRE, or ad libitum control. The trial of adults aged 60 years and older is titled “Health, Aging and Later-Life Outcomes Pilot Trial” (HALLO-P, U01AG073240, NCT05424042, Kritchevsky SB—Contact PI). This trial involves a 9-month intervention of 20% CR, delivered in person or remotely, versus TRE, with a target enrollment of 120 participants who have obesity or overweight with at least one comorbidity. These two planning studies will be used to refine the interventions and to develop longer-term, larger-scale trials to evaluate the effects of long-term CR and/or TRE on various biomarkers of aging.

As new aging biomarkers continue to emerge, it will be critical to capitalize on biobanks of biological samples obtained from geroscience trials, as well as to explore opportunities to gain additional insight from novel intervention studies and observational cohorts.

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Part II

Protein Restriction

Chapter 2

Protein Restriction in Aging and Disease



Sebastian Brandhorst

Abstract Protein restriction has emerged as a promising dietary intervention to promote health and combat age-related diseases. Extensive research conducted in animal models, including mice and primates, as well as data from selected clinical trials, has shed light on the molecular, cellular, and systemic responses to protein restriction. In this review, I summarize the key findings from preclinical and clinical studies, focusing on the effects of protein restriction on aging and disease prevention. Additionally, I discuss current strategies for implementing protein restriction and its potential for improving therapeutic outcomes in various pathological conditions.

2.1 Introduction

Aging and Age-Related Diseases Aging is an inevitable biological process that affects all living organisms. Aging is associated with a gradual decline in physiological function and an increased susceptibility to age-related diseases, such as cardiovascular disorders, neurodegenerative conditions, cancer, and metabolic syndromes. These diseases not only impair the quality of life but also pose a significant social and economic burden on individuals and healthcare systems worldwide. Age-related diseases have emerged as major public health concern since due to their prevalence and to the global increase in life expectancy, the burden of age-related diseases is projected to escalate in the coming decades. Thus, effective strategies for disease prevention, early detection, and intervention are urgently needed to mitigate the individual and societal consequences.

Organismal aging is a complex and multifactorial process influenced by a combination of genetic, environmental, and lifestyle factors. The interplay between

S. Brandhorst (✉)
Leonard Davis School of Gerontology, University of Southern California,
Los Angeles, CA, USA
e-mail: brandhor@usc.edu

these factors gives rise to a wide range of molecular, cellular, and physiological changes that contribute to the aging phenotype. Key hallmarks of aging include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, and immune system dysregulation (Lopez-Otin et al. 2013). These interconnected mechanisms shape the aging process and predispose individuals to age-related diseases (Lopez-Otin et al. 2013). Advancements in our understanding of the biology of aging have opened new avenues for exploring interventions that can promote healthy aging and delay the onset of age-related diseases. By deciphering the intricate mechanisms underlying the aging process, scientists have identified potential strategies, including dietary modifications, for disease intervention and prevention (Longo and Finch 2003; Brandhorst and Longo 2019; Fontana et al. 2010, 2014). Furthermore, insights gained from studying model organisms, such as mice, primates, and other non-human species, have paved the way for translational research in humans (Lee et al. 2021; Di Francesco et al. 2018; Ingram et al. 2006).

Dietary Interventions and Protein Restriction Diet plays a crucial role in maintaining overall health and well-being throughout the life span. In recent years, dietary interventions including caloric restriction, intermittent fasting, fasting-mimicking diets, time-restricted eating, or isocaloric modification of specific dietary components (such as protein restriction) have gained attention as potential strategies to promote healthy aging and prevent age-related diseases (Di Francesco et al. 2018; Solon-Biet et al. 2015a). Among these interventions, protein restriction has emerged as a promising approach that has been extensively studied in various model organisms and human populations (Fontana and Partridge 2015; Minor et al. 2010; Mirzaei et al. 2016; Pallavi et al. 2012). The manipulation of protein intake, specifically reducing its quantity or altering its composition, holds the potential to modulate molecular, cellular, and systemic processes that influence aging and disease progression. However, some dietary approaches may combine lowered caloric intake and the modification of dietary composition. For example, the fasting-mimicking diet combines a low protein diet with low caloric intake and a composition that mimics fasting (Brandhorst et al. 2015). Therefore, separating the effects of reduced caloric intake from specific composition changes like reducing proteins is important and can be achieved by introducing isocaloric experimental conditions. Speakman and colleagues performed a detailed meta-analysis comparing published effects of dietary interventions on life span in mice and rats and concluded that although there is evidence for life span extension from PR alone, the effects are substantially reduced compared with those reported associated with CR (Speakman et al. 2016). Although the following chapter outlines the general health benefits that have been reported for protein restricted dietary regimens, it should be noted that a careful evaluation of the exact feeding paradigm remains paramount for all studies. In addition, while mice and primates are opportunistic omnivores, the role of plant vs. animal-derived protein in health and life span extension has not been established in detail. Another major limitation of the experiments designed to evaluate protein

sources is that often only one type of animal protein (such as casein) is compared with one type of vegetable protein (typically soya); this clearly is not an accurate reflection of the complex composition of most human diets.

Proteins are essential macronutrients composed of amino acids, which are the building blocks for numerous biological processes including in tissue repair, enzymatic reactions, immune function, hormone regulation, and many other physiological functions. However, excessive protein intake, particularly from animal sources, has been implicated in the development of chronic diseases, including cardiovascular disorders, diabetes, certain cancers, and age-related conditions. Therefore, balancing protein intake to meet nutritional requirements while avoiding potential adverse effects is an important consideration in promoting healthy aging.

Protein restriction elicits a range of cellular and systemic responses that have implications for health and longevity. At the cellular level, protein restriction can influence metabolic adaptations, promoting mitochondrial efficiency and reducing oxidative stress. It may also modulate inflammation and immune function, leading to a more balanced and responsive immune system. Systemically, protein restriction has been associated with improvements in body composition, metabolic health, insulin sensitivity, and life span extension in various model organisms. Understanding these responses is crucial for assessing the potential benefits and limitations of protein restriction in promoting healthy aging.

While much of the initial research on protein restriction has been conducted in model organisms, human studies have shed light on its effects and translational potential. Clinical trials and epidemiological studies have explored the impact of protein restriction on various health outcomes, including muscle mass and strength, metabolic health, cognitive function, and disease prevention. These studies provide valuable insights into the feasibility, safety, and effectiveness of protein restriction in real-world settings.

2.2 Health Benefits of Protein Restriction in Mammalian Animal Models

2.2.1 Systemic Responses

Protein restriction can elicit systemic responses that have been associated with life span extension and improvement in health span, which refers to the period of life characterized by health and functionality. These responses involve the delay of aging processes, activation of anti-aging pathways, reduction in age-related diseases, and modulation of aging-related biomarkers. It influences cellular senescence, reducing the accumulation of senescent cells and their associated detrimental effects. Protein restriction also affects age-related changes in tissue morphology and function, preserving tissue integrity and maintaining organ function. Additionally, protein restriction can extend life span and improve health span through enhanced

stress resistance, reduced inflammation, decreased oxidative stress, and metabolic adaptations. Collectively, these systemic responses contribute to the promotion of healthy aging and may have implications for extending life span and maintaining optimal health. In mammals, protein restriction causes systemic responses related to body composition and weight regulation, including a reduction in adiposity, preservation of lean body mass, and alterations in energy balance. These responses contribute to the maintenance of a healthy body weight and may have implications for the prevention of obesity and related metabolic disorders, well-recognized risk factors for aging-related diseases and accelerated aging.

Protein restriction also influences insulin sensitivity; improves glucose homeostasis, lipid metabolism, and overall metabolic profile; and may have implications for the prevention and management of metabolic disorders such as type 2 diabetes. Notably, health benefits associated with protein restriction in mammals include reduced blood pressure and systemic inflammation, improvement in neurological function and neuroprotection, and reduced risk of cardiovascular diseases and cancer, as well as neurodegenerative diseases.

Changes in Body Composition Protein restriction has been shown to decrease adipose tissue mass, particularly white adipose tissue (WAT) (Lacroix et al. 2004; Dommerholt et al. 2021), due to decreased lipid synthesis and increased lipolysis and the subsequent mobilization/utilization of stored fat for energy production (Trautman et al. 2022). For example, in mice, the nutritional deprivation of leucine decreases adiposity by stimulating lipolysis in WAT and upregulating of UCP-1 in the brown adipose tissue (Cheng et al. 2010). The decrease in WAT mass may be associated with improved metabolic health and a decreased risk of obesity-related complications. Despite a decrease in overall body weight, moderate protein restriction tends to preserve lean body mass, including muscle mass (Wei et al. 2017).

Cognitive Health Protein restriction promotes synaptic plasticity, the central mechanism underlying learning and memory processes in the brain, through the enhanced production and/or release of neurotransmitters, including acetylcholine and glutamate, essential for neuronal communication (Mattson 2005, 2012; Mattson and Arumugam 2018). Importantly, the dietary restriction of protein has also been associated with an increased formation of new neurons, known as neurogenesis, in specific brain regions, such as the hippocampus (Brandhorst et al. 2015). Neurogenesis plays a crucial role in learning, memory, and overall brain health. By promoting neurogenesis, protein restriction may enhance cognitive function and provide resilience against age-related cognitive decline. As outlined in greater detail below, protein restriction can induce potent systemic anti-inflammatory effects that also effect the central nervous system, by decreasing the production of pro-inflammatory cytokines and inhibiting the activation of microglial cells. By dampening neuroinflammation, protein restriction helps protect against neuronal damage and neurodegenerative diseases (Rangan et al. 2022; Parrella et al. 2013). In combination, these systemic responses contribute to the prevention of age-related cognitive decline, Alzheimer's disease, and other neurodegenerative conditions.

Alterations in Energy Balance Energy intake in mammals is regulated by neuropeptides and signaling molecules in the hypothalamus. The increased expression of neuropeptide Y (Npy) and agouti-related peptide (Agrp) stimulates caloric intake, whereas the upregulation of pro-opiomelanocortin (Pomc) and cocaine and amphetamine-regulated transcript (Cart) reduces caloric intake (Wilding 2002; Schwartz 2010; Heisler and Lam 2017). The mammalian target of rapamycin (mTOR) expression is increased under energy surplus and responds to amino acid availability (Beugnet et al. 2003; Kapahi et al. 2010). The protein leverage hypothesis proposes that a dominant appetite for protein in conjunction with a decline in the ratio of protein to fat and carbohydrate in the diet drives excess energy intake and could therefore promote the development of obesity. Consistent with this, moderate protein-restricted diets result in hyperphagia in rodents (Solon-Biet et al. 2014; White et al. 1994, 2000) and in some (Griffioen-Roose et al. 2012, 2014) but not all human studies (Martens et al. 2014; Du et al. 2000). Severe protein restriction below a certain threshold leads to a reduction in food intake in rodents (White et al. 2000; Du et al. 2000). Ghrelin and leptin are two important hormones involved in the regulation of appetite and energy balance (Klok et al. 2007). Protein restriction can modulate the secretion and signaling of these hormones. It may lead to decreased ghrelin levels, reducing appetite and promoting satiety. Additionally, protein restriction can increase leptin sensitivity, enhancing the hormonal signaling related to energy balance and weight regulation. Protein restriction thereby activates AMP-activated protein kinase (AMPK), a key cellular energy sensor that modulates various metabolic adaptations, such as enhanced glucose uptake, fatty acid oxidation, and mitochondrial biogenesis to help maintain energy balance and improve metabolic health during protein restriction (Minokoshi et al. 2002). While a number of studies indicate that increased protein consumption increases satiety and thereby naturally reduces food intake (Solon-Biet et al. 2014; Keller 2011; Huang et al. 2013), several studies now demonstrate that very-low-protein diets can decrease body fat and improve glucose tolerance (Wu et al. 2021). Additionally, protein restriction can enhance energy expenditure through increased thermogenesis (Laeger et al. 2014), mitochondrial activity (Simpson et al. 2017; Solon-Biet et al. 2015b), and fat oxidation (Wu et al. 2021).

Metabolic Adaptation Protein restriction in mice induces a range of metabolic adaptations that contribute to improved metabolic health and optimize energy utilization based on the bioavailability of carbohydrate, lipid, or proteins (Pezeshki and Chelikani 2021). Mammalian cells undergo metabolic reprogramming that involve metabolic adaptations that prioritize the utilization of alternative energy sources, enhance mitochondrial function, and promote metabolic flexibility to cope with reduced protein availability, which also may play a role in the observed benefits of protein restriction on aging and disease prevention.

Dietary modifications that limit the bioavailability of protein lead to changes in amino acid metabolism and utilization (Gerhart-Hines et al. 2007). Adaptations that help maintain amino acid balance and optimize cellular functions include the

conservation of essential amino acids for vital cellular processes, such as protein synthesis and enzymatic activities, while stimulating the recycling and reutilization of amino acids through autophagy and/or the ubiquitin-proteasome system-mediated degradation of cellular components (Henagan et al. 2016; Uriarte et al. 2021).

Protein restriction promotes the utilization of fatty acids as an alternative energy source, which in turn helps preserve glucose and amino acids for other essential cellular functions (Gerhart-Hines et al. 2007). This metabolic adaptation plays a crucial role in maintaining energy balance and is regulated by various molecular mechanisms. Protein restriction increases the expression and activity of peroxisome proliferator-activated receptors (PPARs), a group of nuclear receptors that activate genes involved in fatty acid oxidation and promote the breakdown of fatty acids (Contreras et al. 2013). For example, protein restriction increases the expression of PPARs and their target genes, including carnitine palmitoyltransferase I (CPT-I), which is responsible for the transport of long-chain fatty acids into the mitochondria, where they undergo beta-oxidation to generate energy (Contreras et al. 2013). Another mechanism by which protein restriction promotes fatty acid oxidation is through the activation of adenosine monophosphate-activated protein kinase (AMPK). AMPK is a cellular energy sensor that regulates various metabolic processes, including fatty acid oxidation. Protein restriction activates AMPK, leading to the phosphorylation and activation of enzymes involved in fatty acid oxidation, such as acetyl-CoA carboxylase (ACC) and hormone-sensitive lipase (HSL) (Srivastava et al. 2012). The activation of these enzymes promotes the breakdown of fatty acids and their subsequent oxidation. In addition, protein restriction affects hormone signaling pathways involved in lipid metabolism. Protein restriction reduces circulating levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-C), commonly referred to as “bad cholesterol” (Solon-Biet et al. 2015b; Trevino-Villarreal et al. 2018; Fontes et al. 2018). Simultaneously, it can increase high-density lipoprotein cholesterol (HDL-C), known as “good cholesterol” (Fontes et al. 2018). This response contributes to the metabolic adaptations observed during protein restriction, including a decrease in adipose tissue mass and improvements in lipid metabolism that, combined, result in a lower risk of atherosclerosis and cardiovascular complications.

Enhanced Mitochondrial Function Mitochondria play a critical role in energy production, metabolism, and cellular homeostasis (Kyriazis et al. 2022). Protein restriction has been demonstrated to improve mitochondrial efficiency and function, contributing to overall cellular and metabolic health. Protein restriction stimulates mitochondrial biogenesis (the formation of new mitochondria within cells) through various signaling pathways, including the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) pathway (Perrone et al. 2010). Protein restriction upregulates PGC-1 α expression and activity, leading to increased mitochondrial biogenesis. The formation of new mitochondria improves cellular energy production capacity and enhances overall mitochondrial function. Protein restriction improves mitochondrial quality control mechanisms, ensuring the removal of damaged or dysfunctional mitochondria through a process called mitophagy (Zhang

et al. 2022). Mitophagy eliminates impaired mitochondria and maintains a healthy mitochondrial pool. Protein restriction activates mitophagy pathways, such as the PINK1-Parkin pathway, promoting the selective degradation of damaged mitochondria and preserving mitochondrial integrity (Ding and Yin 2012; Wang et al. 2022). Protein restriction influences mitochondrial dynamics, the processes of mitochondrial fusion and fission, which regulate mitochondrial morphology and function. It promotes mitochondrial fusion, leading to larger and more interconnected mitochondria. This fusion event enhances mitochondrial bioenergetics optimizes mitochondrial network organization and improves overall mitochondrial functionality (Kyriazis et al. 2022; Putti et al. 2015).

Altered Glucose Metabolism Protein restriction influences glucose metabolism by modulating insulin sensitivity and glucose uptake (Green et al. 2022). Protein restriction improves insulin sensitivity, which is the ability of cells to respond to insulin and efficiently take up glucose from the bloodstream. Protein restriction reduces insulin secretion, leading to decreased levels of circulating insulin. This reduction in insulin levels improves insulin sensitivity and enhances the cellular response to insulin, facilitating glucose uptake by peripheral tissues such as skeletal muscle and adipose tissue (Yanagisawa 2023). On the other hand, protein restriction modulates gluconeogenesis (the process by which the body produces glucose from non-carbohydrate sources) by reducing the availability of amino acids, which are essential substrates for glucose production. This decrease in amino acid availability results in decreased gluconeogenesis, helping to maintain stable blood glucose levels (Toyoshima et al. 2010). Protein restriction also affects the systemic glycogen metabolism, which involves the breakdown (glycogenolysis) and synthesis (glycogenesis) of glycogen, the storage form of glucose in the liver and muscles (Krebs 2005). Protein restriction reduces glycogenolysis, thereby preserving glycogen stores and preventing excessive glucose release into the bloodstream. Additionally, protein restriction enhances glycogen synthesis, facilitating the storage of glucose as glycogen. Another mechanism by which protein restriction can influence glucose metabolism is by modulating the expression and activity of glucose transporters, such as GLUT4, which play a vital role in facilitating glucose uptake into cells. Protein restriction upregulates GLUT4 expression, particularly in the skeletal muscle and adipose tissue, leading to increased glucose transport into these tissues (Luo et al. 2019; Stone et al. 2014). This enhanced glucose uptake helps maintain glucose homeostasis and improves insulin sensitivity. As mentioned above, protein restriction affects lipid metabolism, which intersects with glucose metabolism. By promoting lipid oxidation and reducing lipid synthesis, protein restriction helps prevent the accumulation of lipids and spares glucose for other metabolic processes, contributing to improved glucose metabolism.

Inflammation and Immune Function Protein restriction in mice elicits cellular responses related to inflammation and immune function, influencing the overall immune response and systemic inflammation (Alwarawrah et al. 2018). It attenuates inflammatory signaling, modulates immune cell populations, improves immune

cell function, and suppresses the senescence-associated secretory phenotype (SASP). It decreases the levels of pro-inflammatory cytokines and markers of systemic inflammation, such as C-reactive protein (Brandhorst et al. 2015; Wei et al. 2017), and the suppression of nuclear factor-kappa B (NF- κ B) activation, a key transcription factor involved in the expression of pro-inflammatory cytokines and chemokines (Zhenyukh et al. 2018). By dampening NF- κ B signaling, protein restriction helps attenuate the production of pro-inflammatory cytokines, chemokines, and other inflammatory mediators. Protein restriction has been associated with decreased production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), which play crucial roles in initiating and sustaining inflammatory processes in the body (Sharma et al. 2019; Wanders et al. 2014). Another notable consideration is that protein restriction can impact the composition and function of the gut microbiota, which is intricately linked to inflammation (Rangan et al. 2019; Wu et al. 2022). The gut microbiota plays a crucial role in immune regulation and the maintenance of intestinal barrier function. Protein restriction was shown to promote the growth of beneficial bacteria and reduce the abundance of pro-inflammatory microbial species, thereby influencing immune and inflammatory responses (Rangan et al. 2019). These responses contribute to a more balanced immune response, reduced systemic inflammation, and potentially play a role in the beneficial effects of protein restriction on immune-related diseases.

Oxidative Stress Mitigation The cellular responses to protein restriction in mice include a reduction in oxidative stress, activation of adaptive stress response pathways, and preservation of mtDNA integrity and collectively contribute to improved cellular health and redox balance (Pamplona and Barja 2006). Protein restriction has been associated with antioxidant effects and reduces the production of reactive oxygen species (ROS) and subsequent accumulation of oxidative stress, thereby protecting against oxidative damage to blood vessels and cardiac tissues and contributing to cardiovascular health. These protective effects of protein restriction are based on enhanced cellular antioxidant defense mechanisms, such as the expression and activity of antioxidant enzymes (Richie et al. 1994; Ren et al. 2021; Cullinan and Diehl 2004). This response helps maintain redox balance and protect cells from oxidative damage. Furthermore, protein restriction can induce the activation of adaptive stress response pathways such as the sirtuin pathway and the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Cullinan and Diehl 2004; Nogueiras et al. 2012). These pathways play crucial roles in cellular adaptation to oxidative stress and modulate the expression of antioxidant enzymes and detoxification proteins, as well as maintenance of mitochondrial homeostasis.

2.2.2 *Hormonal Regulation*

Protein restriction affects hormonal signaling involved in metabolic regulation (Green et al. 2022). It leads to changes in the levels of hormones such as insulin, glucagon, and growth factors. These hormonal adaptations in response to reduced protein intake help regulate energy balance, glucose metabolism, and nutrient partitioning. The growth hormone (GH)-insulin-like growth factor 1 (IGF-1) axis is a tightly controlled hormonal system that influences growth, development, metabolism, and various physiological processes (Tatar et al. 2003; Brown-Borg 2009). Protein intake serves as a key regulator of this axis, influencing GH secretion and IGF-1 production.

In mammals, protein intake stimulates the release of GH from the anterior pituitary gland through multiple mechanisms. First, specific amino acids, especially branched-chain amino acids (BCAAs), stimulate GH release from the pituitary gland. Leucine, in particular, has been shown to have a strong stimulatory effect on GH secretion (Nair and Short 2005). Additionally, protein intake increases the plasma concentration of insulin, which inhibits the release of somatostatin, a hormone that suppresses GH secretion (Brown-Borg 2009). By reducing somatostatin levels, insulin indirectly promotes GH release. GH acts on target tissues, particularly the liver, to promote the synthesis and secretion of IGF-1. Here, protein intake provides the necessary amino acids for the liver to synthesize IGF-1, an autocrine and paracrine hormone with potent anabolic effects that upon binding to the IGF-1 receptors (IGF1R) in various tissues exerts its biological effects as a master regulator of cell growth, protein synthesis, and tissue development (Tatar et al. 2003). Notably, the GH/IGF-1 axis is tightly regulated through negative feedback mechanisms. As such, increased levels of IGF-1 in circulation exert negative feedback on the hypothalamus and pituitary gland, resulting in the reduced secretion of GH. Conversely, low levels of IGF-1 enhance GH secretion to stimulate IGF-1 production and restore homeostasis (Brown-Borg 2009). Although complex, research indicates that the extreme ranges of IGF-1 levels are associated with all-cause mortality: both high and low levels of IGF-1 increase mortality risk (thus following a U-shaped distribution), with a specific 120–160 ng/ml range being associated with the lowest mortality (Rahmani et al. 2022).

In summary, protein intake is a key regulator of the GH-IGF-1 axis in mammals. It stimulates GH secretion and provides the necessary amino acids for the liver to synthesize IGF-1. The GH/IGF-1 axis plays a crucial role in growth, development, and metabolism, and maintaining the balance of this hormonal system is essential for optimal physiological function.

2.2.3 *Cellular and Molecular Responses*

Protein restriction influences various molecular pathways downstream of the GH-IGF-1 axis that are intricately linked to aging and disease processes (Fontana et al. 2010; Bartke et al. 2013). The mechanistic target of rapamycin (mTOR) signaling pathway, specifically mTOR complex 1 (mTORC1), a key regulator of cell growth and metabolism, is one of the primary targets affected by protein restriction (Fontana et al. 2010). The inhibition of mTORC1 by protein restriction triggers a cascade of molecular events that contribute to the health-promoting effects of protein restriction. For instance, reduced mTORC1 activity promotes autophagy, a cellular recycling process crucial for maintaining cellular homeostasis and removing damaged components, thereby improving cellular quality control (Dossou and Basu 2019). During periods of limited protein availability, cells rely on autophagy to obtain amino acids for protein synthesis and energy production. Protein restriction induces the activation of autophagy-related genes and increases the formation of autophagosomes, the structures responsible for sequestering cellular material for degradation. This upregulation of autophagy during protein restriction helps remove damaged or unnecessary proteins, contributing to improved cellular quality control and proteostasis (Dossou and Basu 2019). Additionally, protein restriction has been found to enhance the clearance of protein aggregates and improve the folding of newly synthesized proteins. This is achieved through the activation of molecular chaperones and the unfolded protein response, which ensure proper protein folding and prevent the accumulation of misfolded proteins (Hetz et al. 2020). The enhanced proteostasis observed during protein restriction contributes to cellular health and mitigates the detrimental effects of protein aggregation, which are often associated with aging and neurodegenerative diseases.

Cellular senescence is a state of permanent cell cycle arrest that contributes to tissue aging and age-related diseases (Campisi et al. 2011; Kumari and Jat 2021). Protein restriction has been shown to modulate cellular senescence in mice, providing an additional potential mechanism for the anti-aging effects of protein restriction in multiple tissues. Reduced mTOR activity associated with protein restriction prevents the accumulation of senescent cells by limiting the replicative capacity and proliferation of senescent cells (Weichhart 2018). Moreover, protein restriction downregulates pro-inflammatory cytokines and chemokines, which are known to promote cellular senescence and the senescence-associated secretory phenotype (SASP) (Herranz et al. 2015; Laberge et al. 2015). The SASP is a pro-inflammatory secretome produced by senescent cells, which contributes to tissue dysfunction and systemic inflammation. By suppressing the SASP, protein restriction may attenuate the detrimental effects associated with cellular senescence. Together, these molecular responses contribute to the anti-aging effects of protein restriction and highlight its potential as a dietary intervention to mitigate age-related cellular dysfunction and promote healthy aging.

2.3 Protein Restriction in Humans

Not surprising, given the large percentage of overweight or obese subjects following unhealthy dietary meal patterns, in many Western societies, the estimated daily protein consumption is nearly double the recommended amount at approximately 90–100 g per day and consisting of up to 80% animal-based protein sources (Gardner et al. 2019). Notably, this excessive protein intake may induce insulin resistance, type 2 diabetes, as well as adverse health outcomes due to the overstimulation of the GH-IGF-1/mTOR pathway. In a weight loss trial, postmenopausal women with obesity consuming a relatively high protein diet (1.3 g/kg/day) prevented improvements in insulin sensitivity compared to women consuming a normal protein diet (0.8 g/kg/day) who lost the same amount of body weight (Smith et al. 2016). High protein intake, therefore, counteracts the beneficial effects of weight loss on insulin resistance, and the associated compensatory hyperinsulinemia potentiates pro-aging effects despite reductions in abdominal and liver adiposity. On the other hand, protein restriction in humans likely holds therapeutic potential for the prevention and risk reduction but also management of various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. However, due to the lack of comprehensive long-term clinical trials in humans, questions remain about the optimization of outcomes across various cohorts, including but not limited to sex, age, weight, etc. and relative risks such as safety and efficacy associated with their long-term implementation.

Cancer Risk Reduction and Cancer Therapy Protein restriction may help reduce the risk of certain types of cancers due to effects on cellular processes involved in tumor development, such as cell proliferation, angiogenesis, and immune surveillance. Protein restriction modulates hormone signaling, inhibits mTOR pathway activation, and reduces inflammation, all of which can contribute to the prevention of cancer initiation and progression. In a study investigating the relationship between diabetes-, cancer-, and all-cause-related mortality based on Cox proportional hazard ratios in the US representative NHANES dataset involving 6381 individuals aged 50 years and over that compared low (<10% of calories from protein)-to-moderate- (10–19% of calories from protein) and high (>20% of calories from protein)-protein-intake groups, higher protein levels were linked to a 74% increased risk of all-cause mortality and more than fourfold increase cancer-related mortality in subjects aged 50–65 years but with inverse effects in those 66 years and older (Levine et al. 2014). In fact, individuals aged >65 years who consumed a moderate protein intake and those consuming a high-protein diet had a 28% reduction in all-cause mortality (likely due to changes in nutritional requirements associated with macro-nutrient absorption and prevention of frailty/sarcopenia) and a 60% reduction in cancer mortality compared with those consuming low-protein diets, which was not affected when controlling for other fat or carbohydrate intake or the protein source.

IGF-I concentrations decrease with aging in humans, but in this dataset, individuals aged ≥ 50 years who consumed a high-protein diet also had higher IGF-I

concentrations. Elevated circulating IGF-I concentrations are associated with increased risk of developing certain malignancies (Giovannucci et al. 2003; Smith et al. 2000), and thus, it is possible that the benefits of the high-protein intake in those aged ≥ 65 years rely on maintaining “healthy” IGF-I concentrations in line with the u-shaped distribution of mortality risk and IGF-I levels (Rahmani et al. 2022), which could help maintain a healthy weight and preserve muscle mass, thereby preventing frailty and fall-associated hospitalization (Chang and Lin 2015). Similarly, in a prospective cohort study of over 85,000 women (aged 34–59 years at baseline) and nearly 44,500 men (aged 40–75 years at baseline), diets high in either animal-based sources of fat and protein were associated with a higher risk for mortality and cancer for men and women compared to vegetable-based sources of fat and protein (Fung et al. 2010). Unlike animal protein, plant protein has not been associated with increased IGF-1 levels (Allen et al. 2002; Holmes et al. 2002).

A study population from the NIH American Association of Retired Persons (AARP) Diet and Health Study cohort of 322,263 men and 223,390 women aged 50–71 years at baseline, with a 10-year follow-up, further supports these findings: men and women in the highest compared with the lowest quintile of red meat intakes had elevated risks for overall mortality and cancer mortality (Sinha et al. 2009). Additional evidence for a link between low-protein diets and cancer comes from the Japanese island of Okinawa where the traditional diet is very low in protein (9% of total calories), or about 20% reduced in overall calories consumed compared to the population of mainland Japan, and associated with a reduction in various forms of cancer, such as lymphoma and cancer of the prostate, breast, and colon compared to age-matched other Japanese or Americans (Willcox et al. 2007).

Another potential advantage of applying protein-restricted interventions, such as a fasting-mimicking diet, is the enhancement of the effectiveness of existing cancer therapies (Brandhorst and Longo 2016). It can sensitize cancer cells to treatments such as chemotherapy and radiotherapy by reducing their growth and enhancing their susceptibility to cell death (Caffa et al. 2020; Di Biase et al. 2016; Di Tano et al. 2020; Vernieri et al. 2018). Additionally, protein restriction may mitigate treatment-related side effects and improve overall patient outcomes (Caffa et al. 2020; Vernieri et al. 2018; de Groot et al. 2020).

Cardiovascular Disease Prevention and Management Protein restriction positively influences blood pressure regulation (Elliott 2003; Appel 2003) and endothelial function and reduces inflammation and oxidative, which helps mitigate the inflammatory processes that contribute to the development of cardiovascular diseases, and improves lipid profiles by lowering levels of triglycerides and LDL cholesterol while increasing HDL cholesterol. These systemic responses contribute to the prevention of atherosclerosis, hypertension, and other cardiovascular conditions. Furthermore, incorporating protein restriction as part of a comprehensive treatment plan may aid in disease management and improve cardiovascular outcomes. Increasing protein intake by 10% (or 5 g of protein) while decreasing carbohydrate intake by 10% (or 20 g carbohydrates) was correlated with a 5% increase in incidences of cardiovascular disease (CVD) in a Swedish study of 43,396 women

with an average follow-up of 15.7 years; interestingly, individuals substituted carbohydrates mostly with animal protein, thereby changing their overall protein/carbohydrate intake ratio (Lagiou et al. 2012).

In a cohort of 29,017 postmenopausal women without previous diagnosis of cancer, coronary artery disease (CAD), or diabetes, nutrient density models based on mailed questionnaires were used to estimate risk ratios from a simulated substitution of total and type of dietary protein (Kelemen et al. 2005). For women in the highest intake quintile, CAD mortality decreases by 30% from an isocaloric substitution of vegetable for animal protein. CAD mortality is associated with red meats and dairy products (Kelemen et al. 2005). Although no significant correlation between overall protein intake levels and ischemic heart disease (IHD) or stroke events was measurable among 43,960 middle-aged (53 ± 10 years) men during an 18-year follow-up, comparison of protein source groups provided further insight into the effects of animal-based protein compared with plant-based protein: an inverse correlation between plant-based protein intake and IHD or stroke incidence in the top compared with the bottom quintile, as well as a negative correlation between animal-based protein intake and IHD and stroke, has been demonstrated (Preis et al. 2010a, b). In the NHS and HPFS cohorts, after adjusting for major life-style and dietary risk factors, animal protein intake was not associated with all-cause mortality but was associated with higher cardiovascular mortality, whereas plant-derived protein was associated with lower all-cause mortality and cardiovascular mortality for both men and women (Fung et al. 2010; Song et al. 2016). Analyses of the NHS cohort of 84,136 women aged 30–55 years, with no known cancer, diabetes mellitus, angina, myocardial infarction, stroke, or other cardiovascular disease, showed that higher intakes of red meat, red meat excluding processed meat, and high-fat dairy were significantly associated with elevated risk of CAD. Vegetable protein was significantly associated with an 18% decreased risk when comparing the lowest and the highest intakes across quintiles. Higher intakes of poultry, fish, and nuts were significantly associated with lower risk of CAD (Bernstein et al. 2010).

Neurodegenerative Disease Prevention and Intervention Metabolic disorders such as obesity, dyslipidemia, and insulin resistance resulting from unhealthy dietary patterns and subsequent increased systemic inflammation can accelerate brain aging (Mattson and Arumugam 2018; Cunnane et al. 2020; Fontana et al. 2021). In line with this, in an analysis conducted in 1352 subjects with a mean age of app. 54 years, hypertension, diabetes, smoking, and obesity were associated with an increased rate of progression of vascular brain injury and global and hippocampal atrophy and decline in executive function a decade later (DeBette et al. 2011). Interestingly, white matter brain hyperintensity burden selectively increases in obese subjects with high visceral fat accumulation, independent of common obesity comorbidities such as hypertension, through increases in proinflammatory cytokines (Lampe et al. 2019). In Ecuador, a group of subjects exhibits a rare case of symmetrical dwarfism, known as Laron syndrome, characterized by very low (≤ 20 ng/mL) circulating IGF-I concentrations due to growth hormone receptor defi-

ciency (GHRD) (Nashiro et al. 2017). This cohort provides unique information on the role of the GH/IGF-I axis, regulated by proteins, on human aging and disease. Although only studied in a small cohort of 13 subjects with Laron syndrome compared to 12 unaffected relatives, the GHRD group displayed enhanced cognitive performance and greater task-related activation in frontal, parietal, and hippocampal regions that appear similar to the cognitive performance of young adults (Nashiro et al. 2017). These studies suggest that the reduction of dietary protein content and the resulting decrease in GH-IGF-1 signaling could be used as potential interventions to delay cognitive decline and delay the onset of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Longo and Mattson 2014). Among the neurological diseases, epilepsy has the oldest relationship with dietary modification as fasting and lower meat intake were among the earliest treatment regimens for this disease (Magiorkinis et al. 2014). Protein restriction promotes neuroplasticity, enhances mitochondrial function, and reduces inflammation and oxidative stress in the brain, and these systemic responses suggest that therapeutic protein restriction may help preserve cognitive function and delay the progression of neurodegenerative diseases.

2.4 Strategies for Preventing Disease and Improving Therapeutic Outcomes

Personalized nutrition approaches may be adopted to optimize the benefits of protein restriction for disease prevention and improving therapeutic outcomes. These strategies involve tailoring dietary interventions based on an individual's unique characteristics, including their health status, age, genetics, lifestyle, and nutritional requirements. For example, daily protein intake recommendations should be adjusted based on the subject's age due to changes in protein uptake in older people. Although many adults consume protein at or above the recommended daily allowance, 20–24% of women and 5–12% of men ≥ 51 years and older consume less protein than the estimated average requirement of 0.66 g/kg BW/day (Volpi et al. 2013; Berner et al. 2013). Adding subject-specific guidance by implementing well-educated dietitians to optimize nutritional support, as well as motivation, can increase adherence to the dietary intervention.

- (a) Nutritional assessments: Conducting comprehensive nutritional assessments, such as Mini Nutritional Assessment (Guigoz 2006) and the Mini-Nutritional Assessment-Short Form (MNA®-SF) for older adults >65 years or older (Doroudi et al. 2019; Kaiser et al. 2009), can help determine an individual's specific nutrient needs, including protein requirements, based on factors such as age, sex, body composition, and physical activity level, to guide the development of personalized protein restriction plans.
- (b) Individualized macronutrient ratios: Personalized nutrition approaches consider the optimal macronutrient ratios for each individual, taking into account

their specific health goals, metabolic profile, and dietary preferences. Individuals with certain health conditions, such as kidney disease, may require stricter protein restriction guidelines compared to others. For example, for patients with estimated glomerular filtration rate of 60 mL/min without diagnosed nephrotic syndrome, restricting daily protein intake to approximately 0.6 g/kg has been recommended (Ikizler et al. 2020), although these guidelines are now being carefully reviewed (Obeid et al. 2022).

- (c) **Monitoring and adjustments:** Regular monitoring of biomarkers, such as blood glucose, lipid profiles, and markers of inflammation, can help assess the effectiveness of protein restriction and guide necessary adjustments to the dietary plan. This monitoring ensures that the personalized approach remains aligned with the individual's health goals and prevents any potential adverse effects.

2.5 Long-Term Implications and Sustainability

Long-term implications and sustainability of protein restriction strategies are crucial for ensuring the maintenance of health benefits and adherence to dietary interventions (Nichols-English and Poirier 2000; Gibson and Sainsbury 2017).

- (a) **Education and behavioral support:** Providing education and behavioral support are essential components of long-term success. Informing individuals about the rationale, potential benefits, and practical implementation of protein restriction helps establish a solid foundation for sustained adherence to the dietary plan.
- (b) **Variety and flexibility:** Promoting dietary variety and flexibility within the framework of protein restriction can enhance long-term sustainability. Encouraging the consumption of a diverse range of protein sources, including plant-based proteins, helps maintain nutritional adequacy, accommodate personal preferences, and facilitate adherence over an extended period.
- (c) **Gradual transition and lifestyle integration:** Facilitating a gradual transition to protein restriction and integrating it into an individual's lifestyle promotes sustainability. Gradually reducing protein intake allows the body to adapt to the dietary changes and reduces the likelihood of adverse effects. Integration into daily routines, meal planning, and social situations helps individuals incorporate protein restriction seamlessly into their lives.

In summary, modernized strategies for preventing disease and improving therapeutic outcomes through protein restriction should involve personalized nutrition approaches, combination therapies, and the consideration of long-term implications and sustainability. By tailoring protein restriction to individual needs, combining it with synergistic interventions, and providing support for long-term adherence, these strategies aim to optimize the benefits of protein restriction and improve overall health outcomes.

2.6 Conclusion

Protein restriction is a promising intervention with high translational potential for disease prevention, to improve therapeutic outcomes, and to promote healthy aging. Studies in mice, primates, and humans have provided valuable insights into the molecular, cellular, and systemic responses to protein restriction and highlight the potential benefits of protein restriction in modulating aging processes and mitigating age-related diseases. These responses encompass diverse aspects, such as nutrient sensing pathways, metabolic adaptations, cellular signaling, gene expression, muscle mass, metabolic health, cognitive function, autophagy, and proteostasis to ensure cellular recycling and protein homeostasis, combined contributing to improved cellular function, systemic disease prevention, and longevity. However, challenges remain in optimizing protein restriction strategies, addressing individual variability, and translating findings into clinical practice. Further research is warranted to unravel the underlying mechanisms and refine personalized approaches to maximize the benefits of protein restriction by tailoring dietary interventions to individual needs and optimizing macronutrient ratios. Combining protein restriction with other therapies, such as exercise, pharmacological interventions, or nutritional supplements, may yield optimal effects and improve therapeutic outcomes.

Implementing protein restriction as a long-term dietary strategy requires careful consideration of nutritional adequacy and sustainability. Education, behavioral support, and gradual transitions are key components in promoting long-term adherence to protein restriction and integrating it into individuals' lifestyles. With a large portion of the US and other Western countries' population being overweight/obese, undoubtedly, weight loss interventions will reduce disease risk and improve health/life span. However, differentiating health benefits related to weight loss/anti-obesogenic interventions vs. actually affecting systemic biological aging remains a complication of many interventions. Nonetheless, the health benefits stemming from recommending healthy dietary patterns, such as the low-moderate protein Mediterranean diet, to the general public likely outweighs any concerns associated with the limited data derived from controlled clinical trials. The following nutritional and implementation strategies should be considered:

- (a) Adequate nutrient intake: When implementing protein restriction, it is essential to ensure adequate intake of other essential nutrients to meet the body's requirements for vitamins, minerals, essential fatty acids, and amino acids that may be limited due to reduced protein intake.
- (b) Gradual and individualized approach: Protein restriction should be approached gradually and tailored to individual needs and health conditions. Sudden and drastic reductions in protein intake may have adverse effects, especially for individuals with specific dietary requirements or medical conditions.
- (c) Long-term sustainability is crucial when implementing protein restriction as a dietary intervention. Compliance with dietary changes is more likely when they are practical, enjoyable, and align with cultural and personal preferences.

Promoting a variety of protein sources, largely focused on plant-based proteins, can help diversify the diet and maintain sustainability.

Working with healthcare professionals such as registered dietitians or nutritionists can help develop personalized protein restriction plans that consider these strategies.

In conclusion, protein restriction represents one of the most promising interventions with high potential for disease prevention and therapeutic strategies. An extensive body of research in animal models highlights its molecular, cellular, and systemic responses, implicating it in various physiological processes. Moving forward, personalized approaches, combination therapies, and a focus on long-term implications and sustainability will contribute to unlocking the full benefits of protein restriction and improving health outcomes for individuals seeking disease prevention and therapeutic intervention.

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Part III
Intermittent Fasting: Time Restricted
Eating

Chapter 3

Time-Restricted Eating: A Circadian Intervention for the Prevention and Management of Metabolic Diseases in Animal Models and Humans



Shaunak Deota and Emily N. C. Manoogian

Abstract Many fasting interventions of various durations, cadence, and magnitude of caloric restriction have demonstrated health benefits in both animal models and humans. All these interventions are focused on the duration of fasting and caloric restriction. Time-restricted feeding/eating (TRF/TRE) is unique in that it is based on circadian biology. TRF/TRE is a consistent daily eating window aligned to an individual's circadian patterns of behavior (such as sleep/wake cycles) and does not require any form of caloric restriction. Typically, it includes an eating window of 6–11 h aligned to the active phase resulting in 13–18 h of fasting each day, mainly during the sleep/rest phase. A wide variety of health benefits have been observed in both animal models and human trials. Like any behavioral intervention, the study design, implementation, and population being assessed are keys to data interpretation and can explain the difference in outcomes between studies. In this chapter, we will discuss the origins of TRF/TRE, current findings and mechanisms, critical factors to consider, how TRE can be applied to shift work, and the future of the field.

3.1 Introduction

Fasting occurs on multiple time scales, from within a day (i.e., time-restricted eating/feeding (TRE/TRF), 10-hour eating/14-hour fast), within a week (intermittent fasting such as the 5:2 diet: 2 days fasting in a week, or alternate day fasting, fasting every other day), or prolonged fasting (fasting for many days), all of which are discussed in this book. This chapter will discuss daily fasting, specifically time-restricted feeding/eating (TRF for animal models, TRE for humans).

TRE is a dietary regimen in which all calorie intake is restricted to a consistent period during the 24-hour day, resulting in a daily fasting window of 13–18 h

S. Deota · E. N. C. Manoogian (✉)

Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA

e-mail: emanoogian@salk.edu

(eating window of 6–11 h). There is no explicit limit on energy intake during eating hours. TRE allows water and medication (not supplements) consumption outside of the designated eating window, but in some cases, non-caloric beverages such as unsweetened tea or black coffee are permitted. The time of the fasting window should be customized to an individual based on their schedule and sleep time. TRF simply refers to non-human animal models. TRF requires at least an 8-hour eating window for animals to consume the same amount of food as controls.

Unlike other forms of fasting, TRE is solely focused on the daily timing of eating and does not require any sort of caloric restriction or specific diet. Fasting has typically been a focus of the nutrition field (with an emphasis on calories in and out); however, TRE emerged from circadian biology. Circadian biology is the study of 24-hour rhythms in cell function, physiology, and behavior. For instance, almost anything you would get checked at the doctor's office (heart rate, body temperature, enzyme production, metabolism, hormone release, blood pressure, glucose levels, etc.) has an approximately 24-hour rhythm. Even behaviors such as sleep/wake cycles, eating patterns, activity patterns, and cognitive ability have a daily pattern. This means you are physically and mentally a different person at different times of the day.

All these rhythms are controlled endogenously by the circadian system in your body, but they also incorporate external inputs such as light and food. Both light and food can shift the circadian system, with light having a more direct effect on the brain and behavior, and food having a more direct effect on peripheral organs.

The idea of TRF/TRE was built from over a decade of research in mice that found changes to circadian rhythms in peripheral organs and metabolism when food was consumed at different times of the day. In 2000, Damiola et al. found that by restricting feeding to rest phase/daytime hours (when mice are normally sleeping), the circadian rhythms in peripheral tissues shifted phase and uncoupled from central clocks in the brain (Damiola et al. 2000). In 2001, Stokkan et al. showed that by restricting food availability to 4 or 8 h during the day, the clocks in the liver shifted within 2 days, but the clocks in the brain did not shift (Stokkan et al. 2001). These studies were later supported by Vollmers et al. in 2009 who found that both endogenous circadian clocks and the timing of feeding influence gene expression in the liver (Vollmers et al. 2009). In 2007, Kohsaka et al. found that mice provided *ad libitum* access to food on a high-fat diet (used to induce obesity) changed their eating patterns from eating primarily at night to spreading their calories across the day and night (Kohsaka et al. 2007). This led to a very important and simple question. What happens if mice are fed a high-fat diet (HFD) but only have access to it at night, when they would normally eat? In 2012, Hatori et al. found that by restricting access to a HFD for 8 h at night, mice ate the same number of calories as mice with *ad libitum* access, but they gained 70% less body fat and did not develop fatty liver as the controls did (Hatori et al. 2012). This was the first use of the term time-restricted feeding (TRF). Since then, many TRF studies have been performed using eating windows ranging from 8 to 12 h and multiple diets (high-fat, high-sugar, combinations) to show consistent and significant health benefits. Based on these findings, clinical trials of TRE began and found similar results (Fig. 3.1).

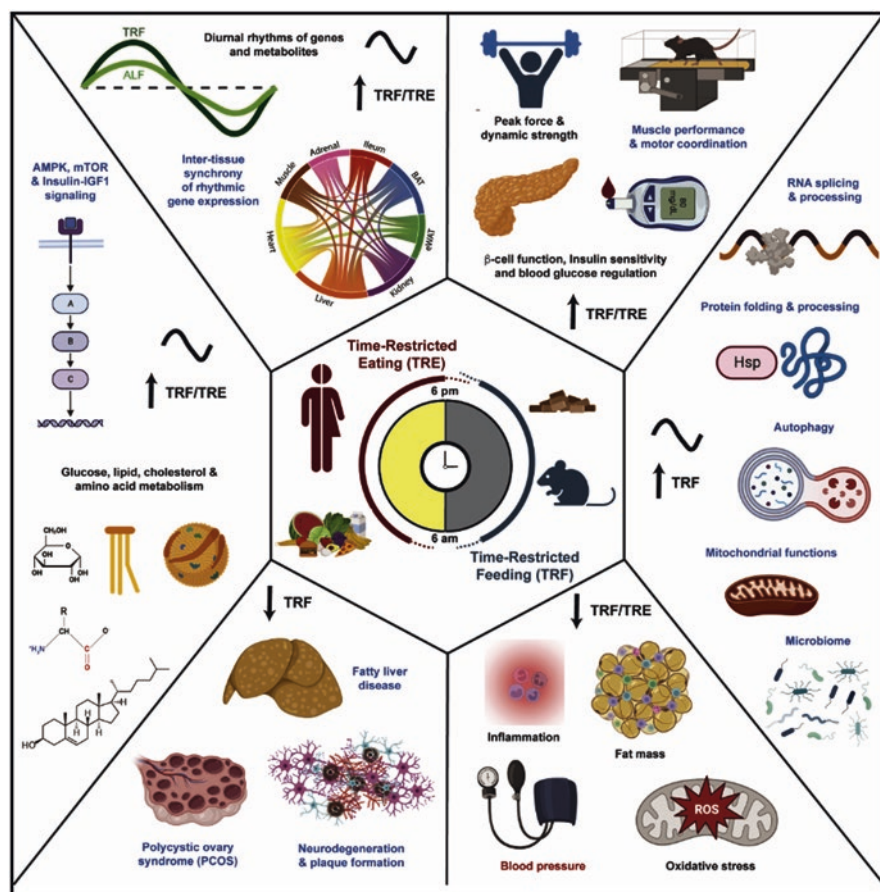


Fig. 3.1 Pleiotropic benefits of TRF and TRE in mice and humans respectively. The most common eating windows for TRF and TRE studies are indicated (center). Physiological benefits seen in both rodent and human studies are indicated in black text, evidence from human studies only is indicated in red text and evidence from rodent studies only is indicated in blue text. The wave symbol (~) indicates a change in rhythmicity. The up (↑) and down (↓) arrows indicate an increase or decrease in the biological function by TRF/TRE respectively. Image created with help from [Biorender.com](https://www.biorender.com)

In this chapter, we will discuss the health benefits, important considerations, mechanisms, and current findings for both TRF and TRE.

3.2 Time-Restricted Feeding (TRF)

3.2.1 *Physiological Benefits of TRF in Preclinical Rodent Models*

Since the consumption of HFD causes loss of daily eating rhythms in male mice and potentiates diet-induced obesity and insulin resistance, most of the research focuses on the role of TRF in protection from metabolic diseases. TRF paradigms having 8–10-hour feeding windows during the active/dark phase in diet-induced obese mice prevent HFD-dependent increase in body weight gain and adiposity, despite having similar calorie consumption (Hatori et al. 2012; Chaix et al. 2014; Sundaram and Yan 2016; Hua et al. 2023; Woodie et al. 2018). TRF also reduces the hypertrophy of adipocytes in multiple white and brown adipose tissue depots (Hatori et al. 2012; Chaix et al. 2014). Concomitant with a reduction in the size of adipocytes, there is less infiltration of pro-inflammatory macrophages in the white adipose tissues and less production of cytokines such as TNF α , IL-6, and IL-1 β (Hatori et al. 2012; Chaix et al. 2014). Moreover, TRF protects from fatty liver disease, reduces serum AST and ALT levels, and increases FGF21 and adiponectin (Hatori et al. 2012; Chaix et al. 2014; Hua et al. 2023). Furthermore, TRF improves type 2 diabetes by augmenting glucose tolerance and potentiating insulin sensitivity, as measured by a reduction in the serum leptin and fasting insulin levels, and enhanced mTOR and insulin-IGF1 signaling (IIS) in the liver (Hatori et al. 2012; Chaix et al. 2014). Interestingly, the metabolic benefits of TRF are present independent of the type of diet used to cause metabolic syndrome such as high fructose, high sucrose, or high fat and cholesterol, and linearly scale with the duration of fasting from 12 to 16 h (Chaix et al. 2014). Not only does TRF prevent metabolic diseases, but it also acts as a therapeutic intervention to improve health and reduce the symptoms of metabolic syndrome in obese mice models, indicating that it may also provide similar benefits in humans (Chaix et al. 2014). While most of these beneficial effects are observed in young male mice, our lab recently showed that TRF could improve liver health and augment glucose tolerance even in middle-aged mice and in females as well (Chaix et al. 2021; Duncan et al. 2016).

One of the major benefits of TRF is the restoration of daily physiological rhythms. Due to the dampening of feeding rhythms in diet-induced obesity, mice lose their daily rhythms in body temperature, energy expenditure, and nutrient metabolism. TRF-driven feeding-fasting cycles improve and sustain the daily respiratory exchange ratio (RER) and energy expenditure rhythms in mice, independent of sex and age (Hatori et al. 2012; Chaix et al. 2014, 2021; Hua et al. 2023; Woodie et al. 2018; Duncan et al. 2016). TRF also restores diurnal rhythmicity in gastric

vagal afferents (GVA) mechanosensitivity in obese mice, possibly potentiating satiety signaling (Kentish et al. 2018).

While most research has focused on the beneficial effects of TRF on liver and adipose functions, there are pleiotropic benefits on several other organ systems (Fig. 3.1). TRF improves gut microbial rhythms in the ileum and colon, alters bile acid and GLP-1 signaling, and improves the rhythmicity of intestinal metabolites (Zarrinpar et al. 2014; Dantas Machado et al. 2022; Xia et al. 2023). Interestingly, fecal microbiota transplant from TRF mice protects from fatty liver disease by modulating tryptophan derivatives and potentiating serotonin signaling (Xia et al. 2023). TRF also reduces disease severity, increases the number of colonic crypts, and prevents infiltration of leukocytes and macrophages in the crypts in the mouse model of DSS-induced colitis (Song et al. 2022). Additionally, TRF attenuates the symptoms of autoinflammatory arthritis and affects the expression of cell surface receptors and adhesion molecules in peripheral neutrophils and monocytes in K/BxN serum-transfer arthritis, an experimental mouse model of human autoimmune joint inflammation (Ella et al. 2022).

In normal and db/db mice, TRF improves heart functions and restores daily rhythms in heart rate and blood pressure by suppressing sympathetic activity and reducing cortisol levels during the light/inactive phase in mice (Hou et al. 2021; Tsai et al. 2013). Moreover, TRF reduces atherosclerotic lesion size and macrophage infiltration in plaques and lowers plasma cholesterol in APOE*3-Leiden.CETP mouse model of dietary hyperlipidemia (Panhuis et al. 2023). Furthermore, TRF improves muscle functions in obese mice as measured using grip strength and treadmill tests and enhances motor coordination as measured by rotarod test (Hatori et al. 2012; Chaix et al. 2014, 2021). This effect is both age and sex specific and independent of the duration of TRF. Both TRF and exercise have additive effects in improving glucose tolerance, insulin sensitivity, and IIS in the liver of young mice (Vieira et al. 2022). However, in 20-month-old obese males, exercise only improves physical performance, while TRF improves RER rhythms, glucose tolerance, and cardiac functions (Schafer et al. 2019).

Several studies have shown the role of TRF in improving brain health, especially in the context of neurodegenerative diseases. TRF improves spatial working memory and long-term synaptic potentiation (LTP) magnitude in obese mice without weight loss (Davis et al. 2021). TRF also reduces neuroinflammation, improves memory and cognitive functions, restores activity-sleep rhythms, increases amyloid- β peptide clearance, and reduces plaque deposition and disease progression in Alzheimer's disease mice models (Whittaker et al. 2023). Moreover, TRF improves locomotor activity, motor performance, and sleep rhythms and reduces heart rate variability and disease markers in Q175 (Wang et al. 2018) and the BACHD (Whittaker et al. 2018) mice models of Huntington's disease.

TRF has also been shown to improve reproductive functions in female mice. While females are resistant to diet-induced obesity and protected from adipose tissue inflammation, HFD causes ovarian follicular loss and affects estrus cycling. Ovariectomy (OVX), a model to mimic menopause, predisposes female mice to body weight gain and dampens daily feeding rhythms. TRF in both OVX and

ovary-intact mice protects from obesity, prevents ovarian follicle loss, restores estrus cyclicity, and improves fertility in obese female mice via FGF21 signaling on GnRH neurons (Chung et al. 2016; Hua et al. 2020). TRF also improves glucose and lipid metabolism in a mouse model of polycystic ovary syndrome (PCOS), ameliorates hyperandrogenemia, reduces cyst formation, and promotes corpus luteum formation (Han et al. 2022). In the context of cancer, TRF reduces breast tumor growth and metastasis to the lung in orthotopic models in obese, postmenopausal female mice via suppression of hyperinsulinemia (Das et al. 2021). TRF also delays tumor growth and progression and reduces tumor size in MMTV-PyMT female mouse model of breast cancer (Sundaram and Yan 2018). Moreover, TRF prevents lung metastasis in the obese Lewis lung carcinoma mouse model (Yan et al. 2019) and reduces pro-neoplastic lesions and cell senescence in the livers of aged rats (Serra et al. 2019).

Aligning feeding rhythms with endogenous circadian rhythms is also important to improve and maintain health during aging, shiftwork, and circadian disruptions. TRF reduces obesity and improves glucose tolerance in daytime sleep disruption in rats—a model for night-shift work (Salgado-Delgado et al. 2010). TRF also protects against obesity and restores daily RER rhythms in mice subjected to long photoperiod days, independent of the melatonergic system (Small et al. 2023). Moreover, TRF improves glucose tolerance and insulin sensitivity in mice exposed to circadian disruption by continuous light via preserving clock gene expression and promoting PAR bZIP transcription factor activity at chromatin to improve insulin production and secretion (Brown et al. 2021). Not only in the shorter duration but lifelong TRF also improves health span and extends median life span in both male and female mice, under isocaloric as well as calorie restriction conditions (Acosta-Rodríguez et al. 2022; Mitchell et al. 2019; Duregon et al. 2023).

3.2.2 Critical Factors to Consider

Since the past decade, more than 100 studies have been performed to assess the role of TRF in improving health using rodent mice models. While most of these studies have a general consensus that TRF improves metabolism and can alleviate several metabolic and age-associated diseases, several critical factors need to be considered to understand the context in which these studies were performed and how to better translate the findings from preclinical rodent models to humans.

3.2.2.1 Feeding Time: Day vs. Night

One of the main points to consider in any TRF study is the feeding time. Since mice are nocturnal, they are active during the night and consume most of their calories during that period. This means that aligning feeding times to the active phase in mice for TRF intervention should ensure that food access is provided only during

the night/dark phase. Even in the dark phase, early TRF reduces weight gain and adiposity much better than late TRF (Regmi et al. 2021). However, several TRF studies provide food during the day/light phase due to the ease and feasibility of animal maintenance. Due to this daytime-restricted feeding (DRF), mice have increased anticipatory activity during the dark-to-light transition and may have disrupted daily sleep rhythms due to feeding at the wrong time, essentially mimicking late-night eating in humans. Although the DRF paradigm has some beneficial effects on reducing inflammation, improving physical performance, and extending health span and life span in a few of the studies (Acosta-Rodríguez et al. 2022; Duregon et al. 2023; Sherman et al. 2011, 2012; Xin et al. 2023), the endogenous feeding rhythms are not in alignment with the daily activity rhythms in mice, and most of the beneficial effects observed may be due to the extended fasting window (Pak et al. 2021). Moreover, DRF may reduce total food consumption and induce a day-time calorie restriction (CR)-like paradigm (Sherman et al. 2011, 2012). On the other hand, intentional CR only during the dark phase, which is in alignment with the endogenous circadian rhythms in mice, has a more pronounced effect on metabolic health, health span, and life span (Acosta-Rodríguez et al. 2022; Mihaylova et al. 2023), indicating the importance of both the fasting duration and meal timing in improving health.

3.2.2.2 Feeding Window and Calorie Consumption

One of the critical facets of TRF is that the total food consumption should not be different than the *ad libitum* fed animals and should be completed within a period of 6–10 h during the active phase. While most of the studies performing 8- to 10-hour TRF intervention in mice show no differences in total food consumption, a few studies have indicated that TRF reduces total food intake. One of the reasons for this reduction in food intake is a shorter TRF feeding window of <8 h (Sherman et al. 2011, 2012). It is important to provide enough feeding time to mice for isocaloric food consumption and determine the feeding window accordingly to avoid the additional confounding factor of calorie restriction.

3.2.2.3 Health at Baseline and Study Population Characteristics

While most TRF studies on obese mice show improved metabolic health as compared to the *ad libitum* fed mice, such dramatic differences are not observed in healthy, normal chow-fed mice. One of the reasons for this might be that normal chow-fed mice have intact daily feeding rhythms, and they anyway consume most of their food during their habitual active/dark phase. Thus, further restricting food only to the active phase may not provide any distinct additional benefits for preventing weight gain or adiposity. However, there are still some benefits in chow-fed mice on TRF such as improved daily rhythms in RER and better rotarod performance (Hatori et al. 2012). Moreover, in the disease models, TRF-dependent

physiological benefits are seen even under normal chow feeding conditions, with further exaggerated effects observed under HFD consumption (Wang et al. 2018; Sundaram and Yan 2018; Yan et al. 2019, 2020; Hou et al. 2019). Thus, the health of the animals and their daily feeding rhythms at baseline dictate the extent of TRF's beneficial effects.

3.2.3 *Molecular Mechanisms of TRF Action*

At the organismal level, information about the fed and fasted states is conveyed to the brain and peripheral organs by various neural and hormonal signals. In TRF, the level of satiety hormone leptin decreases due to improved leptin sensitivity (Hatori et al. 2012; Chaix et al. 2014), and the level of appetite hormone ghrelin increases due to prolonged fasting (Sundaram and Yan 2016; Desmet et al. 2021). Interestingly, the central melanocortin system is required to attain the beneficial effects of TRF, and the Melanocortin 4 receptor (Mc4r) knockout mice are only partially protected by TRF from weight gain and obesity despite having improved glucose tolerance (Sorrell et al. 2020). Moreover, even in young and middle-aged female mice on HFD, TRF does not prevent weight gain and adiposity, but these mice are protected from fatty liver disease and have improved glucose tolerance and muscle functions (Chaix et al. 2021). Together, these results indicate that TRF-dependent beneficial effects are independent of a reduction in body weight and fat mass, at least in the female mice. Another fasting-induced hormone that has been linked to TRF action is FGF21. FGF21 levels are higher under several nutritional states such as low protein diet, ketogenic diet, as well as TRF. However, the liver-specific knockout of FGF21 in obese mice partially abrogates the metabolic benefits of TRF such as blunted weight gain, improved glucose tolerance, protection from fatty liver disease, and better reproductive functions (Hua et al. 2020, 2023).

Several studies have identified the genes and pathways that are altered by TRF in multiple tissues and linked them to understand how TRF works. Due to daily consistent feeding-fasting rhythms in sync with the circadian rhythms, TRF increases the amplitude of several core clock genes in the liver. TRF also increases energy expenditure and adipocyte thermogenesis by potentiating creatine metabolism (Hepler et al. 2022). Moreover, TRF improves the diurnal rhythms in AMPK, mTOR, and insulin-IGF1 signaling, which are dampened in the livers of obese mice (Hatori et al. 2012; Chaix et al. 2014, 2021). These diurnal rhythms in intracellular signaling pathways ultimately cause rhythmic glucose, lipid, ketones, amino acid, nucleotide, cholesterol, and bile-acid metabolism in the liver as measured by time-series lipidomics and metabolomics (Hatori et al. 2012; Chaix et al. 2014, 2021; Xia et al. 2023; Mehus et al. 2021), similar to what is observed in flies (Livelio et al. 2023). Interestingly, TRF partially improves diurnal rhythms in most of these nutrient signaling and metabolism pathways even in mice lacking the core clock genes *Arntl* or *Nr1d1/Nr1d2* in the liver and in the whole body *Cry1/Cry2* double knockout (CDKO) mice (Chaix et al. 2019). This indicates that daily, consistent

alternation of feeding-fasting signals may be the major driver of TRF benefits and the clock genes may not play a direct role in mediating TRF action.

Since TRF brings about pleiotropic health benefits, it is not surprising to expect the involvement of a coordinated response across multiple organ systems. To this end, our lab performed a time-series transcriptome analysis from 22 different brain regions and peripheral organs to generate a diurnal transcriptome atlas of 9-hour TRF in young male mice on HFD (Deota et al. 2023). Surprisingly, about 80% of genes were found to be differently expressed or rhythmic in at least one of the tissues under TRF. TRF increased the expression and rhythmicity of genes involved in RNA processing, protein folding, fatty acid metabolism, mitochondrial functions, and autophagy across multiple organs. This is in line with the TRF studies in flies that have linked augmented lipid metabolism, mitochondrial functions, and autophagy to improved heart and muscle functions and life span extension (Gill et al. 2015; Villanueva et al. 2019; Ulgherait et al. 2021). TRF also increased gene expression rhythmicity in most tissues and altered clock gene phase and amplitude in a tissue-specific manner. Interestingly, HFD consumption desynchronized rhythmic gene expression across tissues and dampened the diurnal peaks. However, TRF resynchronized rhythmic gene expression across tissues and consolidated into two distinct peaks corresponding to the fasting and the feeding phases. Genes involved in fatty acid oxidation, autophagy, DNA repair, and cell cycle were expressed majorly during the fasting phase, while genes involved in chromatin regulation, transcription, splicing, rRNA processing, protein folding, and glycosylation were predominantly expressed in the feeding phase across most tissues. Finally, TRF led to increased expression and rhythmicity of fatty acid uptake and synthesis genes in the gut and adipose tissues during the feeding phase and higher expression and rhythmicity of fatty acid oxidation, ketone body metabolism, and autophagy genes in the BAT, liver, muscle, and heart during the fasting phase. These results indicate that TRF compartmentalizes catabolic and anabolic processes into distinct fasting and feeding phases respectively; improves nutrient uptake, storage, and utilization across tissues; and promotes metabolic elasticity and flexibility (Zhou et al. 2023).

3.2.4 Future Outlook in TRF Research

In the past decade, multiple studies have now unequivocally proven that the alignment of timing of feeding with endogenous circadian rhythms is critical for improving and maintaining health, and protection from various age-associated diseases in mice models. However, several outstanding questions remain in the field of TRF. One of the main questions is identifying the molecular mechanisms of TRF action and how such a pleiotropic multi-organ response is achieved. Since age and sex can affect the outcomes of any feeding interventions, more research is needed to identify if the observations from young male mice are also applicable in females, as well as in middle-aged and older mice. A few of the recent studies have indeed tried to examine the molecular changes across multiple tissues and identified

tissue-specific mechanisms for TRF action in young male mice, but there is still a lack of comprehensive understanding of the molecular changes in the transcriptome, proteome, and metabolome induced by TRF across age and sex, the intracellular signaling mechanisms conveying the nutrition timing to the endogenous clock, and how the duration of TRF affects outcomes. One of the ways to investigate the molecular mechanisms is to mimic TRF-like conditions using *in vitro* cell/organoid cultures and induce feeding-fasting oscillations of several metabolites such as glucose and hormones such as insulin and glucagon (Gagliano et al. 2021).

While TRF-dependent changes in diurnal gene expression are known, the upstream signals that orchestrate the assembly of transcription factors and chromatin modifiers, post-transcriptional mechanisms regulating RNA processing and protein translation, and the protein modifications affecting activity and turnover are still not known. While there are a few reports providing some insights, e.g., in the hippocampus, TRF-dependent increase in BHBA may inhibit HDACs and cause higher histone H3 acetylation (Landgrave-Gómez et al. 2016), the field lacks an understanding of the non-autonomous cellular mechanisms for TRF action.

It is also important to identify the disease stages and the populations for which TRF may or may not work. For example, TRF may not work efficiently by itself in older populations as well as in several late-stage diseases, and combinations with other drug interventions might be needed to attain the additive effects. Since TRF potentiates several nutrient signaling mechanisms and affects the expression of drug metabolism genes, it will be important to test the combination of drugs and TRF in various disease contexts such as type 2 diabetes, NASH and liver fibrosis, autoimmune disorders, gut diseases, and cancer. Such drug-nutrition combination interventions have already provided some success in a few preclinical cancer models (Blaževič et al. 2023; Hopkins et al. 2018). This is important since about 60% of adults aged 18 and over reported taking at least one prescription medication in 2021, with 36% reporting taking three or more (Mykyta and Cohen 2023), and the interaction of various drugs with TRF intervention needs to be tested thoroughly before the concepts and understandings can be translated from the pre-clinical models to humans.

3.3 Time-Restricted Eating (TRE)

When TRF research expanded to clinical trials, the term “time-restricted feeding” was still used. However, it quickly became clear that humans do not like being told they feed; thus, the term was changed to time-restricted eating (TRE). This section will discuss temporal eating patterns, how TRF was translated to clinical trials in humans, critical considerations when assessing a TRE study, and how TRE can be used in shift workers who experience chronic circadian disruption.

Table 3.1 Definitions of temporal eating pattern components

Temporal eating component	Definition	Examples	Diets
Duration	The time from first to last calorie on a given day.	8 am to 7 pm would be 11 h of eating duration (13 h fast).	TRE, IF (16:8)
Variability	Day-to-day change in time of dietary intake, especially first and last calorie	Low variability would be eating first and last calories within 15 15-min window each day (e.g. 9–9:15 am and 6:45–7 pm). High variability would be having the first calories at 8 am one day and 10 am the next, or the last calories at 7 pm one day and 10 pm another day.	TRE
Phase	The time of the eating interval and/or meal size and quality relative to other factors such as sleep and light exposure.	If sleep is from 11 pm to 7 am, eating from 7 am to 5 pm would be early, 9 am–7 pm would be mid-day, and 12 pm–10 pm would be late. For calories, a big breakfast and a smaller dinner could be a recommendation.	TRE, Big breakfast diet
Frequency	Number of caloric consumption events each day.	Could be anything from 1 meal a day to 16+ snacks in a day. Commonly includes 3–6 meals/snacks, or many small meals/snacks in a day. Ex. 3 meals a day (breakfast, lunch, dinner) 6 meals: Breakfast, snack, lunch, snack, dinner, snack 12+: Series of small snacks/meals	• 3-meals/day • 6-meal/day, • Frequent snacks.

3.3.1 Temporal Eating Patterns

When discussing the timing of eating, it is important to consider all components of temporal eating patterns. These include duration, phase, variability, and frequency (Table 3.1). TRE is unique in that it is the only dietary intervention that incorporates more than one of these components (Table 3.1). TRE incorporates duration and variability and should incorporate phase (optimize the time of day to early or midday of the sleep/activity phase). Although TRE usually does not incorporate frequency of dietary intake, it can easily be combined.

3.3.1.1 Duration

Eating duration refers to the daily eating/fasting window (e.g., 9:00 a.m.–7:00 p.m. would be a 10-hour eating window). When characterizing eating duration over longer periods, two methods can be used: (1) average daily eating interval and (2) 95% eating window. An average daily eating window simply takes the eating window from each day and averages them. For example, an average eating window over

3 days would equal 10 h if calories were consumed from 9:00 a.m.–7:00 p.m. on day 1, 12:00 p.m.–10:00 p.m. on day 2, and 5:00 a.m. to 3:00 p.m. on day 3. A 95% eating window looks at the window of time that food was consumed over 7+ days and excludes the 2.5% of the earliest and latest entries. For example, if over 1 week, 95% of caloric intake events were consumed between 8:00 a.m. to 6:00 p.m., which would be a 10-hour eating window. However, if over a week, the eating window for each individual day was 10 h, but the timing of the eating windows varied dramatically such as 9:00 a.m.–7:00 p.m. on day 1, 12:00 p.m.–10:00 p.m. on day 2, 5:00 a.m. to 3:00 p.m. day 3, 8:00 a.m. to 6:00 p.m. day 4, 9:30 a.m.–7:30 p.m. day 5, 8:45 a.m.–6:45 a.m. day 6, and 11:00 a.m.–9:00 p.m. day 7; then the full eating window would be 5:00 a.m.–10:00 p.m. (17 h), and with outliers removed, the 95% eating window would be 9:00 a.m. to 9:00 p.m., which would be 12 h. The incorporation of variability into the eating window can lead to very different results.

The average daily eating window can underestimate the duration of the eating cues affecting the circadian system, a full eating window (no outliers removed) likely overestimates the eating window, and the 95% eating window accounts for variability and removes outliers. Accounting for variability is crucial because the circadian system is anticipatory.

Long eating durations have been associated with many negative health outcomes. A study that collected data from 2413 women who had breast cancer and participated in the prospective Women's Healthy Eating and Living study found that women who fasted less than 13 h each night (aka daily eating duration of 11 h or more) had an increased risk of breast cancer recurrence compared to women who fasted for ≥ 13 h per night (Marinac et al. 2016). A randomized control trial in 20 adults found that BMI was positively correlated with the extended duration of the eating window (Chow et al. 2020). Another study found similar results in 85 participants with overweight or obesity had an average eating window of 14 h, with only 13.1% of participants having an eating window < 12 h (Popp et al. 2021).

3.3.1.2 Variability

Eating pattern variability refers to the day-to-day differences in the timing of dietary intake. Typically, the focus is on the timing of the first and last bout of dietary intake. TRE incorporates this by setting a set window that must be kept consistent. There is still some variation when sticking to a set eating window as an individual can choose to have their first food/beverage after the eating window starts, but usually, with a 10-hour or less eating window, this variation is minimal. There is also a possibility of eating outside the window, but this is typically a rare occasion. Studies have shown that TRE interventions have decreased variation in the timing of both first and last dietary (Wilkinson et al. 2020). Moreover, in a post hoc temporal eating pattern analysis of a caloric restriction randomized control trial (CALERIE 2 Trial), it was found that the decreased variability of first-last calorie intake correlated to weight loss and amount of caloric restriction achieved. Duration and phase also influenced outcomes but to a lesser extent (Fleischer et al. 2022).

It is important to note that intermittent fasting diets including 16:8, 5:2 and alternate day fasting do not require consistent eating times. This explains different outcomes from these diets and highlights the circadian consideration of TRE.

3.3.1.3 Phase

Phase of dietary intake refers to the time of day that the diet is consumed in relation to an individual's endogenous circadian rhythms, typically referenced by sleep/wake cycles. There can be a phase of dietary consumption as well as a phase of macronutrient and calorie distribution.

Research from animal models has demonstrated that even a consistent, short eating window can have diverse and sometimes negative health effects when provided at the wrong time of day (i.e., daytime feeding for a nocturnal animal). As mentioned earlier (Sect. 1), it leads to a dissociation of circadian rhythms in the brain and peripheral organs (Damiola et al. 2000; Stokkan et al. 2001; Longo and Panda 2016; Cui et al. 2022). Daytime feeding (12 h) in mice has also been shown to disrupt the gut microbiota and bile acid profiles (Cui et al. 2022). Adamovich et al. demonstrated that feeding times regulate the phase and levels of triglyceride accumulation in the liver, with TRF at night decreasing accumulation of liver triglycerides by 50% compared to ad lib fed animals (Adamovich et al. 2014). Even the cerebellar proteome is altered in the daytime vs. nighttime-fed mice and may be associated with food anticipation (Bertile et al. 2021). Daytime-restricted feeding in rats (also nocturnal) has demonstrated increased LDL-cholesterol production (Rivera-Zavala et al. 2017) and increased hepatic glucocorticoid receptors, altered the urea cycle (Luna-Moreno et al. 2012), and promotes gluconeogenesis in the liver (Pérez-Mendoza et al. 2014). Other groups have found benefits of daytime-restricted eating, such as enhanced endurance, but only in comparison to ad lib fed mice, and not to nighttime-restricted mice (Xin et al. 2023). Studies that have compared both daytime- and nighttime-restricted eating have found significant health benefits in nighttime-restricted eating (Acosta-Rodríguez et al. 2022). These studies reveal that the duration of the feeding/fasting window and the phase of feeding both significant impact physiology independently.

In humans, research has consistently shown that delayed and late-night eating negatively impacts metabolism and is associated with increased weight and compromised energy metabolism (Yeh and Brown 2014; Mirghani 2021; McHill et al. 2014, 2017, 2023). Lab studies have revealed that late-night eating leads to elevated blood glucose that can remain high throughout sleep at least in part due to decreased insulin sensitivity during sleep and leads to increased lipid storage (Vujović et al. 2022). This study also showed that late eating increased waketime hunger and decreased wake time energy expenditure (Vujović et al. 2022). A delayed eating phase can include both breakfast skipping and late eating. Large-scale observational surveys, such as The National Health and Nutrition Examination Survey (NHANES), have found that breakfast skipping and late-night eating are associated with increased rates of cardiovascular disease and all-cause mortality (Rong et al. 2019).

It has even been shown that within weight loss programs, those who had delayed eating patterns lost less weight (Garaulet et al. 2013).

3.3.1.4 Frequency

Frequency of dietary intake refers to the number of caloric intake events in a day. It has traditionally been thought to be three meals a day, but NHANES data has shown that three meals a day have been replaced with a combination of snacks and meals of varying frequency (Kant and Graubard 2015; Kant 2018). There is no consensus on ideal meal frequency. Observational studies have indicated that more frequent meals are associated with lower BMI, potentially by eliminating binge eating (Ha and Song 2019; Toschke et al. 2009). However, controlled clinical trials have found fewer meals a day to be more beneficial. For instance, a randomized control trial that compared three- and six-meal diets in adults with type 2 diabetes found that three-meal diets were superior for weight loss and decreased HbA1c over 12 weeks (Jakubowicz et al. 2019).

Time-restricted feeding/eating research in animal models and humans has typically not regulated the number of eating events during the eating window; only duration, variability, and phase are addressed. In a recent study that compared fasting protocols and caloric restriction in mice, the duration and frequency of food distribution were assessed by comparing one-time allotments of food (which was consumed within 2 h) to evenly spread food across 12 h. This resulted in a large difference in meal frequency, yet there was no difference in health outcomes or life span between these two groups (Acosta-Rodríguez et al. 2022).

3.3.2 *Translating TRF to Clinical Trials in Humans*

Once TRF had been established in mice, the next question was if it applied to humans. First, there needed to be an understanding of when people ate. Since nutritional studies have focused on the quality and quantity of food, but not on the timing of food, temporal eating patterns in humans were greatly oversimplified or unknown. To better understand the temporal dynamics of when people ate, Gill and Panda (2015) created a smartphone app called myCircadianClock (mCC) to answer this question (Manoogian et al. 2022a; Gill and Panda 2015). Unlike other nutritional logging that requires detailed food records including portions and sometimes ingredients, the mCC app just asked for the name of the item and a picture. All entries were then time-stamped to be able to assess temporal data (Gill and Panda 2015). They found that in 156 adults in San Diego, CA, USA, the 95% eating window was greater than 14.75 h for more than half of participants. They also noted that less than 25% of calories were consumed before noon and more than 35% of calories were consumed after 6:00 p.m. Moreover, they found an average 1-hour delay in the onset of eating on weekends, demonstrating metabolic jet lag. These data indicated

that in the real world, adults were eating over long periods, consuming more calories in the latter half of their day, and shifting eating patterns on weekends (Gill and Panda 2015).

Long-eating windows in free-living adults have been identified by many labs in various cities in the USA and internationally. In India, using a camera phone-based approach, Gupta et al. found that in 94 healthy adults, more than half of the participants had an eating window of 15 h or more. They also found a similar distribution of calories as the San Diego population (29% of calories before noon, 60% of calories after 6:00 p.m.) (Gupta et al. 2017). However, there were no significant differences in the timing of the first and last meals on weekdays and weekends (Gupta et al. 2017). In Lausanne, Switzerland, Phillips et al. also found that 56% of adults ($n = 213$) had an eating window ≥ 14 h (Phillips et al. 2021). Similarly, when screening for a randomized controlled trial at the University of Minnesota, Chow et al. found that 70% of adults with overweight had an eating window of ≥ 14 h (Chow et al. 2020). Popp et al. (New York, NY) found that 87% of their 85 participants with overweight or obesity had an eating window ≥ 12 h, with 14 h 4 mins as the median (Popp et al. 2021). NHANES that used data over eight cycles, (2003–2018) from adults, also found that the average daily eating window was ≥ 13 h for 35% of adults and late-night eating was common with 59% of participants consuming calories after 9:00 p.m. (Farsijani et al. 2023). The consistently high prevalence of long eating durations across multiple cultures and cities demonstrates that temporal eating patterns are disrupted across the board and should be addressed.

The first TRE human trial was an extension of the Gill and Panda (2015) study. In a small pilot, they found that in eight individuals who had obesity and an eating window of ≥ 14 h, a 10-hour TRE intervention for 16 weeks led to weight loss and reported improvements in energy levels, sleep quality, and decreased hunger (Gill and Panda 2015). From there, a series of studies came out demonstrating that TRE (6–10-hour eating windows) had a variety of cardiometabolic health benefits (Manoogian et al. 2022b) (see Chap. 4).

3.3.3 *Critical Factors to Consider*

There have been over 100 studies now published on TRE in humans, and although there is largely a consensus that it is beneficial (Fig. 3.1), there are still conflicting reports for some outcomes or the magnitude of outcomes. These differences can be explained by understanding the critical components of these studies. These include eating window assessment, baseline eating window, duration, and phase of the eating window, whether the eating window is personalized or set to a specific clock time, implementation (participant engagement and monitoring) of the intervention, the duration of the intervention, and the patient population being assessed. Each of these components significantly impact the study and outcomes and must be considered when interpreting results.

3.3.3.1 Method of Eating Window Assessment

Accurate assessment of eating patterns is crucial for any intervention that alters it. To understand an individual's eating pattern, including eating duration, it is necessary to monitor all dietary intake for at least 7 days, preferably 2 weeks. This is due to the large variability in eating times (especially first and last intake) for both day-to-day and weekday-weekends. Recalls of average eating times are known to be largely inaccurate. Thus, real-time monitoring over many days is needed to assess the baseline eating window. Surveys that ask for dietary recall or 24-hour dietary assessments are insufficient to accurately characterize the eating window.

3.3.3.2 Eating Window vs. Average Daily Eating Duration

Measuring an eating window (95% eating window) vs. average eating duration will provide different information (as described in Sect. 3.1.1). As mentioned, clinical trials that have assessed 95% eating window consistently find that 50% or more of the population have eating windows of 14 h or more (Manoogian et al. 2022a). However, when the average daily eating duration is assessed, the average is found to be around 12 h (Manoogian et al. 2022b). The difference in outcomes is due to variability in eating times. As TRE requires a consistent eating window, assessing the eating window as opposed to the average daily eating duration is more appropriate.

3.3.3.3 Baseline Eating Window

For TRE studies, it is vital to only include participants who have long eating windows at baseline. It is necessary to establish that they are not already practicing a form of TRE and that there is room for change. For example, you would not perform a weight loss study on participants that have normal or lower weight. In the same vein, it does not make sense to include participants that have a 12-hour or shorter eating window in TRE studies, as 10–11-hour eating windows have been used as interventions. A study that investigated 8-hour TRE in comparison with caloric restriction in adults found that there was no significant difference between groups. However, it is important to note that all participants started with an average eating duration of 10 h.

3.3.3.4 Duration of the TRE Intervention

TRE clinical trials have tested eating window durations of 4–12 h, most commonly 8–10 h. There have been no additional benefits found from decreasing the eating window to shorter than 8 h, except for slightly greater weight loss due to combined increased calorie reduction (Cienfuegos et al. 2020). Eating windows shorter than 8 h are also much more difficult to implement and have had much more frequent

adverse events such as headaches, nausea, moodiness, hunger, and social schedule disruption. On the other hand, a TRE intervention with a 12-hour eating window found no significant changes to weight or cardiometabolic health (Phillips et al. 2021). However, TRE interventions with an eating window of 8–10 h are feasible with minimal adverse events, are largely well accepted by adults from diverse locations, and have found significant cardiometabolic health benefits.

3.3.3.5 Phase of the Eating Window

Although TRE mainly focuses on the duration and variability of eating/fasting, the phase at which that occurs can also heavily influence outcomes. Clinical trials have taken different approaches to when the eating window will occur: early, midday, or late. Although times are specific to each study, early typically starts between 6:00 and 8:00 a.m. and ends 2:00 and 4:00 p.m., whereas late is usually 12:00 pm to 8:00 pm. Midday is not usually called that but is typically 9:00–10:00 a.m. start and 5:00–8:00 p.m. end (also depending on TRE window duration). In all those examples, the same eating window was applied to all participants, regardless of individual differences in sleep/wake times. Even though it is the same time on the clock, it can result in very different phases of the eating window. For example, say there are two participants in a study with a TRE window of 8:00 a.m. to 4:00 p.m. If one participant wakes up at 6:00 a.m. and goes to bed at 10:00 p.m., this may be a midday approach in which they awake for 2 h before they consume any calories. However, if the second participant is someone who wakes up at 7:30–8:00 on weekdays and sleeps in till 9:00 a.m. or later on weekends and goes to bed around midnight, this is a very early phase and would likely be much more difficult physically and feasibly. These differences in phases will occur in all studies that set the same eating window for all participants.

Studies that have compared early and late eating patterns have found similar results for most outcomes with some additional or larger benefits in early eating TRE compared to late TRE (Hutchison et al. 2019). Late TRE studies have also more frequently found mild or insignificant benefits (Lowe et al. 2020). However, there have been no studies to show that early TRE is superior to midday TRE. From a circadian perspective, consuming calories during the active part of the day and fasting before bed is ideal. For feasibility, midday is more likely to be maintained long term as it still allows individuals to eat dinner.

3.3.3.6 Personalized Eating Windows

The alternative approach to pre-set eating windows is to personalize the eating window for each participant. This can be done by assessing sleeping patterns and/or by letting them self-select an eating window that works for them within certain boundaries (i.e., the eating window must end at least 3 h before typical bedtime). The self-selection option also allows for participants to incorporate important meals

such as family dinners that would be very difficult to change and are necessary to consider for long-term adherence.

3.3.3.7 Implementation: Participant Engagement and Monitoring

For any behavioral intervention, the method of implementation plays a large role in the success of the intervention. To make any behavioral change, feedback, resources, and support are required. These may include self-monitoring, educational materials (blogs, videos, handouts, etc.), support groups, clinical research team check-ins, reminders, and regular feedback (in person or virtual). Studies that have had limited to no direct participant engagement have found less change or null results (Lowe et al. 2020). However, increasing engagement through a smartphone app, in-person or virtual check-in, and resources have had much greater success in both outcomes and intervention adherence (O’Neal et al. 2022).

Self-monitoring has also been shown to be a large contributor to the success of many health interventions. Daily logging of dietary intake not only helps the participant monitor themselves, but when logged on a smartphone app, dietary intake can be monitored in real time by the researchers as well (Manoogian et al. 2022a). This allows for real-time personalized feedback for participants and then a chance to contact participants if there are any concerns.

3.3.3.8 Duration of the Intervention

TRE interventions have ranged from less than a week to a year, with most conducting the intervention for 6–12 weeks. Depending on study outcomes, the minimum duration of intervention can vary. For instance, HbA1c approximates glucose over 3 months. Thus, an intervention shorter than 3 months would be insufficient. From a circadian perspective, it may take up to a couple of weeks for the clocks in the brain and periphery to align, which is likely needed for many effects of TRE. In addition, to assess long-term feasibility and outcomes, a longer period of 6 months to multiple years is needed.

As the full mechanisms of how TRE affects different aspects of physiology, the minimum duration of an intervention needed to see full results is not understood. Thus, there is no one correct duration of a TRE intervention but should be considered when interpreting results.

3.3.3.9 Patient Population

Most TRE clinical trials have focused on adults with obesity/overweight or prediabetes. There are a few that have looked at healthy adults, especially in the context of exercise (endurance and strength). More recently, TRE research has explored many other disease states including metabolic syndrome, type 2 diabetes, cancer,

polycystic ovarian syndrome, and affective disorders. There have not yet been studies to assess the long-term (many years to a lifetime) effects of TRE in healthy individuals of any age.

As the patient population is so diverse, it is not surprising that different outcomes are observed across studies. For instance, you would not expect to see a change in blood pressure or HbA1c in a participant that has overweight or obesity, but no hypertension or elevated glucose at baseline. Similarly, you would not expect weight loss in participants who do not have elevated weight at baseline. Different health factors/markers can take different time spans to change, i.e., fasting blood glucose can have significant changes based on what was consumed the day before, but HbA1c would take a full 3 months or more to see the full effect.

The other key factor to assess is the participant's eating window at baseline. Unfortunately, this eating window can either be shorter than 12 h, which indicates that they are already doing a form of TRE, or are not assessed at all (Manoogian et al. 2022b). This would be the same as putting someone on an exercise plan that they are already on or only making small modifications. In either case, you would expect minimal or no differences. For any intervention trial, it is necessary for there to be room for change to expect to see a change in outcomes.

It is important to keep all these factors in mind when interpreting results as differences in outcomes may be easily explained, and even expected, based on these considerations.

3.3.4 TRE in Shift Work

Over 20% of the population internationally does some form of shift work. Although the shift work schedules vary greatly, they all lead to circadian disruption (Kervezee et al. 2018). Circadian disruption and misalignment are known to have many negative health consequences such as increased rates of sleep disruption, cancer, metabolic disorders (diabetes, weight gain, etc.) cardiovascular disease, affective disorders, and even decreased cognitive ability (Scheer et al. 2009; Vetter et al. 2016; Straif et al. 2007; Blakeman et al. 2016; Chellappa et al. 2018; Castanon-Cervantes et al. 2010; Khan et al. 2018; Knutsson and Bøggild 2010). Nurses have been the most widely researched group for a long time, noting increased rates of weight gain, coronary artery disease, irritable bowel syndrome, metabolic disease, menstrual dysfunction, chronic fatigue, and breast cancer (Scheer et al. 2009; Vetter et al. 2016; Nojkov et al. 2010; Ferri et al. 2016; Kang et al. 2019; Sharma et al. 2017; Garrido et al. 2021; Gangwisch et al. 2014; Wegrzyn et al. 2017). Recent studies on police officers have demonstrated both negative health consequences and how their eating patterns are affected by different shift schedules (Koshy et al. 2019; Kosmadopoulos et al. 2020). Shift work disrupts the circadian system through a combination of mistimed sleep, activity, light, and food consumption. Each of these factors usually helps the circadian system align with the environment, but shift work sends conflicting cues regularly.

Despite the increased need for health interventions to help offset these negative outcomes, shift work is almost always an exclusion criterion for clinical research due to potential confounding effects. There are also limitations to what aspects of their schedule can be improved given that sleep, activity, and light cannot be altered. However, the timing of dietary intake, which is likely to be responsible for the negative metabolic outcomes of shiftwork, is something that can be changed.

3.3.4.1 TRE Clinical Trials in Shift Workers

Most clinical trials in shift workers are observational. These studies provide a critical understanding of the challenges shift workers face and can identify potential interventions. Yet there is still a great need for interventions to decrease the negative impact of circadian disruption in shift workers. TRE provides a unique opportunity to address this challenge, as the timing of dietary intake can be altered while still on a shift work schedule. Although there has been some work on shift work in animal models (see Sect. 2.1), human randomized controlled trials are extremely limited. The first randomized controlled trial to assess TRE in free-living adults doing shift work was in firefighters doing 24-hour shifts (Manoogian et al. 2021). The 24-hour shift in firefighters was the simplest shift work schedule to try TRE as they are mainly awake and consume all their meals during the day and they try to sleep at night between calls. One hundred fifty firefighters were randomized to either a standard of care intervention consisting of advice to follow the Mediterranean diet (SOC) or the same dietary advice with personalized 10-hour TRE (TRE). The primary intervention lasted for 3 months, with follow-ups every 3 months up to a year. Feasibility was the primary outcome with other cardiometabolic and quality-of-life measures as secondary outcomes. The study found that 10-hour TRE was feasible with no adverse events. Participants who had hypertension or elevated HbA1c at baseline also showed significant improvements at 3 months in the TRE group compared to SOC (Manoogian et al. 2022c). Benefits in emotional health were also observed in the TRE group compared to SOC (Manoogian et al. 2022c). This study demonstrated that TRE is a feasible, safe, and effective behavioral intervention for shift workers on a 24-hour schedule.

Short-term (usually less than a week) shift work simulations are also being done in labs to assess the effects of circadian disruption and the potential for interventions like TRE to improve outcomes. Much more needs to be done on larger scales of participants, duration of intervention/follow-up, and different schedules to understand the feasibility, safety, and efficacy of TRE to improve the health of shift workers. Moreover, observational and potential intervention trials should be done on other patient populations that are also commonly excluded from clinical trials such as children, adolescents, women who are pregnant or breastfeeding, and those with multiple complex disease states.

3.3.5 TRE Summary

In the past 10 years, the field of TRE has grown exponentially. There is a general consensus that it is safe and provides health benefits in the groups tested. As with all research, it is important to keep context in mind and consider critical factors in study design and participant population to accurately interpret results.

Clinical trials to date indicate that TRE is a novel lifestyle tool that can be combined with other healthy lifestyle habits or serve as a more feasible option than more traditional methods that are not always achievable (eat less, move more). TRE is not a replacement for dietary advice on quality and quantities of food but does help optimize the third and often forgotten component of dietary health *when* we eat.

3.4 Conclusion

Unlike other fasting interventions, TRF/TRE is based on circadian biology and thus affects unique pathways and elicits different physiological effects. TRF/TRE can be combined with other health interventions such as exercise, fasting diets, medications, or caloric restriction, but these combinations are not required to see benefits. When interpreting data from this rapidly growing field, in both mice and humans, it is crucial to consider critical factors in study design and patient populations. Circadian disruption is well-known as a result of shift work but also applies to the larger population as well. Erratic eating patterns, jet lag, and child care are all common causes of circadian disruption that most adults experience. Multiple studies from cities around the world have found that long eating durations, high variability in eating times, and late-night eating are common among adults. Thus, TRE may serve as both a preventative and treatment method to address a wide range of cardio-metabolic and other diseases associated with circadian misalignment for all adults.

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Chapter 4

Time-Restricted Eating: Effects on Body Weight and Cardiometabolic Health



Courtney M. Peterson

Abstract Time-restricted eating (TRE) is a form of intermittent fasting that involves eating within a consistent ≤ 10 -hour daily window. More than 100 clinical trials have been conducted in the past decade. In this detailed and thorough review, we review the effects of TRE on body weight and cardiometabolic health. The overwhelming majority of studies report that a wide range of TRE windows are effective for losing weight. TRE is also effective for improving glycemic control in patients with type 2 diabetes or prediabetes and may also improve blood pressure in patients with hypertension. In non-diabetic adults with obesity or overweight, earlier eating windows appear to improve glycemic control and blood pressure, whereas later windows do not. Overall, the weight of evidence suggests TRE is feasible, reduces body weight, and can improve glycemic control, blood pressure, and oxidative stress in adults with cardiometabolic disease and/or when the eating window is earlier in the day. More research is needed on appetite-related hormones, lipid metabolism, and heart rate. TRE may therefore be an effective treatment for obesity, diabetes, prediabetes, metabolic syndrome, and hypertension.

4.1 Introduction

Intermittent fasting is a broad class of eating strategies that alternate periods of eating and extended fasting. In animals, intermittent fasting reverses diet-induced obesity and diabetes, improves cardiometabolic health, reduces cancer incidence and slows tumor growth, reduces the risk of stroke, delays the progression of neurodegenerative conditions, slows cellular aging, and even increases life span (Harvie and Howell 2017; Patterson and Sears 2017; Antoni et al. 2017; Crupi et al. 2020; Mattson et al. 2017; Longo and Mattson 2014; de Cabo and Mattson 2019). On a

C. M. Peterson (✉)

Department of Nutrition Sciences, University of Alabama at Birmingham,
Birmingham, AL, USA

e-mail: cpeterso@uab.edu

molecular level, these effects stem at least partially from the metabolic switch from carbohydrates to fatty acids as the predominant fuel source (Mattson et al. 2017; Longo and Mattson 2014; de Cabo and Mattson 2019; Anton et al. 2018), which in turn triggers wide-sweeping effects. Extended fasting increases lipolysis, promotes cellular stress resistance, favorably alters endocrine hormones, decreases inflammation and oxidative stress, upregulates autophagy, improves circadian rhythms, reduces anabolic signaling, slows cellular proliferation, and induces anti-aging and regenerative effects (Mattson et al. 2017; Longo and Mattson 2014; de Cabo and Mattson 2019; Anton et al. 2018).

There is a wide variety of types of intermittent fasting. Most forms of intermittent fasting involve either (a) 24-hour or longer water-only fasts; (b) *modified fasting* or *intermittent energy restriction*, where a small number of calories are eaten on specific days; or (c) extending the length of the daily overnight fast. In this review, we focus on daily intermittent fasting, which is known as *time-restricted feeding* (TRF) in animals and *time-restricted eating* (TRE) in humans. We summarize what is known about the effects of TRE on body weight and cardiometabolic health in humans, with an emphasis on randomized controlled trials and studies in populations with elevated cardiometabolic risk. We discuss the effects on body weight, body composition, energy expenditure, food intake, appetite, glycemic control, lipids, blood pressure, heart rate, and other aspects of cardiometabolic health.

4.2 Background

4.2.1 Definition

TRE is defined as eating within a consistent ≤ 10 -hour daily window, which is equivalent to fasting for ≥ 14 h/day. Although TRE is often defined as fasting for ≥ 12 h/day in both animals and humans, we use a 14-hour definition in humans to be consistent with the recommendations of an international expert panel on intermittent fasting (*publication forthcoming*). TRE is a simple prescription to extend the daily fasting period between dinner and breakfast the following morning. For this reason, it is sometimes called *prolonged nightly fasting*. During the daily fasting period, people can freely drink water and zero-calorie beverages, such as black coffee, unsweetened tea, and diet sodas. No calories are allowed during the fasting period, though some studies allow caffeine, whereas others do not.

A key pillar of TRE is that the timing of the eating period is consistent and does not vary day to day. This principle stems from animal research showing that eating at inconsistent times desynchronizes circadian rhythms among organs and tissues in the body—a phenomenon known as *circadian misalignment*. Because circadian rhythms regulate several cardiometabolic pathways, ranging from insulin secretion to cholesterol metabolism, circadian misalignment causes cardiometabolic dysfunction (Asher and Schibler 2011). It is therefore important that the eating period is at

approximately the same time of day. This consistent daily eating period is referred to as the *eating window*.

4.2.2 *Current Eating Habits in the USA*

Unfortunately, most Americans have a long daily eating period and eat at irregular times. According to data from the National Health and Nutrition Examination Survey (NHANES), a nationally representative sample of thousands of Americans, the median American eats over a 12-hour period, typically consuming three meals and two snacks (Kant and Graubard 2014). The median breakfast time is around 8:00 a.m., while the median time of the last meal or snack is around 8:00 p.m. However, if the daily eating window is instead defined as the time period in which people eat 95% of all their meals and snacks over a 2–3-week period (to capture the erratic nature of modern eating habits), the eating window may be even longer. A study in 156 young adults mostly under 30 years old used a smartphone app called myCircadianClock (mCC) and found that the median eating window is 14.75 h (Gill and Panda 2015). Moreover, the study reported that most calories are consumed later in the day and that many young adults have erratic eating patterns, with no distinct breakfast-lunch-dinner pattern.

4.2.3 *Counting Time, Not Calories*

A key feature of TRE is that no calorie counting is required. Unlike other forms of intermittent fasting that involve calorie counting, *TRE is a simple rule to count time instead of calories*. In fact, most clinical trials on TRE do not give participants instructions on how much to eat (diet quantity) or what to eat (diet quality). Simply by watching the clock and not eating outside their eating window, participants tend to spontaneously eat less and lose weight (as reviewed herein).

4.2.4 *Common Eating Windows in Humans*

In humans, the most popular length of the eating window is 8 h long. Eight-hour TRE is also known as the *16:8 diet* since it involves 16 h of daily fasting. Eating windows are also characterized by their time of day. For instance, TRE can be practiced by eating an early dinner (*early TRE*), eating breakfast late or skipping breakfast altogether (*midday TRE*), or skipping both breakfast and lunch (*late TRE*). Among eating windows, midday windows are the most popular, particularly from 10:00 a.m. to 6:00 p.m. and 12:00 to 8:00 p.m. Another subset of TRE approaches is eating only one meal per day, known as *one meal a day* (OMAD). OMAD is

usually practiced by skipping both breakfast and lunch, though it can also be practiced by eating only lunch or breakfast. Eating windows for OMAD are typically 1–4 h long.

4.2.5 Overview of the Literature

TRE rose to prominence following a seminal 2012 study published in *Cell Metabolism* reporting that time-restricted feeding prevents cardiometabolic diseases and improves circadian rhythms in mice independent of calorie intake (Hatori et al. 2012). Over the past decade, the number of human studies on TRE has exponentially increased. As of May 2023, there are more than 130 peer-reviewed manuscripts describing about 100 interventional trials. Of these, there are about 60 unique randomized controlled trials. Most studies are pilot studies, meaning they are single-arm, have small sample sizes, and/or are short in duration. About two dozen studies have enrolled at least 50 total participants, and 10 studies have enrolled over 100 participants. About 80% of studies are 4–12 weeks long. The most popular eating window tested is an 8-hour window (72%), followed next by 9–10-hour windows (20%). The overwhelming majority of studies tested a midday or self-selected TRE window. A smaller number of studies have tested early TRE (about 20 studies) or late TRE (6 studies), which we define here as starting to eat at or after 4:00 p.m. Close to half of studies (45%) are in adults with overweight or obesity. A few studies have tested the effects of TRE in populations with specific cardiometabolic phenotypes, such as type 2 diabetes (five studies), prediabetes (two studies), metabolic syndrome (three studies), non-alcoholic fatty liver disease (NAFLD; three studies), and hypertension (one study). To date, there are no studies focusing on dyslipidemia or type 1 diabetes.

Deriving clear conclusions from the literature is somewhat challenging, as there is considerable heterogeneity in the TRE interventions and study protocols. For instance, TRE interventions vary dramatically in the length and time of day of the eating window, whether the eating window is self-selected or imposed, whether the timing of the eating window is relative to absolute clock time or the sleep-wake cycle, the amount of counseling or other support given, the length of the intervention, the degree of adherence to TRE, and the dietary instructions given to the TRE and control groups. Complicating the comparisons of eating windows across studies, a majority of studies do not assess participants' eating windows at baseline. In this review, we emphasize higher-quality studies, particularly randomized controlled trials (RCTs), isocaloric feeding studies, studies with larger sample sizes and/or longer durations, studies in patients with elevated cardiometabolic risk, and studies where the TRE group fasts for at least 3–4 h longer than the control group and/or baseline. Whenever possible, we derive conclusions based on either the majority of studies or patterns in the literature. We also highlight areas of the literature where the results are conflicting or unclear or where notable statistical trends have been reported, to indicate where future research is needed.

4.3 Adherence

A priori, TRE may be more feasible than most types of intermittent fasting for two reasons: (1) the length of the fasting period is shorter, and (2) there is no calorie counting. Indeed, studies have found that 6–8-hour TRE is easier to follow and more acceptable than alternate-day fasting (Turner-McGrievy et al. 2022), and fewer participants drop out (14% vs. 43%) (Erdem et al. 2022). Adherence to TRE is usually high, averaging 5.0–6.2 days/week (~70–90% adherence) (Erdem et al. 2022; Adafer et al. 2020; Gabel et al. 2018; Lowe et al. 2020; Cienfuegos et al. 2020; Zhang et al. 2022; Jamshed et al. 2022; Wilkinson et al. 2020; He et al. 2022; Parr et al. 2020a; Che et al. 2021; Fanaroff et al. 2023; Anton et al. 2019; Martens et al. 2020; Domaszewski et al. 2023; Manoogian et al. 2022; Kesztyüs et al. 2019, 2021; Przulj et al. 2021; Lin et al. 2023; Liu et al. 2022a; Wei et al. 2023). One systematic review of 23 studies reported a mean adherence of 5.6 days/week (80% adherence) (Adafer et al. 2020). Adherence does not appear to vary by the study population. Adults with obesity typically adhere 80–90% of the time in studies testing 8–14-week interventions (Erdem et al. 2022; Gabel et al. 2018; Lowe et al. 2020; Cienfuegos et al. 2020; Zhang et al. 2022; Jamshed et al. 2022). Similar levels of adherence have been reported for patients with metabolic syndrome (Wilkinson et al. 2020; He et al. 2022), type 2 diabetes (Parr et al. 2020a; Che et al. 2021), or hypertension (Fanaroff et al. 2023); the elderly (Anton et al. 2019; Martens et al. 2020; Domaszewski et al. 2023); and 24-hour shift-working firefighters (Manoogian et al. 2022).

However, data are conflicting on whether the length (Erdem et al. 2022; Cienfuegos et al. 2020) and timing (Zhang et al. 2022; Baum Martinez et al. 2022; Wijayatunga et al. 2020; Xie et al. 2022) of the eating window affect adherence. Although it is widely expected that midday and self-selected TRE windows are more feasible than early TRE, data so far are mixed. One study in the USA reported higher adherence to midday TRE than early TRE, but no quantitative data were provided (Wijayatunga et al. 2020), while three studies outside the USA reported similar adherence (Baum Martinez et al. 2022; Xie et al. 2022) or even better adherence to early TRE (Zhang et al. 2022). Of note, the largest two RCTs on early TRE reported a mean adherence of 6.0 (86%) and 5.9 (84%) days/week over 14 weeks (Jamshed et al. 2022) and 1 year (Liu et al. 2022a), respectively, which is in line with studies on midday or self-selected TRE windows.

It is also important to determine who can adhere well to TRE and who struggles to adhere. So far, several behavioral factors have been uncovered. Qualitative investigations (Bjerre et al. 2021, 2022; Lee et al. 2020; O'Connor et al. 2021; 2022; Jefcoate et al. 2023; O'Neal et al. 2022) have found that people who adhere well to TRE typically have regular schedules, are able to align their eating window with their daily routines, report greater improvements in health, report increases in energy levels, and have good social support. By comparison, the minority who struggle with TRE tend to have irregular routines, frequent social situations that discourage TRE, a lack of social support, difficulty self-monitoring, sluggishness,

and/or increased hunger. Common barriers include work schedules, family life, and social events. A small fraction of people also struggle to maintain good diet quality during the eating window.

A key unanswered question is whether TRE is sustainable in the long term. Preliminary evidence suggests that after an initial adaption period of a couple to a few weeks (Sutton et al. 2018), a majority of participants find it easy to adjust (Lee et al. 2020) and want to continue practicing TRE on their own after the study is over (Gill and Panda 2015; Jamshed et al. 2022; He et al. 2022; Fanaroff et al. 2023; Sutton et al. 2018; Anic et al. 2022). To date, four RCTs have tested TRE for 1 year (Lin et al. 2023; Liu et al. 2022a; Wei et al. 2023; Moro et al. 2021). In the three studies reporting adherence data, participants adhered to both early TRE (Liu et al. 2022a; Wei et al. 2023) and midday TRE windows (Bjerre et al. 2021) about ~6 days/week, suggesting that TRE may be sustainable as a lifelong practice.

4.4 Body Weight and Energy Metabolism

The first studies in humans found that participants who adhered to TRE spontaneously lost 1–4% of their body weight within weeks without trying to eat less or healthier (Adafer et al. 2020; Gabel et al. 2018; Cienfuegos et al. 2020; Wilkinson et al. 2020; Che et al. 2021; Anton et al. 2019; Kesztyüs et al. 2019; Chow et al. 2020; Gill and Panda 2015; McAllister et al. 2020; Chen et al. 2021; Moon et al. 2020; Cai et al. 2019). Since then, dozens of studies have investigated TRE’s effects on body weight. Below, we review data on how different TRE windows affect body weight. Thereafter, we discuss the results of recent meta-analyses and the effects on body composition, energy expenditure, food intake, appetite, and appetite-related hormones (Fig. 4.1).

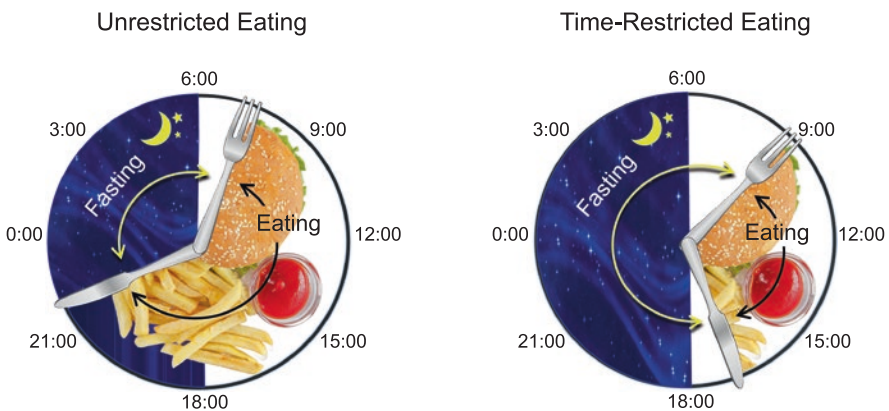


Fig. 4.1 Graphical Schematic of Time-Restricted Eating. Time-restricting eating involves eating within a consistent ≤ 10 -hour daily window and fasting for the rest of the day. The most popular eating window is an 8-hour window

4.4.1 One Meal a Day (≤ 4 -Hour Windows)

Two studies have tested the effects of OMAD on body weight. Both studies found that OMAD decreases body weight. Meessen et al. conducted an 11-day crossover RCT and found that eating one meal/day between 5:00 and 7:00 p.m. lowered body weight by 0.9 kg relative to eating three meals/day (Meessen et al. 2021). Stote et al. conducted an 8-week crossover RCT in which healthy adults were given isocaloric macronutrient-matched diets to eat (Stote et al. 2007). Eating one meal/day between 5:00 and 9:00 p.m. lowered body weight by 1.4 kg over 8 weeks relative to eating three meals/day. However, OMAD in the morning may be even more effective for losing weight than OMAD in the evening. A crossover RCT by Singh et al. found that eating one meal in the morning between 8:00 and 9:30 a.m. decreased body weight by 0.8 kg over 4 weeks relative to eating one meal in the evening between 8:00 and 9:30 p.m. (Singh et al. 2020).

4.4.2 Four- to Six-Hour TRE

Five RCTs have tested the effects of very short eating windows (4–6 h) on body weight for at least 4 weeks. Four- to six-hour windows are effective for losing weight in adults with overweight, while studies in lean healthy populations are mixed. Cienfuegos et al. randomized 58 adults with obesity to a 4-hour window from 3:00 to 7:00 p.m., a 6-hour window from 1:00 to 7:00 p.m., or to a control group who were instructed to maintain their weight and their habitual eating patterns (Cienfuegos et al. 2020). Both 4-hour and 6-hour TRE reduced body weight by about 3.2% over 8 weeks, with no difference between windows. In another RCT, Zhang et al. randomized 60 adults with overweight to either a 6-hour early TRE window from 7:00 a.m. to 1:00 p.m., a 6-hour midday TRE window from 12:00 to 6:00 p.m., or continue their usual eating habits (Zhang et al. 2022). After 8 weeks, 6-hour early TRE and 6-hour midday TRE reduced body weight by 4.6% and 3.7%, with no statistically significant difference between TRE windows. A third RCT in 360 adults with overweight compared five different intermittent fasting diets and found a 6-hour TRE window from 1:00 to 7:00 p.m. decreased body weight by 8% over 12 weeks relative to baseline (Erdem et al. 2022). In healthy populations, however, the results are mixed. A small RCT in 18 young, healthy women found that a 6-hour early TRE window starting an hour after waking up decreased body weight by 3% over 4 weeks compared to eating over a 12-hour period (Mayra et al. 2022a). However, another small RCT in 18 young, healthy, resistance-training men found that practicing 4-hour late TRE by starting to eat any time after 4:00 p.m. did not affect body weight (Tinsley et al. 2017). However, TRE was not practiced consistently since participants were instructed to follow the diet only 4 days/week. Nonetheless, most evidence suggests that short eating windows of 4–6 h are effective for losing weight.

4.4.3 *Eight-Hour TRE*

About 50 studies have tested the effects of 8-hour TRE on body weight for at least 4 weeks. About 30 studies are robust RCTs, while the remaining are single-arm, unrandomized, or lack a non-intermittent fasting control group. Of the approximately 30 RCTs, 16 were in people with overweight or obesity, 10 were at least 12 weeks long, and 9 used an active comparator as the control group. The overwhelming majority of studies report that 8-hour TRE reduces body weight.

4.4.3.1 Non-RCTs

About 90% of non-RCTs (18 out of 20 studies) report that 8-hour TRE reduces body weight. This appears to be true irrespective of whether the eating window is early in the day (Li et al. 2021; Karras et al. 2021a, b), in the middle of the day such as by skipping breakfast (Gabel et al. 2018; Smith et al. 2017; Kirkham et al. 2022; Feyzioglu et al. 2023; Schroder et al. 2021), or self-selected (He et al. 2022; Anton et al. 2019; Przulj et al. 2021; Anic et al. 2022; Park et al. 2021; Kim and Song 2023; Witt et al. 2023; Fagundes et al. 2023; Khan et al. 2022; Lao et al. 2023). It also holds for healthy individuals (Anic et al. 2022; Karras et al. 2021a; Smith et al. 2017; Park et al. 2021; Kim and Song 2023; Witt et al. 2023; Fagundes et al. 2023; Khan et al. 2022); people with overweight or obesity (Gabel et al. 2018; Anton et al. 2019; Przulj et al. 2021; Karras et al. 2021b; Schroder et al. 2021; Lao et al. 2023), metabolic syndrome (He et al. 2022), or PCOS (Li et al. 2021; Feyzioglu et al. 2023); and breast cancer survivors (Kirkham et al. 2022). The two exceptions were one study in adults with multiple sclerosis (Wingo et al. 2022) and one study in patients with obesity and hypertension, which reported a clinically but not statistically significant decrease in body weight (Fanaroff et al. 2023). The magnitude of the weight loss effect is moderate: adults with obesity typically lose between 2.5 and 4.0% of their body weight over 12 weeks (Gabel et al. 2018; He et al. 2022; Przulj et al. 2021; Schroder et al. 2021; Lao et al. 2023).

4.4.3.2 RCTs in Overweight Populations

Among RCTs in people with overweight, about three-quarters report that 8-hour TRE reduces body weight relative to typical eating habits. Lin et al. randomized 90 adults with obesity to one of three groups for 1 year: 8-hour midday TRE from 12:00 to 8:00 p.m., 25% caloric restriction, or to maintain their usual eating habits and baseline weight (Lin et al. 2023). Eight-hour midday TRE reduced body weight by 4.9% over 12 months relative to the control group. In another RCT, Liu et al. randomized 77 college-age women with obesity to one of four groups: 8-hour midday TRE from 10:00 a.m. to 6:00 p.m., exercise, the same TRE window with exercise, or usual eating patterns. Eight-hour midday TRE reduced body weight by 4.2% in 8 weeks relative to habitual eating patterns (Liu et al. 2023a). Two RCTs by

Domaszewski et al. in 116 (Domaszewski et al. 2023) and 46 (Domaszewski et al. 2022) older adults with overweight found that an 8-hour midday TRE window from 12:00 to 8:00 p.m. reduced body weight by 2.0 and 2.2%, respectively, over 6 weeks relative to usual eating patterns. Smaller RCTs have reported similar effects (Chow et al. 2020; Kotarsky et al. 2021). Only two RCTs have reported an exception, including one very small RCT reporting that 8-hour early but not midday TRE tended to reduce body fat ($p = 0.053$) (Wijayatunga et al. 2020) and an RCT in 50 adolescents with obesity where 80% of adolescents ate until 9:00–11:00 p.m. at night and where the control group also lost weight (Vidmar et al. 2021).

Nine RCTs have tested TRE against an active comparison group, such as calorie restriction (CR), rather than comparing TRE to habitual eating patterns (Lowe et al. 2020; Jamshed et al. 2022; Lin et al. 2022, 2023; Liu et al. 2022a; Wei et al. 2023; Cai et al. 2019; Kunduraci and Ozbek 2020; Queiroz et al. 2022). In studies using active comparators, the results are conflicting. Lowe et al. randomized 116 adults with overweight either to an 8-hour TRE window from 12:00 to 8:00 p.m. or to eat three meals/day (Lowe et al. 2020). Practicing TRE by skipping breakfast was not better than eating three meals/day at consistent times. It is unclear whether requiring the control group to stop eating at erratic times (which is also a key principle of TRE), reduce their snacking, and eat a breakfast meal influenced the conclusions or not. Five moderate-sized RCTs have compared the effects of TRE combined with CR versus CR alone, and three reported that TRE enhanced weight loss (Jamshed et al. 2022; Liu et al. 2022a; Wei et al. 2023; Lin et al. 2022; Kunduraci and Ozbek 2020). Jamshed et al. found that combining 8-hour early TRE between 7:00 a.m. and 3:00 p.m. with CR reduced body weight by an additional 2.1% over 14 weeks relative to eating over a 12-hour period with the same CR prescription (Jamshed et al. 2022). Kunduraci and Ozbek conducted an RCT in 70 adults with metabolic syndrome and found that a self-selected 8-hour window combined with CR enhanced weight loss by 1.9% over 12 weeks relative to CR alone (Kunduraci and Ozbek 2020). Lin et al. conducted an RCT in 63 women with overweight and found that combining 8-hour midday TRE from 10:00 a.m. to 6:00 p.m. with CR enhanced weight loss by 1.7% over 8 weeks relative to CR alone (Lin et al. 2022). The two moderate-sized RCTs that reported no effects on body weight tested an 8-hour early TRE window from 8:00 a.m. to 4:00 p.m. with CR versus CR alone in 139 adults with obesity (Liu et al. 2022a) and 88 adults with NAFLD (Wei et al. 2023). However, both studies had notable shortcomings: participants ate over a 10.4-hour period at baseline, which arguably already constitutes TRE; the fasting duration was extended by only ~2.5 h, which is smaller than in most studies; and participants were provided with meal replacement shakes to substitute for meals, so the participants were not truly free-living.

A key question in the field is whether intermittent fasting is more effective for losing weight than CR. Only two RCTs have compared TRE alone versus CR, and they reported conflicting results. Lin et al. found that 8-hour midday TRE from 12:00 to 8:00 p.m. and 25% CR were equally effective for losing weight at the 1-year mark (Lin et al. 2023). Participants reported better adherence to TRE than to CR. An even larger study by Cai et al. randomized 271 overweight patients with

NAFLD to either an 8-hour self-selected TRE window, alternate-day modified fasting, or 20% CR (Cai et al. 2019). TRE was more effective for losing body weight than CR but was as effective as alternate-day modified fasting. However, both intermittent fasting groups were given heart-healthy meals to eat, whereas the CR group was not, which may have biased the results in favor of TRE.

4.4.3.3 RCTs in Healthy Adults

In healthy adults, more than 70% of RCTs report 8-hour TRE is effective for losing body weight and/or body fat compared to habitual eating habits or a control schedule (Xie et al. 2022; Domaszewski et al. 2020, 2022; Moro et al. 2016, 2020; Tinsley et al. 2019; Tovar et al. 2021; Stratton et al. 2020; Correia et al. 2021, 2023; Brady et al. 2021). A 1-year study by Moro et al. found that resistance-training adults who ate over an 8-hour period from 1:00 to 9:00 p.m. lost 6.7% of their body weight relative to those who ate over a 13-hour period (Moro et al. 2021). Xie et al. randomized 90 healthy adults to an 8-hour early TRE window between 6:00 a.m. and 3:00 p.m., an 8-hour midday TRE window between 11:00 a.m. and 8:00 p.m., or to continue their usual eating habits for 5 weeks (Xie et al. 2022). Eight-hour early TRE reduced body weight by 3.1% relative to the control group, whereas midday TRE did not (-0.8% ; $p > 0.05$), though the difference between TRE windows was not statistically significant. Notably, a couple of studies in healthy, exercising adults found that combining 8-hour midday or self-selected TRE with advice to eat a eucaloric diet modestly decreases body fat relative to a control eating schedule (Moro et al. 2016, 2020; Tinsley et al. 2019; Tovar et al. 2021). The small number of RCTs reporting no effects on body weight were 4 weeks long and had small sample sizes (Stratton et al. 2020; Correia et al. 2021, 2023).

Collectively, most studies report that 8-hour TRE is effective for losing weight relative to habitual eating patterns, regardless of the time of day or study population.

4.4.4 9–10-Hour TRE

About a dozen studies have tested the effects of 9–10-hour windows on body weight for at least 4 weeks. The effects of 9–10-hour TRE on body weight are somewhat less clear.

Among single-arm studies, five out of seven reported that 9–10-hour TRE reduces body weight relative to baseline (Gill and Panda 2015; Wilkinson et al. 2020; Kesztyüs et al. 2019, 2021; Zhao et al. 2022). The two studies reporting no effects had among the smallest sample sizes ($n < 25$) and the shortest durations (4 and 7 weeks, respectively) (Parr et al. 2020b; Gonzalez et al. 2021). Four RCTs have investigated the effects of 10-hour TRE on body weight. The two RCTs comparing 10-hour TRE to usual eating habits reported favorable effects, whereas the two RCTs using an active comparator for the control group did not. Che et al. conducted an RCT in 120 adults with type 2 diabetes and found that a 10-hour TRE window from 8:00 a.m. to 6:00 p.m. reduced body weight by 2.9% over 12 weeks

relative to usual eating habits (Che et al. 2021). Similarly, Haganes et al. conducted an RCT in 131 women with overweight and found that a self-selected 10-hour TRE window ending by 8:00 p.m. decreased body weight by 2.3% over 7 weeks relative to usual eating habits (Haganes et al. 2022). However, the two RCTs comparing TRE to an active comparator reported no effects on body weight. Manoogian et al. found that a self-selected 10-hour TRE window combined with a Mediterranean diet did not reduce body weight in 24-hour shift-working firefighters relative to a Mediterranean diet alone (Manoogian et al. 2022). Thomas et al. found that a 10-hour TRE window starting within 3 h of waking up combined with 35% CR did not improve weight loss relative to 35% CR alone (Thomas et al. 2022). However, there was only a 1.3-hour difference in the fasting duration between groups.

In sum, 9–10-hour TRE likely reduces body weight, although the evidence is weaker.

4.4.5 *Meta-Analyses*

Pooling the results across all types of eating windows, meta-analyses consistently report that TRE reduces body weight (Chen et al. 2021, 2023; Moon et al. 2020; Elortegui Pascual et al. 2023; Liu et al. 2022b, 2023b; Wang et al. 2022; Huang et al. 2023; Silva et al. 2023). Most report that TRE decreases body weight by ~1–3% over a few weeks. The largest meta-analysis included 17 RCTs ($n = 899$ participants) and found that TRE reduced body weight by -1.60 kg (95% CI, -2.27 to -0.93 kg) over a few weeks (Liu et al. 2022b). Three other meta-analyses limited to adults with overweight and/or obesity reported similar reductions in body weight of between 1.28 and 1.48 kg (Liu et al. 2022b; Chen et al. 2023; Huang et al. 2023). Collectively, these results suggest that TRE decreases body weight to a modest-to-moderate degree over the short term (~1–3 months). The size of this weight-loss effect concurs with estimates of the energy deficit. Jamshed et al. estimated the energy deficit induced by 8-hour early TRE using differential equation modeling of the weight loss trajectories (Jamshed et al. 2022). In their trial, 8-hour early TRE reduced body weight by an additional 2.3 kg over 14 weeks, which was equivalent to an energy deficit of 214 kcal/day in the intention-to-treat analysis and 350 kcal/day in adherent participants. Thus, TRE induces a moderate energy deficit and is effective for losing weight relative to unrestricted eating.

4.4.6 *Body Composition*

4.4.6.1 *Body Fat*

Since fasting for 12–36 h increases fat oxidation (Anton et al. 2018; Klein et al. 1993), it has been hypothesized that intermittent fasting selectively burns fat while preserving lean tissue. Indeed, three RCTs conducted in respiratory chambers report

that TRE increases 24-hour fat oxidation and improves metabolic flexibility (Ravussin et al. 2019; Bao et al. 2022; Andriessen et al. 2022). Further, four studies in exercise-training adults found that TRE decreases fat mass while leaving fat-free mass unchanged (Moro et al. 2016, 2020; Tinsley et al. 2019; Tovar et al. 2021), suggesting that TRE may induce selective fat loss. However, larger RCTs have not found any evidence of selective fat loss, as decreases in body fat mirror decreases in body weight. In one of the largest studies to use dual x-ray absorptiometry, Jamshed et al. conducted an RCT in 90 adults with obesity and found that 8-hour early TRE did not increase the percent of body weight lost as fat (Jamshed et al. 2022). Moreover, the TRE group followed the expected “three-quarters rule” for fat loss. This result has been replicated by one other study (Teong et al. 2023). Thus, TRE does not selectively burn body fat. Further, there is no clear evidence that TRE decreases visceral or ectopic fat depots. Data on whether TRE decreases visceral adipose tissue are mixed (Gabel et al. 2018; Lowe et al. 2020; Cienfuegos et al. 2020; Jamshed et al. 2022; Wilkinson et al. 2020; He et al. 2022; Domaszewski et al. 2022, 2023; Moro et al. 2021; Chow et al. 2020; Kirkham et al. 2022; Kotarsky et al. 2021), while TRE does not appear to affect intrahepatic lipid content or liver stiffness in patients with NAFLD (Wei et al. 2023; Cai et al. 2019).

4.4.6.2 Fat-Free Mass

Importantly, extending the daily fasting period does not negatively affect fat-free or lean mass, as measured by dual x-ray absorptiometry (Gabel et al. 2018; Jamshed et al. 2022; Martens et al. 2020; Liu et al. 2022a; Cai et al. 2019; Meessen et al. 2021; Tinsley et al. 2017; Wingo et al. 2022; Kotarsky et al. 2021; Moro et al. 2016, 2020; Tinsley et al. 2019; Tovar et al. 2021; Domaszewski et al. 2020; Thomas et al. 2022; Teong et al. 2023; Richardson et al. 2023; Simon et al. 2022). This is true for a wide range of TRE windows and for study populations including adults with obesity, resistance-training adults, and elite cyclists. Furthermore, TRE also does not appear to impair fat-free mass accretion from resistance training, myofibrillar protein synthesis, muscular strength, or endurance (Meessen et al. 2021; Tinsley et al. 2017, 2019; Moro et al. 2016, 2020; Correia et al. 2021; Gonzalez et al. 2021). However, there have been a small number of exceptions (Lowe et al. 2020; Cienfuegos et al. 2020; Chow et al. 2020; Parr et al. 2023a). In particular, Lowe et al. found that skipping breakfast and eating between 12:00 and 8:00 p.m. decreased appendicular lean mass but not total fat-free mass relative to eating three meals/day (Lowe et al. 2020). However, the effect was clinically insignificant, and no data on protein intake were collected to assess protein balance.

4.4.6.3 Bone

A small number of RCTs have also assessed the effects of TRE on bone, and all reported no clinically significant effects on bone mass or bone mineral density relative to usual eating habits or a weight-loss-matched comparison group (Lowe et al.

2020; Jamshed et al. 2022; Martens et al. 2020; Lin et al. 2023; Meessen et al. 2021; Liu et al. 2023a; Kotarsky et al. 2021; Queiroz et al. 2022; Richardson et al. 2023; Lobene et al. 2021). One pilot study by Lobene et al. in 20 adults with overweight reported that a self-selected 8-hour TRE window increased bone mineral content, but the effect was clinically insignificant (<20 g), and there were no changes in markers of bone turnover or bone mineral density (Lobene et al. 2021).

4.4.7 Energy Expenditure

TRE does not affect total energy expenditure, resting metabolic rate, or physical activity. Three studies have measured the effects of TRE using a respiratory chamber, and all report that TRE does not affect 24-hour energy expenditure when both calorie intake and meal frequency are matched (Ravussin et al. 2019; Bao et al. 2022; Andriessen et al. 2022). Studies universally report that TRE does not affect resting metabolic rate (Lowe et al. 2020; Parr et al. 2020a; Martens et al. 2020; McAllister et al. 2020; Fagundes et al. 2023; Queiroz et al. 2022; Moro et al. 2016; Tinsley et al. 2019; Stratton et al. 2020; Ravussin et al. 2019; Bao et al. 2022; Andriessen et al. 2022; Nas et al. 2017; Gabel et al. 2019; Jones et al. 2020) or physical activity levels as measured by accelerometry (Cienfuegos et al. 2020; Liu et al. 2022a; Chow et al. 2020; Meessen et al. 2021; Tinsley et al. 2017; Kotarsky et al. 2021; Vidmar et al. 2021; Thomas et al. 2022; Teong et al. 2023; Gabel et al. 2019; Jones et al. 2020; Kirkham et al. 2023; Hutchison et al. 2019; Parr et al. 2023b). However, TRE may affect other aspects of energy metabolism, such as energy excretion. A randomized crossover controlled feeding study by Bao et al. found that 5.5-hour early TRE from 8:00 a.m. to 1:30 p.m. increased fecal energy excretion by a modest 31 kcal/day and tended to also increase urinary energy excretion by 7 kcal/day relative to eating over an 11-hour period (Bao et al. 2022). The total negative energy balance was equivalent to about 2.6% of energy intake, inducing a small calorie deficit.

4.4.8 Food Intake

Therefore, the main mechanism driving weight loss is likely lower energy intake. The first human studies found that TRE causes participants to spontaneously consume 10–30% fewer calories without consciously trying (Gabel et al. 2018; Cienfuegos et al. 2020; Wilkinson et al. 2020; Moro et al. 2021; Gill and Panda 2015; Brady et al. 2021). Since then, about two dozen RCTs have measured the effects of TRE on food intake. Among RCTs with at least 50 participants, about half report that TRE reduces energy intake relative to a control eating schedule (Cienfuegos et al. 2020; Zhang et al. 2022; Che et al. 2021; Lin et al. 2023; Xie et al. 2022; Haganes et al. 2022; Zeb et al. 2020), while the other half do not (Lowe et al. 2020; Jamshed et al. 2022; Manoogian et al. 2022; Liu et al. 2022a; Wei et al. 2023;

Vidmar et al. 2021; Lin et al. 2022; Kunduraci and Ozbek 2020; Thomas et al. 2022). RCTs reporting favorable effects report that TRE reduces energy intake by 200–550 kcal/day, with larger effects reported for longer fasting durations (i.e., 4–6-hour TRE). Indeed, one cross-sectional study reported that each 1-hour decrease in the daily eating duration was associated with eating 53 fewer kcal per day (Taetzsch et al. 2021). To date, no statistically significant differences in energy intake have been reported between early TRE and midday TRE (Zhang et al. 2022; Xie et al. 2022). However, because a couple of studies report 80–150 kcal/day numerically lower values for early TRE versus midday TRE, much larger and better-powered RCTs will be needed to definitely resolve this question. Studies reporting no effects on energy intake all compared TRE to an active comparator group, such as CR. For instance, Lin et al. found that 8-hour TRE from 12:00 to 8:00 p.m. was equally effective as 25% CR in reducing energy intake over 1 year (Lin et al. 2023), suggesting that TRE is an effective substitute for calorie counting.

Aside from decreasing energy intake, TRE does not affect other aspects of food intake. Specifically, TRE does not affect macronutrient composition in any population (Cienfuegos et al. 2020; Zhang et al. 2022; Jamshed et al. 2022; Parr et al. 2020a; Che et al. 2021; Lin et al. 2023; Liu et al. 2022a; Wei et al. 2023; Baum Martinez et al. 2022; Moro et al. 2021; McAllister et al. 2020; Mayra et al. 2022a; Tinsley et al. 2017; Witt et al. 2023; Wingo et al. 2022; Kotarsky et al. 2021; Lin et al. 2022; Kunduraci and Ozbek 2020; Tinsley et al. 2019; Correia et al. 2023; Haganes et al. 2022; Kirkham et al. 2023; Parr et al. 2023b; Antoni et al. 2018; McAllister et al. 2022; Isenmann et al. 2021; Steger et al. 2023a). Similarly, TRE does not affect diet quality as measured via the Healthy Eating Index (HEI) (Martens et al. 2020; Kunduraci and Ozbek 2020; Thomas et al. 2022; Steger et al. 2023a) or via individual metrics, such as saturated fat, cholesterol, sugar, fiber, sodium, soda, or processed foods (Cienfuegos et al. 2020; Parr et al. 2020a; Che et al. 2021; Manoogian et al. 2022; Kesztyüs et al. 2019; Mayra et al. 2022a; Antoni et al. 2018; Steger et al. 2023a; Malaeb et al. 2020) or alcohol intake (Cienfuegos et al. 2020; Parr et al. 2020a; Manoogian et al. 2022; Antoni et al. 2018; Steger et al. 2023a). Data are mixed on whether TRE reduces the frequency of meals and snacks but leans null (Parr et al. 2020a; Martens et al. 2020; Xie et al. 2022; Chow et al. 2020; Kim and Song 2023; Tinsley et al. 2019; Steger et al. 2023a; Malaeb et al. 2020).

4.4.9 Subjective Appetite

Most studies report that TRE affects subjective appetite patterns over the course of the day (Xie et al. 2022; Cai et al. 2019; Stratton et al. 2020; Thomas et al. 2022; Hutchison et al. 2019). Early TRE decreases hunger in the middle of the day and decreases or has no effects on hunger in the mid-evening (~8:00 pm) but increases hunger 1–2 h before bedtime (Zhang et al. 2022; Sutton et al. 2018; Ravussin et al. 2019; Bao et al. 2022; Nakamura et al. 2021). In aggregate, most studies suggest that early TRE lowers mean hunger levels when averaged across the day (Zhang

et al. 2022; Queiroz et al. 2022; Ravussin et al. 2019; Bao et al. 2022). Midday TRE decreases hunger in the mid-evening, though data are conflicting on whether it increases or decreases hunger in the morning before breakfast (Martens et al. 2020; Parr et al. 2020b). Data are evenly mixed on whether early TRE is more effective than midday TRE at reducing hunger (Zhang et al. 2022; Queiroz et al. 2022; Wehrens et al. 2017; Vujovic et al. 2022) and whether midday TRE decreases hunger more than a control eating schedule (Zhang et al. 2022; Queiroz et al. 2022; Wehrens et al. 2017; Vujovic et al. 2022). By contrast, OMAD in the evening increases self-reported hunger right before dinner relative to eating three meals/day (Stote et al. 2007). Data on self-selected TRE windows is mixed (Przulj et al. 2021; Haganes et al. 2022). Interestingly, Haganes et al. found that hunger is temporarily worse in the first week of adapting to TRE, suggesting that it may take time to physiologically adapt to TRE (Haganes et al. 2022). In terms of other eating behaviors, TRE does not affect dietary restraint, emotional eating, uncontrolled eating, or external eating (Cienfuegos et al. 2020; Li et al. 2021; Tinsley et al. 2019; Stratton et al. 2020; Steger et al. 2023a). It also does not trigger body image perception issues or eating disorder symptoms in individuals with no prior history of eating disorders (Cienfuegos et al. 2020; Fagundes et al. 2023).

4.4.10 Appetite-Related Hormones

About a dozen studies have tested the effects of TRE on appetite-related hormones, including incretins and adipokines. Some studies report that early TRE increases ghrelin about 1–3 h before bedtime (Xie et al. 2022; Ravussin et al. 2019; Nas et al. 2017; Vujovic et al. 2022) and midday TRE increases ghrelin levels during the morning hours (Nas et al. 2017; Vujovic et al. 2022). However, early and midday TRE have similar effects on mean 24-hour ghrelin levels (Nas et al. 2017; Vujovic et al. 2022). However, other studies report that TRE does not affect ghrelin levels at all (Sutton et al. 2018; Carlson et al. 2007; Zhao et al. 2022; Hutchison et al. 2019; McAllister et al. 2022). Larger studies are needed to resolve these conflicting findings. Similarly, data on the satiety hormone peptide YY (PYY) and on the incretins glucagon-like peptide-1 (GLP-1) and gastrointestinal peptide (GIP) are mixed (Sutton et al. 2018; Zhao et al. 2022; Ravussin et al. 2019; Hutchison et al. 2019).

Adipokines—such as leptin, adiponectin, and resistin—are hormones secreted from adipose tissue that play important roles in satiety, energy expenditure, and insulin sensitivity. The data on leptin are mixed, with some studies reporting that TRE decreases fasting leptin levels (Zhang et al. 2022; Moro et al. 2016, 2021; Ravussin et al. 2019; Vujovic et al. 2022). Studies that reported no effects had smaller sample sizes (Xie et al. 2022; Sutton et al. 2018; McAllister et al. 2022) or reported a clinically significant decrease (~20%) that did not reach statistical significance (Carlson et al. 2007). Interestingly, two studies found that early TRE decreases leptin more than midday TRE in the short term (Zhang et al. 2022; Vujovic et al. 2022). If confirmed, the putative reduction in leptin levels could reflect reduced

satiety (perhaps from depletion of fat stores due to weight loss) and/or reflect an improvement in leptin resistance. Studies on adiponectin are conflicting, with some reporting that TRE increases fasting adiponectin by ~10–20% (Moro et al. 2016, 2021; McAllister et al. 2020) and others not (Zhang et al. 2022; Carlson et al. 2007; Moro et al. 2020; McAllister et al. 2022; Karras et al. 2022). To date, no effects on resistin have been reported (Xie et al. 2022; Carlson et al. 2007; McAllister et al. 2022).

4.5 Glycemic Control

TRE improves glycemic control in patients with diabetes and prediabetes, though the effects in other populations appear to depend on the timing of the eating window and the amount of weight loss involved.

4.5.1 *Diabetes and Prediabetes*

To date, there have been five studies in people with type 2 diabetes, three in people with prediabetes, and zero in people with type 1 diabetes. Of the five studies in people with type 2 diabetes, two are robust RCTs (Che et al. 2021; Andriessen et al. 2022), and three are small, single-arm studies lasting only 2–4 weeks long (Parr et al. 2020a, 2023b; Arnason et al. 2017). Che et al. conducted the longest RCT and randomized 120 adults with type 2 diabetes to a 10-hour TRE window from 8:00 a.m. to 6:00 p.m. or to continue their usual eating patterns for 12 weeks (Che et al. 2021). TRE reduced HbA1c by 0.9%, fasting glucose by 12 mg/dl, fasting insulin, and HOMA-IR relative to the control group. TRE also simultaneously reduced the dosage of diabetes medications, making the net effect on HbA1c equivalent to a 1.3% reduction. These effects were accompanied by a moderate 2.9% weight loss advantage over the control group. Importantly, no hypoglycemic events were reported in the TRE group. A well-controlled crossover study by Andriessen et al. later demonstrated that TRE can improve glycemic control in type 2 diabetes independent of weight loss (Andriessen et al. 2022). Andriessen et al. conducted a 3-week crossover RCT comparing a 10-hour eating window ending by 6:00 p.m. versus eating over a ≥ 14 -hour period. TRE reduced mean 24-hour glucose levels as measured by continuous glucose monitoring (CGM) by 14 mg/dl and increased time-in-range by about 3 h/day, even though patients were weight-stable. Similarly, there was no increase in hypoglycemic events. Additionally, during a hyperinsulinemic-euglycemic clamp procedure, TRE improved fasting glucose by 18 mg/dl and non-oxidative glucose disposal by 180%, while improvements in whole-body insulin sensitivity fell short of statistical significance (11%; $p = 0.10$). Three small, single-arm studies reported that eating within a 6–9-hour window for

2–4 weeks decreased mean 24-hour glucose by 13 mg/dl, increased time-in-range by 2.5 h/day, and improved postprandial glucose levels and glycemic variability (Parr et al. 2020a, 2023b; Arnason et al. 2017). TRE also improved or tended to improve HbA1c and fasting and peak glucose values (Parr et al. 2020a, 2023b; Arnason et al. 2017). However, data on whether TRE improves fasting insulin in patients with type 2 diabetes are mixed (Parr et al. 2020a; Che et al. 2021; Andriessen et al. 2022).

There are only a couple RCTs in adults with prediabetes, and both report that TRE improves glucose metabolism. Sutton et al. conducted a 5-week crossover controlled feeding study in men with impaired glucose tolerance while matching food intake across groups and keeping the men weight-stable. Eating within a 6-hour early TRE window ending by 3:00 p.m. reduced fasting insulin levels by 5 mIU/l, decreased insulin resistance as measured during an oral glucose tolerance test (OGTT) by 24%, and improved β -cell responsiveness by 13% (Sutton et al. 2018). Early TRE also improved mean insulin levels and tended to improve HOMA-IR ($p = 0.07$), but there were no effects on fasting glucose, glucose tolerance, or mean glucose levels. Thus, early TRE improves both peripheral insulin resistance and β -cell function. Chair et al. conducted a 3-week RCT comparing a self-selected 8-hour eating window to habitual eating patterns in 101 adults with prediabetes (Chair et al. 2022). Eight-hour self-selected TRE decreased fasting glucose at the 3-month follow-up, though not at the 3-week mark. Insulin and HbA1c were not measured.

4.5.2 Metabolic Syndrome and PCOS

A handful of studies have tested TRE in populations with metabolic syndrome or PCOS, though most were uncontrolled. To date, no RCT has compared the effects of TRE alone versus habitual eating patterns in these populations, which makes it challenging to draw clear conclusions.

In patients with metabolic syndrome, three studies have been conducted. Wilkinson et al. and He et al. both conducted 12-week uncontrolled studies and found that self-selected 8–10-hour windows improved or tended to improve fasting glucose, fasting insulin, and HOMA-IR relative to baseline, with negligible effects on HbA1c (Wilkinson et al. 2020; He et al. 2022). In a post hoc analysis of adults with prediabetes ($n = 12$), Wilkinson et al. reported that a 10-hour self-selected window tended to decrease fasting glucose and mean 24-hour glucose by 9 mg/dl ($p = 0.09$) and 10 mg/dl ($p = 0.08$), respectively. Kunduraci et al. compared the effects of a self-selected 8-hour TRE window with 25% CR versus 25% CR alone in 70 patients with metabolic syndrome (Kunduraci and Ozbek 2020). Relative to CR alone, TRE combined with CR did not affect fasting glucose, fasting insulin, HOMA-IR, or HbA1c, though we note that both groups were counseled to consume the same number of calories and were given prescribed dietary menus to follow.

An estimated two-thirds to three-quarters of women with PCOS also have insulin resistance. Two single-arm studies have assessed the effects of TRE in women with PCOS and reported favorable effects on glycemic parameters (Li et al. 2021; Feyzioglu et al. 2023). Practicing 8-hour early or midday TRE for 5–6 weeks reduced fasting insulin, postprandial insulin, HbA1c, and insulin resistance as measured by both HOMA-IR and the AUC ratio during an OGTT. Eight-hour TRE also improved menstrual cycle regularity and reproductive hormones, though data on fasting glucose was mixed.

4.5.3 *Other Populations*

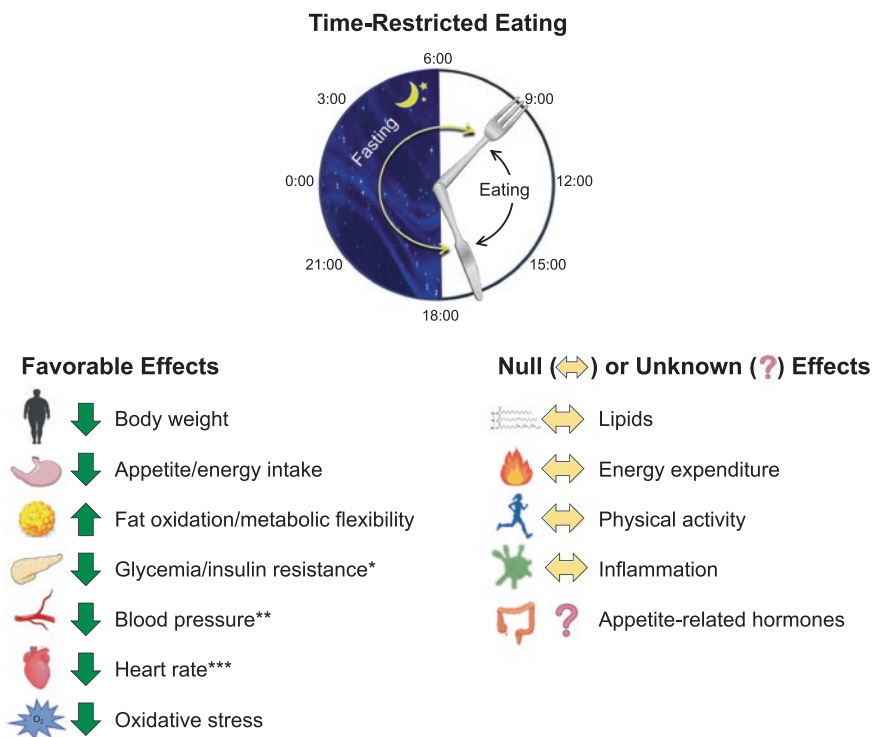
In other populations, TRE appears to improve glycemic parameters only if it involves an early window (Zhang et al. 2022; Xie et al. 2022; Kim and Song 2023; Bao et al. 2022; Jones et al. 2020; Hutchison et al. 2019; Jamshed et al. 2019; Steger et al. 2023b), stopping eating at ~6 pm (Parr et al. 2020b, 2023a; Nakamura et al. 2021), or is accompanied by ~5–7% weight loss relative to the control group (Lin et al. 2023; Moro et al. 2021). RCTs on early TRE nearly always report that it improves fasting glucose, fasting insulin, and/or HOMA-IR in both overweight (Zhang et al. 2022; Hutchison et al. 2019; Jamshed et al. 2019; Steger et al. 2023b) and healthy populations (Xie et al. 2022; Kim and Song 2023; Bao et al. 2022; Jones et al. 2020). For example, Zhang et al. conducted an 8-week free-living RCT in 60 adults with overweight and found that a 6-hour early TRE window from 7:00 a.m. to 1:00 p.m. reduced 24-hour mean glucose levels by 5 mg/dl relative to both a 6-hour midday TRE window from 12:00 to 6:00 p.m. and a control schedule (Zhang et al. 2022). Further, three isocaloric feeding studies found that 5.5–8-hour early TRE windows ending by 4:00 p.m. reduce 24-hour glucose levels by 4–5 mg/dl and decrease glycemic excursions after only 4–14 days (Bao et al. 2022; Jones et al. 2020; Jamshed et al. 2019). Bao et al. also reported decreases in mean postprandial insulin, which were most pronounced after the second and third meals of the day (Bao et al. 2022). Jones et al. also found that 8-hour early TRE from 8:00 a.m. to 4:00 p.m. improved whole-body insulin sensitivity, skeletal muscle glucose uptake, and branched-chain amino acid uptake relative to a calorie-matched control schedule (Jones et al. 2020). The only studies reporting no improvement in any glycemic endpoint were RCTs that used CR as the control group, prescribed the same number of calories for both groups, and/or had smaller than usual differences in the fasting duration between groups (only ~1.3–2.7 h) (Liu et al. 2022a; Wei et al. 2023; Thomas et al. 2022). Similar to early TRE, TRE windows ending at 6:00 p.m. also improve (Parr et al. 2023a; Nakamura et al. 2021) or tend to improve ($p = 0.09$) (Parr et al. 2020b) mean 24-hour glucose levels. In addition, one isocaloric and eucaloric crossover feeding in healthy older adults reported that an 8-hour eating window ending between 6:00 and 7:00 p.m. decreased mean glucose but not insulin levels during an OGTT (Martens et al. 2020).

Interestingly, three RCTs have compared early TRE, midday TRE, and a control schedule, and all report that early TRE is effective at improving fasting glucose, fasting insulin, and/or HOMA-IR relative to the control schedule, whereas midday TRE is not (Zhang et al. 2022; Xie et al. 2022; Kim and Song 2023). Indeed, nearly all RCTs on midday and self-selected TRE windows report no effects on fasting glycemic parameters, glucose tolerance, insulin resistance, β -cell function, or 24-hour mean glucose levels. This is true in both overweight (Lowe et al. 2020; Zhang et al. 2022; Chow et al. 2020; Cai et al. 2019; Fagundes et al. 2023; Kotarsky et al. 2021; Vidmar et al. 2021; Lin et al. 2022; Queiroz et al. 2022; Haganes et al. 2022; Bantle et al. 2023; Naguib et al. 2022) and generally healthy populations (Manoogian et al. 2022; Xie et al. 2022; Meessen et al. 2021; Kim and Song 2023; Moro et al. 2016, 2020; Tinsley et al. 2019; Brady et al. 2021; Richardson et al. 2023; Chiu et al. 2022). The main exceptions are two RCTs lasting 1 year (Lin et al. 2023; Moro et al. 2021) and one study testing 4–6-hour TRE windows ending by 7:00 p.m. (Cienfuegos et al. 2020). Cienfuegos et al. found that both a 4-hour window from 3:00 to 7:00 p.m. and a 6-hour window from 1:00 to 7:00 p.m. simultaneously reduced body weight by 3.2% and decreased fasting insulin and HOMA-IR relative to a weight-stable control group (Cienfuegos et al. 2020). Year-long studies by Moro et al. and Lin et al. reported an 8-hour window in the 12:00–9:00 p.m. range reduced fasting insulin and HOMA-IR, though the effects on fasting glucose were mixed (Lin et al. 2023; Moro et al. 2021). However, Lin et al. found that TRE did not improve glycemic parameters relative to a CR group that lost a similar amount of weight, suggesting that the effects in these two studies were solely due to the substantial weight loss (~5–7%). Finally, late eating windows may actually worsen glycemic control. Two RCTs found that practicing OMAD in the evening for at least 4 weeks elevates fasting glucose, postprandial glucose, and HbA1c; induces insulin resistance; and/or delays first-phase insulin secretion relative to either OMAD in the morning (Singh et al. 2020) or three meals/day (Carlson et al. 2007).

Thus, TRE improves glycemic control in patients with diabetes or prediabetes by improving insulin sensitivity, β -cell function, and non-oxidative glucose disposal. In other populations, only TRE interventions with early windows or involving large weight loss are effective for improving glycemic control.

4.6 Cardiovascular Health

A large number of studies have investigated the effects of TRE on cardiovascular health. Here, we review the effects on lipids, blood pressure, heart rate, inflammatory markers, and oxidative stress. There are a few promising findings, particularly for blood pressure and oxidative stress (Fig. 4.2).



* Type 2 diabetic and prediabetic patients. Otherwise, only eating windows that end by ~6 pm or involve $\geq 5\%$ weight loss.

** Only eating windows that end by ~6 pm and perhaps also hypertensive patients.

*** Affects 24-hour mean heart rate but not resting heart rate. Data on heart rate while exercising is conflicting.

Fig. 4.2 Effects of Time-Restricted Eating on Cardiometabolic Health. Research suggests that time-restricted eating improves several aspects of cardiometabolic health in humans, though some of the effects appear to depend on the study population and the duration and time of day of the eating window

4.6.1 Lipids

A large number of studies have investigated the effects of TRE on lipids in different populations. To date, no study has examined the effects of TRE in populations with dyslipidemia. In adults with overweight or otherwise at increased risk of cardiovascular disease, about three-quarters of RCTs report that TRE has no effects on fasting lipids (Gabel et al. 2018; Lowe et al. 2020; Cienfuegos et al. 2020; Manoogian et al. 2022; Lin et al. 2022, 2023; Liu et al. 2022a, 2023a; Wei et al. 2023; Chow et al. 2020; Fagundes et al. 2023; Kotarsky et al. 2021; Kunduraci and Ozbek 2020; Queiroz et al. 2022; Parr et al. 2020b; Haganes et al. 2022; Thomas et al. 2022; Andriessen et al. 2022; Jamshed et al. 2019). Notable exceptions include four RCTs reporting an increase in fasting LDL cholesterol (Martens et al. 2020; Meessen et al. 2021; Stote et al. 2007; Jamshed et al. 2019), particularly with OMAD (late TRE)

(Meessen et al. 2021; Stote et al. 2007), and four RCTs reporting a decrease in fasting triglycerides (Che et al. 2021; Cai et al. 2019; Hutchison et al. 2019; Chair et al. 2022). Studies reporting a decrease in fasting triglycerides have much larger sample sizes ($n > 100$) (Che et al. 2021; Cai et al. 2019; Chair et al. 2022) or are well-controlled feeding studies (Hutchison et al. 2019). One study reported a very modest 0.03 mM increase in fasting ketones with 6-hour early TRE (Jamshed et al. 2019). However, it is unclear whether this effect is clinically significant, and other TRE studies have not observed any effects (Martens et al. 2020; Fagundes et al. 2023; Mayra et al. 2022b). Overall, recent meta-analyses suggest that TRE does not affect fasting lipids (Liu et al. 2022b; Wang et al. 2022; Chen et al. 2023; Huang et al. 2023; Liu et al. 2023b).

Although TRE likely does not affect fasting lipids, it may affect other aspects of lipid metabolism. An RCT in 137 24-hour shift-working firefighters by Manoogian et al. found that a self-selected 10-hour TRE window decreased VLDL size relative to the control group, which may lower cardiovascular disease risk (Manoogian et al. 2022). Further, TRE may affect 24-hour values of certain lipid species. TRE increases mean 24-hour free fatty acid levels and peak triglyceride levels (Parr et al. 2020b; Nas et al. 2017), although it does not affect mean postprandial triglycerides or free fatty acids (Hutchison et al. 2019; Chiu et al. 2022). Further, an intriguing year-long study in African green monkeys found that 8-hour early TRE from 6:00 a.m. to 2:00 p.m. increased cholesterol efflux capacity, HDL particle size, and HDL apolipoprotein A-1 content, as well as lowered triglycerides by 12 mg/dl (roughly ~20%) (Kavanagh et al. 2023). These improvements were independent of changes in body weight. Further studies in humans are needed to confirm whether TRE affects lipid metabolism.

4.6.2 Blood Pressure

A couple dozen RCTs have tested the effects of TRE on blood pressure in various populations. Only one study has assessed the effects of TRE in adults with hypertension, and it reported that TRE improves blood pressure. Fanaroff et al. conducted a study in 37 adults with hypertension and found that a self-selected 8-hour TRE window reduced systolic blood pressure by 7 mm Hg relative to baseline, falling from 135 to 128 mm Hg over 18 weeks (Fanaroff et al. 2023).

Certain TRE windows may also improve blood pressure in at-risk populations. About a dozen RCTs have tested the effects in populations with overweight or metabolic syndrome, and one-third reported that TRE improved blood pressure. The four RCTs that reported an improvement tested either 6-hour early TRE or 8-hour TRE with a window that ended by 6:00 p.m. (Zhang et al. 2022; Jamshed et al. 2022; Sutton et al. 2018; Lin et al. 2022). These earlier TRE windows reduced systolic and/or diastolic blood pressure by 4–11 mm Hg over 5–14 weeks relative to a control schedule. Importantly, early TRE can reduce blood pressure even if no weight loss occurs. For instance, Sutton et al. conducted a eucaloric and isocaloric

controlled feeding study in men with prediabetes and reported that a 6-hour early TRE window ending by 3:00 p.m. reduced systolic and diastolic blood pressure by 11 and 10 mm Hg, respectively, relative to eating over ≥ 12 h (Sutton et al. 2018). By comparison, RCTs reporting no effects on blood pressure nearly all involve self-selected windows or windows that start after 12:00 p.m. (Lowe et al. 2020; Cienfuegos et al. 2020; He et al. 2022; Chow et al. 2020; Cai et al. 2019; Liu et al. 2023a; Kotarsky et al. 2021; Kunduraci and Ozbek 2020). For instance, Cienfuegos et al. found that 4-hour TRE from 3:00 to 7:00 p.m. and 6-hour TRE from 1:00 to 7:00 p.m. did not affect blood pressure in adults with obesity (Cienfuegos et al. 2020). Zhang et al. randomized 60 adults with overweight to either 6-hour early TRE from 7:00 a.m. to 1:00 p.m., 6-hour midday TRE from 12:00 to 6:00 p.m., or usual eating patterns, and early TRE but not midday TRE decreased blood pressure (Zhang et al. 2022). As further evidence that the timing of the eating window matters, Stote et al. conducted an 8-week crossover isocaloric feeding study in 15 lean, healthy adults and found that OMAD between 5:00–9:00 p.m. worsened systolic and diastolic blood pressure by 7 and 3 mm Hg relative to eating three meals/day (Stote et al. 2007).

Pooling the data across all TRE windows, meta-analyses find that TRE reduces blood pressure. One systematic review and meta-analysis of ten RCTs ($n = 694$ participants) reported that TRE reduces systolic blood by 4 mm Hg and tends to reduce diastolic blood pressure by 2 mm Hg ($p = 0.053$) (Wang et al. 2022). Among RCTs lasting ≥ 12 weeks, TRE did lower diastolic blood pressure, with low heterogeneity. A different meta-analysis of 12 RCTs in people with overweight ($n = 730$ participants) concluded that early TRE reduces diastolic blood pressure by 3 mm Hg, whereas later TRE windows do not (Liu et al. 2023b).

In sum, TRE may lower blood pressure in adults with hypertension. However, in adults with overweight or metabolic syndrome, only earlier windows decrease blood pressure. Several mechanisms could potentially explain these effects, including circadian mechanisms (Scheer et al. 2009; Shea et al. 2011), decreases in insulin levels (Persson 2007; Biston et al. 1996; Bhanot and McNeill 1996), changes in sympathetic tone, and fasting natriuresis (Kolanowski 1977).

4.6.3 Heart Rate

About 18 studies have tested the effects of TRE on resting heart rate, and most report no effects (Gabel et al. 2018; Cienfuegos et al. 2020; Zhang et al. 2022; Jamshed et al. 2022; Martens et al. 2020; Lin et al. 2023; Liu et al. 2022a; McAllister et al. 2020; Meessen et al. 2021; Witt et al. 2023; Lao et al. 2023; Kotarsky et al. 2021; Moro et al. 2020; Brady et al. 2021; Bao et al. 2022; McAllister et al. 2022; Stote et al. 2007; Zimmermann et al. 2023). Controlled feeding studies testing both early TRE (Sutton et al. 2018) and late TRE (Stote et al. 2007) similarly report no

effects. Only one meta-analysis has investigated the effects of TRE on resting heart rate, and it concluded that TRE does not affect resting heart rate (Wang et al. 2022).

However, TRE may lower mean 24-hour heart rate and heart rate while exercising. Bao et al. conducted a crossover RCT in healthy adults and found that an acute bout of 5.5-hour early TRE decreased mean 24-hour heart rate relative to eating over an 11-hour period. The effect was driven by a 3 beats/min decrease over several hours at nighttime and a 4 beats/min decrease while exercising. Overall, though, studies on the effects of TRE on heart rate during exercise are mixed. In a crossover study in healthy older adults, Martens et al. found that TRE lowered heart rate by 3 beats/min during light- and moderate-intensity exercise (Martens et al. 2020). Similarly, two other studies also found that TRE lowered heart rate while exercising (Witt et al. 2023; Kotarsky et al. 2021), with Witt et al. reporting the effects first kicked in around 4 km during a 10-km cycling test. However, three other studies reported no effect of TRE on heart rate during exercise (Meessen et al. 2021; Moro et al. 2020; Brady et al. 2021), including one study that measured heart rate across a wide range of VO_2 consumption levels (Meessen et al. 2021). Thus, although TRE does not affect resting heart rate, further research is needed to determine whether TRE affects 24-hour heart rate and heart rate during exercise.

4.6.4 *Inflammatory Markers*

About 18 studies have investigated the effects of TRE on inflammatory markers (Gabel et al. 2018; Cienfuegos et al. 2020; Zhang et al. 2022; Wilkinson et al. 2020; Martens et al. 2020; Sutton et al. 2018; McAllister et al. 2020; Li et al. 2021; Karras et al. 2021a; Schroder et al. 2021; Lao et al. 2023; Kotarsky et al. 2021; Moro et al. 2016, 2020; Zeb et al. 2020; McAllister et al. 2022; Arnason et al. 2017; Allen et al. 2020). Nearly all studies report that TRE does not affect fasting markers of inflammation, including C-reactive protein (CRP), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) (Gabel et al. 2018; Cienfuegos et al. 2020; Zhang et al. 2022; Wilkinson et al. 2020; Martens et al. 2020; Sutton et al. 2018; McAllister et al. 2020, 2022; Li et al. 2021; Karras et al. 2021a; Schroder et al. 2021; Lao et al. 2023; Kotarsky et al. 2021; Moro et al. 2016, 2020; Zeb et al. 2020; Arnason et al. 2017; Allen et al. 2020). There are a couple notable exceptions. Xie et al. reported that 8-hour early TRE reduced TNF- α and IL-8 relative to usual eating habits, whereas 8-hour midday TRE did not (Xie et al. 2022). However, the differences between TRE groups were not statistically significant. Also, Moro et al. found that 1 year of practicing 8-hour TRE by eating between 1:00–9:00 p.m. reduced IL-1 β , IL-6, and TNF- α in healthy resistance-training adults relative to eating over a 13-hour period, though this effect was likely driven by the 6.7% weight loss observed (Moro et al. 2021). Therefore, TRE does not appear to affect inflammation unless it is accompanied by large weight loss.

4.6.5 Oxidative Stress

A handful of studies have assessed the effects of TRE on oxidative stress, and all but one reported that TRE reduces oxidative stress. In the first study to do so, Sutton et al. conducted a controlled feeding study and found that 6-hour early TRE reduced 8-isoprostane by 14% in 5 weeks relative to eating over a 12-hour period (Sutton et al. 2018). Other studies have also reported positive findings. Cienfuegos et al. reported ~35% reductions in 8-isoprostane from both 4-hour and 6-hour TRE, which were accompanied by about 3.2% weight loss (Cienfuegos et al. 2020). Zhang et al. reported that 6-hour early TRE from 7:00 a.m. to 1:00 p.m. increased superoxide dismutase (SOD)—a marker of antioxidant potential—relative to both 6-hour midday TRE (12:00–6:00 p.m.) and a usual eating habits control group (Zhang et al. 2022). However, changes in 8-isoprostane and malondialdehyde did not differ among the three groups. Although a single-arm study, McAllister et al. found that self-selected 10-hour TRE lowered advanced oxidation protein products by 31% and advanced glycation end products (AGEs) by 25% over 6 weeks but did not affect nitrate/nitrite in resistance-trained firefighters (McAllister et al. 2022). However, another smaller study lacking a proper control group did not find any changes in superoxide dismutase, glutathione, or nitrate/nitrite (McAllister et al. 2020). Taken together, TRE improves a few markers of oxidative stress.

4.6.6 Other Cardiovascular Endpoints

Preliminary data on other cardiovascular endpoints have been null to date. Short-term studies lasting 5–8 weeks report that TRE does not affect endothelium-dependent dilation, arterial stiffness, and carotid artery intima-media thickness (Martens et al. 2020; Sutton et al. 2018; Tinsley et al. 2019). However, longer duration studies are needed, as most diet studies do not see changes in vascular endpoints until the 3- to 6-month mark.

4.7 Conclusions

In conclusion, TRE is a novel and promising intervention for losing weight and improving cardiometabolic health. More than 100 clinical trials have been conducted over the past decade. A wide variety of TRE windows ranging from 1.5 to 8 h long are all effective for losing weight. TRE induces moderate weight loss, typically 2.5–4% over 1–3 months. Preliminary evidence also suggests the weight loss can be sustained at ~5–7% for a year. Although 9–10-h TRE may also reduce body weight, the evidence is weaker. TRE induces weight loss primarily by decreasing energy intake, not by changing energy expenditure or physical activity. Whether

gastrointestinal peptides, incretins, and adipokines also play a role is unclear, and further research is needed. Similarly, it is unclear whether early TRE is more effective than midday TRE for losing weight. Nonetheless, the overwhelming majority of studies report that TRE is effective for losing weight.

TRE may also be an effective treatment for type 2 diabetes and prediabetes. In patients with type 2 diabetes, TRE improves time-in-range and reduces mean 24-hour glucose levels, HbA1c, glycemic excursions, and even the need for anti-hyperglycemic medication. Mechanistically, these effects are driven by improvements in several facets of glucose metabolism, including peripheral insulin sensitivity, β -cell function, and non-oxidative glucose disposal. In adults with overweight, only eating windows ending by 6:00 p.m. and TRE interventions involving ~5–7% weight loss improve glycemic control. Self-selected and midday TRE interventions do not appear to affect glycemic parameters in adults with overweight.

TRE also improves some aspects of cardiovascular health, particularly blood pressure and oxidative stress. Self-selected TRE decreases blood pressure in patients with hypertension. However, in adults with overweight or metabolic syndrome, only earlier windows ending by 6:00 p.m. decrease blood pressure. Although TRE does not affect fasting lipids, resting heart rate, or inflammatory markers, it may affect other facets of lipid metabolism and lower heart rate at certain times of day. Such data underscore the need to move beyond fasting cardiometabolic risk factors, which may fail to uncover improvements in cardiometabolic health, and instead measure 24-hour levels of glucose, insulin, lipids, blood pressure, and heart rate, as well as measure more robust or harder endpoints.

A major question in the field is whether the effects of intermittent fasting on health are solely due to energy restriction. Six well-controlled isocaloric feeding studies now report that earlier eating windows improve glycemic control, blood pressure, and/or oxidative stress, even when food intake is matched to the control group. This demonstrates that the effects of TRE are not solely due to energy restriction or weight loss. Other potential mechanisms may involve the circadian system, increased fat oxidation/metabolic flexibility, reduced oxidative stress, fasting natriuresis, autophagy, and other aging-related pathways.

Importantly, TRE appears to be feasible across a wide range of populations, with participants typically adhering 5–6.2 days/week. TRE is also safe in most populations, as it does not increase the risk of hypoglycemia in diabetes patients or increase disordered eating, and only a few minor side effects such as headaches, increased thirst, and hunger have been reported. However, out of an abundance of caution, TRE is contraindicated in children and women who are pregnant or lactating.

Thus, TRE is a safe and feasible intervention that induces weight loss and can improve cardiometabolic health. Preliminary evidence suggests that earlier windows may be more effective than later windows at improving glycemic control and blood pressure. Nonetheless, TRE is promising as a simple and effective treatment for obesity, diabetes, prediabetes, metabolic syndrome, and hypertension. However, more research is needed in these populations before TRE can be incorporated into clinical treatment guidelines.

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Chapter 5

Time-Restricted Eating: Safety and Efficacy in Youth



Jomanah Bakhsh, Elizabeth Hegedus, Sarah-Jeanne Salvy,
and Alaina P. Vidmar

Abstract Increased pediatric obesity has been accompanied by a rising incidence of life-limiting obesity-related comorbidities. In pediatrics, conventional health and behavior lifestyle interventions comprehensively address nutritional, physical activity, and behavioral topics to achieve clinically meaningful weight loss. There has been increased interest in alternative interventional approaches that can be implemented without requiring additional resources. One simpler and promising approach is based on the timing of eating. Intermittent fasting harnesses aligning circadian rhythms with peripheral neuroendocrine biology to improve cardiometabolic risk. Although the research on intermittent fasting in youth for weight loss purposes is still in its early stages, the simplicity of the approach has promise in this age group. Below, we summarize the available literature on all forms of intermittent fasting in youth. The current research, although limited, supports its feasibility, acceptability, and safety in youth with obesity. Future research is needed to investigate the efficacy of intermittent fasting in youth, discover the optimal timing of the eating window, and find the best methods to administer intermittent fasting across all communities.

J. Bakhsh

Department of Population and Public Health Science, University of Southern California, Los Angeles, CA, USA

E. Hegedus · A. P. Vidmar (✉)

Department of Pediatrics, Center for Endocrinology, Diabetes and Metabolism, Children's Hospital Los Angeles and Keck School of Medicine of USC, Los Angeles, CA, USA
e-mail: avidmar@chla.usc.edu

S.-J. Salvy

Research Center for Health Equity, Cedars-Sinai Medical Center, West Hollywood, CA, USA

5.1 Introduction

From 1963 to 2018, the prevalence of pediatric obesity tripled (Hampl et al. 2023). Model estimates suggest that if this rate continues, over half of youth will live with obesity by 2050 (Hales et al. 2018; Ogden et al. 2020). Increased pediatric obesity has been accompanied by a rising incidence of life-limiting obesity-related comorbidities, such as type 2 diabetes, obstructive sleep apnea, and fatty liver disease (Hales et al. 2018; Ogden et al. 2020). With increasing prevalence and increasing cost of care, obesity in youth is expected to result in extensive financial costs and significant life-limiting complications (Hampl et al. 2023). Acknowledging the increasing prevalence of pediatric obesity, the American Academy of Pediatrics (AAP) released clinical practice guidelines in January of 2023 for the care of children and adolescents with obesity (Hampl et al. 2023). The AAP recommends early and proactive intervention for children and adolescents with obesity that include concurrent intensive health and behavior lifestyle interventions, obesity pharmacotherapy, and bariatric surgery (Hampl et al. 2023). In pediatrics, conventional health and behavior lifestyle interventions comprehensively address nutritional, physical activity, and behavioral topics to achieve clinically meaningful weight loss (Styne et al. 2017; Barlow and Expert Committee 2007).

Due to limited resources in historically marginalized communities, which have the highest rates of pediatric obesity, the implementation of comprehensive behavioral interventions is often challenging (Hoare et al. 2021; Truby et al. 2011; Byrd et al. 2018). Limited access to specialized, multidisciplinary weight management clinics may, in turn, exacerbate health disparities (Byrd et al. 2018). Across all communities, adherence to treatment components is the strongest predictor of weight loss, and the best strategy for a given individual is the one they are willing and able to practice and sustain (Schmied et al. 2023; Dhaliwal et al. 2014; Skelton et al. 2012; Skelton and Beech 2011). Family engagement and support, individual preferences, developmental and social stages, and the larger socio-structural and geographical environments all contribute to youth's ability to receive comprehensive behavioral interventions as intended and sustain change in multiple behaviors conducive to weight loss (e.g., food monitoring, physical activity programing) (Ryder et al. 2019; Kelly et al. 2013, 2018). Intervention flexibility and simplicity, while maintaining effectiveness, are essential to adolescent's psychosocial framework and schedule (Truby et al. 2011).

Based on these challenges, there has been increased interest in alternative interventional approaches that can be implemented as intended without requiring additional resources or specialized training (Phillips et al. 2021; Hart et al. 2020, 2022; Baron et al. 2017). One simpler and more promising approach is based on the timing of eating (Vidmar et al. 2022). Pediatric guidelines have historically focused on dietary/nutrition and physical activity quality and quantity (Hampl et al. 2023; Barlow and Expert Committee 2007). While healthful eating and exercise certainly have health benefits, there is now increasing evidence suggesting that the timing of eating and – to some extent – the timing of physical activity, sedentary time, and

sleep may independently predict health trajectories and disease risk (Vidmar et al. 2022). Intermittent fasting, in the form of time-restricted eating, is one such theoretically driven intervention that harnesses aligning circadian rhythms with peripheral neuroendocrine biology to improve cardiometabolic risk (Fanti et al. 2021). Although the research on intermittent fasting in youth for weight loss purposes is still in its early stages, the simplicity of the approach has promise in this age group. Below, we summarize the available literature on all forms of intermittent fasting in youth (Table 5.1).

5.2 Intermittent Fasting in Youth

5.2.1 *Religious Fasting in Youth*

Religious fasting has been a common practice for centuries, long before intermittent fasting gained popularity for the management of obesity in youth. During Ramadan, for instance, Muslims fast from early dawn to sunset (8–10 h/day) and limit their food consumption to a large meal after sunset and a typically smaller meal before dawn (Trabelsi et al. 2020; Azad et al. 2012; Maughan et al. 2008). Studies examining the effects of Ramadan among youth have primarily focused on the safety of fasting among healthy youth and those with chronic disease that require adjustments to their medication regimens to account for prolonged fasting periods with a paucity of studies investigating the effects of Ramadan fasting on cardiometabolic outcomes or eating behaviors (Zabeen et al. 2014; Deeb et al. 2020).

In 2014, Radhakishun et al. conducted a prospective, observational study of 25 youth with obesity, ages 14 to 18 years to examine the effect of Ramadan fasting on cardiometabolic outcomes over the 6-week period. At week 6, compared to baseline there was no change in BMI, fasting blood glucose, or hemoglobin A1c. There was a significant decrease in total body fat percentage and a significant increase in heart rate, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and c-reactive protein. Follow-up measures were collected 6 weeks after Ramadan ended, and all clinical and biochemical levels had returned to baseline (Radhakishun et al. 2014). In 2019, Loh et al. conducted a systematic review of 17 observational studies (involving $n = 1699$ youth with type 1 diabetes) to investigate the safety of Ramadan fasting in youth with type 1 diabetes, and pooled analyses suggested that youth on continuous insulin infusion via an insulin pump system had less glycemic variability than those on multiple daily injection regimen. Based on these observations, the authors concluded that Ramadan fasting was safe for youth with type 1 diabetes providing appropriate supervision and medical management (Loh et al. 2019). A small number of studies to date have investigated the cardiometabolic effects of Ramadan in healthy youth athletes. In 2020, Trabelski et al. conducted a systematic review to investigate the effects of Ramadan fasting on dietary intake, body mass, and body composition in adolescent athletes who

Table 5.1 Summary of studies conducted on intermittent fasting protocols in youth

Author (date)	Design	Population	Size	Duration	Intervention	Control	Outcome	Key findings
Ramadan fasting								
Radhakrishun et al. (2014)	Retrospective	14–18 years BMI ≥ 95th%	25	6 weeks	Ramadan fasting	None	Body composition, vital signs, and fasting metabolic profile	At week 6, compared to baseline, there was a significant decrease in total body fat percentage and a significant increase in heart rate, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and c-reactive protein
Ali and Abizari (2018)	Prospective cohort	12–18 years	366	6 weeks	Ramadan fasting	Pre/post	Dietary intake, BMI	Dietary intake: At week 6, compared to baseline, meals consumed during Ramadan contained more vitamin A-rich fruits, milk, and milk products with a reduction in foods from roots and tubers, legumes and nuts, and dark green leafy vegetables. BMI: in this cohort, there was a significant weight reduction at week 6 compared to baseline of –1.5 kg that was regained 1 month after Ramadan ended
Loh et al. (2019)	Systematic review	10–18 years T1D On insulin therapy	1699	5 weeks	Ramadan fasting	None	Glycemic variability, hypoglycemia	At week 5, compared to baseline, there was less glycemic variability noted with insulin pump therapy compared to multiple daily injections during Ramadan fasting

Trabelsi et al. (2020)	Systematic review	10–19 years Athletes without obesity exercising at least three times per week	192	6 week	Ramadan fasting	None	Dietary intake, BMI, total fat mass	Twelve articles met the criteria. Continuation of training during Ramadan did not change body mass, total fat, or lean mass from before to the first week or from before to the fourth week of Ramadan. Dietary data showed the intake of energy fat, protein, carbohydrate, and water remained essentially unchanged during as compared to before Ramadan
Diizçeker et al. (2021)	Cross-sectional	14–18 years	287		Ramadan fasting	Non-fasting youth	Risk of disordered eating as measured by EAT-26 and TFEQ-R18	There was no significant difference observed between energy intake, EAT-26, and TFEQ-R18 scores between youth participating in Ramadan fasting and those who were not
Abdelmalek et al.(2022)	Pilot	14–18 years BMI ≥ 95th%	12	2 weeks	Ramadan fasting +45-min exercising sessions 5 days per week	No treatment control group	BMI, Total fast mass	At week 2, compared to baseline, there was a significant decrease in body weight, BMI, and body fat percentage

(continued)

Table 5.1 (continued)

Author (date)	Design	Population	Size	Duration	Intervention	Control	Outcome	Key findings
Alternate day fasting								
Jebeile et al. (2019a)	Pilot	12–17 years BMI ≥ 95th%	30	26 weeks	Phase 1: 0–12 weeks; ADF Phase 2: 12–26 weeks; self-selected either continued ADF or prescribed nutrition plan	None	Feasibility change %BMI _{p95} at week 12	Mean BMI _{p95} was significantly reduced at week 12 by $-5.6\% \pm 1.1$ and week 26 by $-5.1\% \pm 1.9$ compared with baseline
Time-restricted eating								
Vidmar et al. (2019)	Case-series	5–15 years BMI ≥ 95th%	4	16 weeks	TRE (8-hour eating window/16-hour fasting window)	None	Feasibility Change in BMIz	Mean BMIz decreased by $-8.9\% \pm 2.1$ at week 16 compared to baseline
Vidmar et al. (2021a)	Randomized controlled pilot	14–18 years BMI ≥ 95th%	45	12 weeks	TRE (8-hour eating window/16-hour fasting window)	12+ h eating window	Feasibility Change in %BMI _{p95} , dietary intake, eating behaviors	Feasibility: High satisfaction and adherence with TRE 6.1 days per week. Weight reduction: TRE resulted in decrease in %BMI _{p95} of -3.76 ± 5.76 compared to -3.72 ± 3.34 in control ($p = 0.70$). Dietary intake: TRE resulted in mean energy deficit of -441 kcal/day compared to -436 kcal/day in control ($p = 0.99$). Eating behaviors: no negative compensatory eating behaviors were reported in either group

Tucker et al. (2022)	Cross-sectional	8–17 years BMI ≥ 95th%	213	NA	Survey regard youth and parent perceptions of all forms of TRE	NA	Baseline eating window Perceptions of TRE approach in youth	Average daily eating windows: 12.5 ± 1.9 h (7:35 a.m.–8:05 p.m.) and included 5.6 ± 1.6 eating bouts (meals + snacks) Most parents (66%) reported being open to trying an eating window of ≤12 h/day, with 39% reporting being open to trying an eating window ≤10 h and only 26% reporting being open to trying an eating window ≤8 h
Jayakumar et al. (2023)	Secondary Analysis of a Randomized Controlled Pilot (Vidmar et al. 2021a, b)	14–18 years BMI ≥ 95th%	45	12 weeks	TRE (8-hour eating window/16-hour fasting window)	12+ h eating window	PSQ	There was no significant difference in the change in total PSQI score or sleep latency between TRE and control over the study period. There was no association between PSQI score and change in weight or glycemic profile between groups

Abbreviations: *ADF*, alternate day fasting; *BMI*, body mass index; *%BMIp95*, BMI in excess of the 95th percentile; *BMIz*, BMI z-score; *EAT-26*, eating attitudes test-26; *TFEQ-R18*, three-factor eating questionnaire-R18; *ID*, type 1 diabetes; *TRE*, time-restricted eating; *PSQI*, Pittsburgh Sleep Quality Index

continued to train at least three times per week. Twelve articles met inclusion criteria and were rated of moderate quality (Trabelsi et al. 2020). Ten articles evaluated dietary intake; four were rated as strong and the remaining moderate in quality. Twelve articles were included, and overall continuation of training during Ramadan did not change total body fat or lean mass within subjects. Ten articles evaluated dietary intake, and overall energy intake (total fat, total protein, total carbohydrates, and water intake) remained unchanged during Ramadan compared to before Ramadan in this cohort (Trabelsi et al. 2020). In 2022, Abdelmalek et al. conducted a study to investigate the effect of exercising on body composition, physical performance, and metabolic parameters in adolescents with BMI greater than the 95th percentile during Ramadan fasting compared to control. Twelve adolescents participated in a 45-min exercising session 5 days/week compared to the no treatment control group. The results showed a significant decrease in body weight, body mass index, and body fat percentage after Ramadan compared to before Ramadan, as well as compared to the second week of Ramadan (Abdelmalek et al. 2022).

Studies in adults suggest that Ramadan is associated with significant changes in eating habits, energy, and macronutrient intake (Faris et al. 2019; Kul et al. 2014), but few comparable studies have been conducted in children and adolescents. In 2018, Ali et al. conducted a prospective cohort study in 366 youth, ages 12 to 18 (50% female), to examine the effects of Ramadan fasting on dietary intake. Twenty-four-hour dietary recalls were accessed at baseline, week 2, week 6, and 2 weeks after Ramadan fasting ended. When compared to recalls collected at baseline and after Ramadan ended, dietary intake during Ramadan differed significantly. Meals consumed during Ramadan contained more vitamin A-rich fruits, milk, and milk products with a reduction in foods from roots and tubers, legumes and nuts, and dark green leafy vegetables. In this cohort, there was a significant weight reduction at week 6 compared to baseline of -1.5 kg that was regained 1 month after Ramadan ended (Ali and Abizari 2018).

Concerns have also been raised regarding the potential detrimental effects of fasting in inducing disordered eating behaviors and practices in youth. Chia et al. conducted a study using an ecological momentary assessment to examine the effect of Ramadan fasting on disordered eating behaviors in 28 participants during Ramadan compared to 74 non-fasting participants using a mobile app to capture eating behaviors six times per day for 7 days. In this cohort, there was no significant difference in disordered eating behaviors between groups on any variable captured (Chia et al. 2018). In 2021, Düzçeker et al. conducted a cross-sectional study to examine the association between Ramadan fasting and disordered eating behaviors in 238 fasting adolescents compared to 49 non-fasting adolescents. Risk of disordered eating was evaluated using the Eating Attitudes Test-26 (EAT-26) and Three-Factor Eating Questionnaire-R18 (TFEQ-R18). Body image dissatisfaction was rated with Stunkard's Figure Rating Scale (FRS). Nutritional status was assessed using a 24-h dietary recall. There was no significant difference between energy intake, EAT-26, and TFEQ-R18 scores between the groups. The authors concluded that motivation of adolescents to fast during Ramadan was due to spiritual decisions rather than weight control or other factors and Ramadan fasting was not correlated

with disordered eating behaviors or body image dissatisfaction (Düzçeker et al. 2021).

Studies in youth participating in Ramadan fasting are the first studies to explore the effects of fasting practices on cardiometabolic outcomes in pediatric cohorts. Overall, there is a paucity of literature, and thus it is challenging to make any clear conclusions on the effect of this approach on cardiometabolic effects in youth given that most studies are limited by lack of control group, small sample sizes, and lack longitudinal data. In addition, unlike other forms of intermittent fasting, such as time-restricted eating, Ramadan fasting does not align eating times with circadian rhythm, in that eating does not occur during the active phase, and therefore mechanistically may not be the best aligned eating pattern for humans to achieve improvements in cardiometabolic patterns. Further investigation is required to assess how religious fasting practices effect health outcomes in pediatric cohorts.

5.2.2 Alternate Day Fasting in Youth

In 2019, Jebeile et al. conducted the first pilot trial of intermittent fasting in obese youth seeking weight management treatment (Jebeile et al. 2019a). Thirty adolescents, aged 12–17 years, with BMI percentile greater than the 95th percentile obesity, completed a 26-week clinical trial of alternate day fasting (ADF), which includes intermittent energy restriction (IER) involving a very-low-energy diet (500–600 kcal/day) 3 days a week. For the first 12 weeks of intervention, participants followed an ADF regimen alternating a very-low-energy diet (500–600 kcal/day) 3 days a week and an age-appropriate healthy eating plan, emphasizing nutritious food choices, portion control, and reduction of highly processed food products and sugar-sweetened beverages, for the remaining days of the week. After completing the 12-week intervention, participants were given the choice to either continue ADF (3 days/week) or to follow a prescriptive nutrition plan for 14 weeks. Primary outcomes were feasibility (defined as recruitment rate, adherence, retention, and participant selection to remain in the ADF group after week 12) and change in BMI expressed as a percentage of the 95th percentile ($\%BMI_{p95}$) at week 12. Secondary outcomes were diet acceptability, body composition, cardiometabolic risk, vascular structure and function, quality of life as measured by the Pediatric Quality of Life Inventory scale, and eating behaviors as measured by the Dutch Eating Behavior Questionnaire (DEBQ-C). Of the 45 eligible youth, 30 elected to participate (67% recruitment rate; 83% female, mean $\pm$ SD age: 14.5 ± 1.4 years, median BMI of 34.9 kg/m^2); at week 12, 23/30 participants elected to continue in the AED, and 21/30 completed the study. In an intention-to-treat analyses, mean BMI_{p95} was significantly reduced at week 12 by $-5.6\% \pm 1.1$ and week 26 by $-5.1\% \pm 1.9$ compared with baseline. Percent body fat and triglyceride levels also significantly decreased over the study period. DEBQ-C and Pediatric Quality of Life Inventory scores improved throughout the intervention, and most youths who completed the

exit interview reported that ADF was acceptable and easy to follow (Jebeile et al. 2019a).

5.2.3 Time-Restricted Eating Intervention in Adolescents with Obesity

While ADF may be a feasible approach for some youth, it still requires the monitoring of daily energy intake to ensure adherence to different caloric allotments. By contrast, time-restricted eating (TRE) removes the need for counting of daily calorie intake or macronutrient content and strictly focuses on when food is consumed. TRE involves fasting for at least 14 h/day and can be practiced by eating early, in the middle, or late in the day, with avoidance of food for at least 3 hours before sleep onset. The consistency of the selected eating window and the alignment of the eating window with the active phase of an individual's circadian rhythm are important factors to consider supporting health outcomes. While pre-clinical and clinical studies substantiate the effectiveness of TRE in adults with obesity and type 2 diabetes, there is limited data on the use of TRE in youth (Browne and Cuda 2022; Vidmar et al. 2020). In 2019, Vidmar et al. reported a case series of four youth, aged 5–15 years, with various past medical histories, who practiced 8-hour TRE for 4 months. Participants showed that, on average, BMI z-scores decreased by $-8.9\% \pm 2.1$ over the 16-week intervention. Qualitative interviews highlighted high degrees of satisfaction with this approach with no reported barriers to implementation (Vidmar et al. 2019). Tucker et al. conducted a survey-based study to assess the feasibility of utilizing TRE in a pediatric weight management setting. Youth and their parents who were attending a pediatric weight management clinical program were surveyed regarding their views on a TRE approach. Two-hundred and thirteen youth (aged 8–17 years, 58% female, 52% White, 22% Black, 17% Hispanic/Latino, and 47% with a reported psychological disorder) completed an electronic survey. On average, parents reported their child's daily eating spanned 12.5 ± 1.9 h (7:35 a.m.–8:05 p.m.) and included 5.6 ± 1.6 eating bouts (meals + snacks). Most parents (66%) reported being open to trying an eating window of ≤ 12 h/day, with 39% reporting being open to trying an eating window ≤ 10 h and only 26% reporting being open to trying an eating window ≤ 8 h. Interest in TRE was not related to youth's age, sex, or ethnicity. Parents who reported being open to a TRE approach had youth with shorter eating windows and who ate few times over the day (Tucker et al. 2022).

In 2021, Vidmar et al. conducted a 12-week, randomized, controlled, feasibility trial of 45 youths (aged 14–18 years) with obesity, without diabetes (Vidmar et al. 2021a). Participants assigned to TRE were asked to self-select an 8-hour eating window, while the control group was instructed to eat over a 12-hour window. The primary outcome of this study was feasibility. Ninety percent of adolescents in the TRE groups opted to start eating between 10:00 a.m. and 12:00 p.m. Adolescents

were generally adherent to their assigned eating window, with an average of 5.2 days/week for the TRE group and 6.1 days for the control group. These results were compared to the CGM reports as well. TRE was viewed favorably, and 95% reported that they would recommend TRE to others. None of the participant assigned to TRE reported unhealthy eating practices or compensatory behaviors. The TRE group reported that this dietary approach would be feasible for most adolescents. Secondary outcomes included anthropometric measurements, dietary intake, quality of life, physical activity, and binge-eating behaviors. Weight and height were measured by the participant or their parent/guardian under the guidance of the research coordinators. Post-intervention, 28% (10/35) of youth assigned to TRE lost $\geq 5\%$ of their baseline weight vs. 13% (2/15) in the control group. Consistent with intention-to-treat analysis, across the study period, there was a significant decrease in %BMI_{p95} of (-3.76 ± 5.76) with no significant difference when compared to the control group. There was an overall significant 25% reduction in total caloric intake among all participants, with no significant differences between the groups on 24-hour dietary recall collected across the study period compared to baseline (TRE, mean energy deficit -441 kcal/day compared to -436 kcal/day in control). Compared to the baseline, there was no significant change in binge-eating episodes as measured on the binge-eating disorder screener-7 (BEDs-7), excessive eating, or eating-related distress among all participants. Also, none of the participants reported disordered eating symptoms, excessive exercise, or dietary restraint during the exit interview. In summary, this pilot study found that TRE was feasible and safe for adolescents with obesity, and it resulted in clinically meaningful weight loss for one-third of the intervention groups and one-quarter of the control group. This dietary approach did not adversely affect quality of life or physical activity. Additionally, TRE did not result in any unhealthy eating behaviors or impact daily living activities and social engagement (Vidmar et al. 2021a). In 2023, Jayakumar et al. conducted a secondary analysis of the Pittsburgh Sleep Quality Index (PSQI) assessments collected as part of the Vidmar et al. (Vidmar et al. 2021a) pilot study to examine the effects of 8-hour TRE on sleep parameters in youth with obesity (Jayakumar et al. 2023). For youth who completed PSQI surveys at baseline, week 4, and week 12, there was no significant difference in the change in total PSQI score or sleep latency between groups over the study period (Jayakumar et al. 2023). In addition, there was no association between PSQI score and change in weight or glycemic profile between the TRE and control groups (Jayakumar et al. 2023). These results are in alignment with data in adult cohorts investigating the association between TRE and sleep and suggest that an 8-hour TRE approach did not negatively impact sleep quality or efficiency when compared to control.

5.2.3.1 Time of Eating Window in Youth

As mentioned earlier, TRE can be practiced by eating early or late in the day. In adult populations, various lengths of TRE have been studied with minimal differences in efficacy found between 4-, 6-, 8- and 10-hour TRE protocols (Cienfuegos

et al. 2020; Prasad et al. 2021; Wilkinson et al. 2020). Late TRE is tantamount to skipping breakfast, whereas early TRE involves eating earlier in the day and initiating night fasting in late afternoon. For many adult cohorts, the most frequently chosen TRE window is between 9:00 a.m. and 7:00 p.m., which avoids meal skipping and allows for continued integration of social engagement in the evenings and may be a reasonable TRE protocol for youth as well. Adolescents are more likely to shift to an evening chronotype as they start to sleep later and wake up later in the day compared to younger children (Hagenauer and Lee 2012). While the average eating window of American adults has been well defined, less is known in terms of adolescent typical eating timing and duration. Vidmar et al. examined the timing of food consumption in 101 Hispanic adolescents with obesity (Vidmar et al. 2021b). The results indicated the majority of total kilocalories, carbohydrates, and added sugar were consumed between 11:00 a.m. and 7:00 p.m. on weekdays and weekends, and 72% reported that lunch was their first meal of the day. TRE strictly involves adhering to a prescribed eating window thereby preserving one's agency over food choices. Therefore, being able to make preferred food choices, choose a suitable eating window, eat with friends, and go to restaurants without limits on dietary options makes TRE a unique dietary approach that supports sustained behavior change without interfering with adolescents' daily commitments. This could result in better adherence to TRE than conventional dietary interventions. Indeed, adolescence is a period of increasing autonomy and independence reflected in youth food selection and choice of activities and time allocation; therefore, further investigation is needed to categorize eating patterns and frequencies in this age group to determine how meal-timing approaches should be designed and implemented for optimal efficacy (Cienfuegos et al. 2020).

5.2.3.2 Intermittent Fasting and Eating Behaviors in Youth

While larger, rigorous studies are needed, initial evidence suggests that ADF and TRE are feasible and acceptable to youth living with obesity. However, concerns remain around the potential side effects of fasting regimens in inducing disordered eating behaviors and attitudes. These concerns are certainly not limited to intermittent fasting and the object of ongoing discussions in scientific and clinical communities who seek to address obesity without causing harm during the pivotal developmental period of adolescence. Jebeile et al. in 2019 conducted a systematic review and meta-analysis to evaluate the association between obesity treatment with a dietary component and eating disorder risk in children and adolescents (Jebeile et al. 2019b). The results suggested that professionally supervised dietary interventions are associated with improvements in other markers of eating disorder risk, in contrast to adolescents dieting on their own, and promote supervised restraint. In response to Jebeile et al., Vanderwall et al., in a pilot study of IER in youth with obesity, published a commentary discussing concerns that prescribed restrictions may result in unhealthy compensatory practices versus inducing appropriate restraint as intended (Vanderwall and Carrel 2020). In 2022, Gaynson et al.

examined data from the Canadian Study of Adolescent Health Behaviors, a national study of Canadian adolescents and young adults ($N = 2762$; aged 16–30 years; 47% women, 38% men, 52% transgender/non-conforming [TGNC]), to determine the prevalence and characteristics of engagement in self-selected intermittent fasting and associations with eating disorder behaviors and psychopathology. In this sample, 47.7% of women, 38.4% of men, and 52.0% of TGNC reported practicing self-defined intermittent fasting (not prescribed by a health professional or monitored by a clinical or research team) in the past 12 months with over 75% using a TRE type of approach in which they reduced their daily eating window to 8 or 10 h/day and 5–10% practicing an alternate day fasting approach in which they would consume less than 500 to 800 kcal/day for 1–3 days/week. The survey results showed that for young adults practicing various forms of unmonitored fasting, there was an association with increased eating disorder psychopathology. The most consistent relationships between self-prescribed fasting practices and self-reported disordered eating behaviors were in women (Ganson et al. 2022). It is important to highlight that in this cohort, the fasting practices being executed by these young adults were not prescribed or monitored by clinical staff. These findings are in alignment with previous work that has shown that disordered eating behaviors are greater in youth who self-select dieting practices independent of medical advice, without oversight from clinical providers (Jebeile et al. 2019b, 2021, 2023). In contrast, youth with obesity, seeking professional medical support of their disease, often have reduction in their disordered eating behaviors, if present at baseline, over the course of their weight management treatment program (Jebeile et al. 2019b). Studies are needed to identify drivers of disordered eating behavior and attitudes among youth seeking weight management. Regardless of the interventional approach, however, the physical and psychosocial well-being of youth should continuously be monitored through ongoing discussions with the youth and their caregivers.

5.3 Practical Considerations for Clinicians and Researchers

As with any novel nutrition approach, intermittent fasting should be implemented by a care team with experience in the implementation of this intervention in a pediatric cohort to ensure appropriate monitoring and evaluation for any negative compensatory eating behaviors, effects on growth, school performance, and cognitive development overtime. With the growing rates of pediatric obesity, prescriptive nutrition approaches are being implemented more commonly in both clinical and research settings as a treatment tool. In the clinical practice guidelines for the care of youth with obesity, the AAP provides a framework for the implementation success implementation of health and behavior lifestyle interventions in youth with obesity that can be applied to the execution of intermittent fasting approaches in this age group (Hampl et al. 2023). The recommendations outline that to promote adherence, engagement, and sustained participation, interventions must identify and address social drivers of health, engage the entire family, and utilize motivational

interviewing approaches to align treatment expectation across all stakeholders (Hampl et al. 2023). This approach allows for the intervention to be tailored to the ongoing and changing needs of the individual youth, family, and community context (Hampl et al. 2023). The guidelines emphasize the successful lifestyle interventions are longitudinal, interactive, family-based, frequent, and prompt (Hampl et al. 2023). While further research is needed on the safety of prescriptive nutrition approaches in youth with obesity, including intermittent fasting strategies, there is no evidence to date that these approaches negatively impact growth trajectory or puberty and thus are appropriate to trial in collaboration with the family and pediatrician in typically developing children and adolescents. Many obesity medicine clinicians will routinely start youth on daily multivitamins while they are participating in prescriptive nutrition regimens; however, there is no evidence to suggest that intermittent fasting approaches result in inadequate daily nutritional intake (Tang et al. 2022; Lu et al. 2023; Ryan et al. 2018; Jelalian et al. 2014; Lee et al. 2020). Collection of longitudinal, frequent assessments of anthropometric, biochemical, and behavioral metrics in pediatric cohorts allows for close monitoring of any negative compensatory effect to be identified early and correct as needed with minimal risk to the patient and is recommended as part of any protocol implementation in this age group. Adding collection of continuous monitoring via continuous glucose monitors, actigraphy, and mobile health platforms provides an additional layer of protection and allows clinical and research teams to better understand how these interventions effect daily function cohort experiencing constant developmental changes.

Growing evidence in adults has been exploring the use of intermittent fasting approaches in other chronic conditions such as cancer and diabetes, and so there is preliminary findings that intermittent fasting can be modified based on the child's unique health condition and developmental factors. One example of this type of modification would be in youth living with diabetes. Currently, there are two protocols registered with clinical [trials.gov](https://clinicaltrials.gov) exploring the use of time-restricted eating in youth with obesity and type 2 diabetes (NCT04536480) and in youth with type 1 diabetes (NCT05031429). Modification to medication regimens can be made in collaboration with the treating clinicians, and use of continuous glucose monitors can assist with capturing 24-hour glycemic profiles during the intervention period to determine how prolonged fasting effects glycemic control and medication tolerance. Specifically in youth living with diabetes, time spent below 70 mg/dL can be captured and monitored weekly over the study period to ensure there are not changes to this metric while participating in intermittent fasting. Furthermore, if intermittent fasting results in decreased medication requirements (i.e., insulin), closely assessing hypoglycemia parameters will allow for down-titration of one insulin dose to prevent any severe hypoglycemia events during prolonged fasting periods. In addition, in cohorts with diabetes, clear treatment parameters for hypoglycemia are required prior to starting the intervention to ensure the youth is aware that they may have to drink a juice or consume simple sugar product to treat a low blood glucose value even if it is during their fasting window to prevent severe hypoglycemia, and that is accounted for in the treatment protocol. The outcomes are important for

safety monitoring and assessing effect of intermittent fasting on cardiometabolic outcomes in this age group.

When presenting an intermittent fasting approach to a youth and their family, there are some practical topics that often must be discussed prior to starting the intervention that arise as concerns for parents and clinicians. Firstly, as described above in detail, there remains concern that intentional restraint may inadvertently place excess attention on eating habits, body shape, and body size and lead to disordered eating patterns in youth living with obesity. To date, the literature does not support the association between structured supervised weight management programs and worsening of disordered eating patterns. Given the concern regarding the relationship between intermittent fasting approaches and eating behaviors, additional work is needed to determine the best way to screen youth prior to implementing an intermittent fasting approach how to accurately monitor for any compensatory eating behaviors that may arise overtime. This approach should be prescribed by a clinical or research team that is trained in delivering this type of care to youth cohorts and is able to monitor for age-appropriate growth, developmental, and psychological stability over the course of the treatment. Clinicians and scientists should look for warning signs like losing weight more quickly than expected, psychological symptoms like new-onset anxiety or depression, or abnormal laboratory values to help guide further evaluation if concerns arise. In addition, if the youth reports signs of disordered eating like an obsession with food, guilt or shame around eating, or fear of eating around peers, the clinician should discontinue intermittent fasting approach and refer for psychological evaluation. Secondly, there remains controversy regarding the association of skipping breakfast and obesity in youth. Although the literature is sparse, some evidence has suggested that youth who regularly have breakfast have a better diet quality and a higher intake of key food groups, whereas youth who skip breakfast tend to eat more energy-dense food and experience more excess hunger and overeating and that breakfast is beneficial for cognitive and academic performance in school. However, breakfast is the most frequently skipped meal, especially among adolescents, and there remains methodologic limitations of the existing research to fully elucidate this relationship. A systematic review of 43 studies, published in 2016, investigated the effects of breakfast on cognitive performance in youth. The findings suggested that breakfast consumption relative to fasting had a short-term (same morning) positive domain-specific effect on cognition. Tasks requiring attention, executive function, and memory were facilitated more reliably by breakfast consumption relative to fasting, with effects more apparent in undernourished children (Adolphus et al. 2016). Monzavi et al. conducted a systematic review, in 2019, and reported the prevalence of skipping breakfast ranging 10–30%, with an increasing trend in adolescents, mainly in girls, and that there is a relationship with overweight and obesity and skipping breakfast (Monzani et al. 2019). Thirty-nine articles were included and of moderate quality, assessing 286,804 children and adolescents living in 33 countries. There were several important limitations in this investigation in that there was not a universal definition of skipping breakfast and other factors important to define chrononutrition such as eating window, time to first and last calorie, sleep habits, food quality and quantity, physical

activity, and fasting duration were not included in most of the studies (Monzani et al. 2019). Parents often know of this data peripherally and are as confused as scientists on this association, and thus thoughtful conversations about what is known and the limitations are warranted. The field of chrononutrition has provided new language to highlight the importance of not only the skipping of one meal but the association of all eating occurrences throughout the day and other activities such as movement and sleep (Vidmar et al. 2022; Adafer et al. 2020). These factors can be reviewed with families as well as the honest discussion that this work remains novel, and so more work is needed to design precision approaches for each child and adolescent living with obesity (Bomberg et al. 2019; Hulman et al. 2021; Mortazavi and Gutierrez-Osuna 2021).

Taken together from the existing studies on intermittent fasting in youth, this approach is overall well tolerated in youth. Vidmar et al. collected Pediatric Quality of Life Scales (PedsQL) total scores reported by both youth participants (TRE Group: $n = 37$) and parent/guardian and found no negative effect of TRE on PedsQL total score, with a five-point increase in quality of life reported by both youth participants and their parent or guardian (Vidmar et al. 2021a). According to participants' responses to the surveys and interviews, TRE was viewed favorably with 95 % reported that they would recommend TRE to others, and the participants denied any undesirable compensatory behaviors. Five youth reported barriers to follow their 8-hour eating window, which included conflicts with family dinner time, work schedule, and social commitments; however, all participants were willing to continue with their assigned eating window after the study.

Several strategies can be harnessed to promote adherence and sustained engagement to intermittent fasting in youth cohorts. First, engaging youth participants and families at the beginning of the intervention period can facilitate adherence by identifying possible barriers early on and creating solutions. Many families have set dinner schedules, and so discussing a shift to the family dinner schedule is often something families are willing to consider when it is brought up openly by the treatment team. Second, many youth living with obesity experience food insecurity and thus receive the majority of their food intake at school, so ensuring the eating window aligns with food occasions set by the school district can promote adherence and engagement. Those schedules are set by the school districts annually and thus can be utilized to create treatment protocols and reviewed with families at consent. Finally, as the field of chrononutrition continues to expand, there is a desire to integrate rigorous controlled feeding trials with real-life implementation with continuous monitoring devices in pragmatic study designs. This approach allows for weekly variability and flexibility, which has been shown to promote long-term sustainment and adherence in this age group (Ryan et al. 2018; Jelalian et al. 2014; Venditti et al. 2018; Johnston et al. 2019).

5.4 Conclusion

Though the research is still in the preliminary stages, intermittent fasting may be a useful tool for weight management for youth with obesity. Adherence to treatment components is the strongest predictor of weight loss (Styne et al. 2017; Barlow and Expert Committee 2007), and the best strategy for a given individual is the one they are willing and able to practice and sustain. Time-based approaches are simple, straightforward, and non-stigmatizing and can be implemented across all communities regardless of resources without requiring significant change to the home and school environments. Meal-timing approaches do not require additional cost, advanced interventional skills, training, or certifications to be implemented and thus can be rapidly adopted by professionals including in resource-limited areas recommendations.

When determining an appropriate obesity intervention for an adolescent, the provider must take the medical history, current lifestyle habits, motivation, social support, and financial status into consideration. Dietary interventions like intermittent fasting are one of the many treatment options available. Intermittent fasting may be an approach that can be used intermittently or continuously and alone or in combination with other therapies such as obesity pharmacotherapy or bariatric surgery. This flexibility, especially in pediatric cohorts that have variable academic and extracurricular schedules occurring simultaneously with development changes, requires different treatment approaches at different times to promote efficacy and sustained engagement. In addition to its flexibility, another benefit of intermittent fasting is that the treatment is completely free. Research shows that obesity rates are closely related to economic status; those with lower socioeconomic status experience higher rates of obesity (John 2010; Sharifi et al. 2017). Most other nutrition interventions recommend individuals consume a higher quantity of whole foods, which are traditionally more expensive than the typical diet of an adolescent experiencing poverty. Barriers like lack of familial support, food insecurity, and nutritional content of school cafeteria meals may make it impossible for the adolescent to incorporate the intervention recommendations. Specifically, a TRE type of approach may offer an option that has greater alignment with adolescent dietary habits and social and developmental preferences without requiring changes to the home or school environment.

Intermittent fasting approaches may be a useful tool to support weight management in youth with obesity. The current research, although limited, supports its feasibility, acceptability, and safety in youth with obesity. Future research is needed to investigate the efficacy of intermittent fasting in youth, discover the optimal timing of the eating window, find the best methods to administer intermittent fasting across all communities, identify characteristics associated with positive responses, and investigate mechanism by which intermittent fasting operates in this age group.

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Part IV
Intermittent Fasting: Alternate Day
Fasting and 5:2

Chapter 6

Alternate Day Fasting and the 5:2 Diet: Effects on Body Weight and Metabolic Disease Risk Factors



Kelsey Gabel and Krista A. Varady

Abstract The goal of this review is to summarize the effects of alternate-day fasting (ADF; 0–500 kcal fast day, alternated with an ad libitum intake feast day) and the 5:2 diet (2 fast days per week, 5 ad libitum feast days) on body weight and markers of cardiometabolic health in humans. Results reveal that ADF and 5:2 can produce clinically significant weight loss in individuals with overweight or obesity. Clinical trial evidence shows that these diets produce reductions in energy intake of approximately 10 to 30% and mild to moderate weight loss (1–8% from baseline). These regimens may lower coronary heart disease and diabetes risk by decreasing blood pressure, insulin resistance, and oxidative stress. LDL cholesterol and triglyceride levels are also reduced, but improvements in these lipid parameters are not consistent. Favorable changes in the diversity and overall composition of the gut microbiome have also been demonstrated, but more studies will be needed to confirm these findings. These diets do not produce deleterious changes in sleep, reproductive hormones, thyroid hormones, RMR, eating disorder symptoms, or diet quality. While these preliminary data offer promise for the use of ADF and the 5:2 diet as an alternative option to daily calorie restriction for treating obesity and metabolic disturbances, more research will be needed to provide crucial clinical evidence to support the use of fasting in clinical treatment guidelines.

6.1 Introduction

In 2005, only three human trials of intermittent fasting had been published (Varady and Hellerstein 2007). Over the past 15–20 years, interest in the health benefits of fasting has surged in the scientific community and in the general

K. Gabel (✉) · K. A. Varady

Department of Kinesiology and Nutrition, University of Illinois at Chicago,
Chicago, IL, USA

e-mail: kdipma2@uic.edu

public. As a result of this growing interest, more human trial data are becoming available year after year (de Cabo and Mattson 2019; Patterson and Sears 2017). Intermittent fasting can be defined, in the simplest of terms, as periods of eating alternated with periods of not eating. These regimens are unique in that they require very minimal alterations in daily eating patterns in order to lose weight. When following intermittent fasting, participants do not need to count carbohydrates or omit certain food groups from their diet. These regimens also do not require individuals to buy expensive food products to instigate weight loss. In addition, most fasting protocols permit individuals to eat freely during certain periods of the day, which can augment diet tolerability. These distinct features of intermittent fasting may explain why these diets have become so popular in recent years.

The purpose of this review is to summarize the effects of two key forms of intermittent fasting, i.e., alternate-day fasting (ADF) and the 5:2 diet, on body weight and metabolic disease risk variables in human participants. The impact of these diets on other health-related variables, such as sleep and the gut microbiome, will also be discussed. The safety of these diets in humans will also be critically evaluated. The paper will conclude with some advice for clinicians and patients on how to implement these diets in everyday life.

6.1.1 Types of Intermittent Fasting

- **Alternate-day fasting (ADF):** This form of fasting generally consists of a “feast day” alternated with a “fast day.” On the feast day, participants may eat ad libitum, with no restrictions on quantities or types of foods eaten. On fast days, participants may prefer to consume only water, which is referred to as “zero calorie alternate day fasting (ADF).” Alternatively, individuals may prefer to consume 25% of their energy needs (approximately 500 kcal) on the fast day. This version is called “modified alternate-day fasting (ADF).” During modified ADF, the fast-day meal can be eaten all at once or spread throughout the day without impacting the degree of weight loss achieved (Hoddy et al. 2014).
- **The 5:2 diet:** This diet is a modified version of ADF that involves 5 feast days and 2 fast days per week. Akin to ADF, individuals are permitted to eat ad libitum on the feast days. On fast days during the 5:2 diet, ~25% of energy needs (500–800 kcal) are typically consumed, and the fast days can be placed on consecutive or nonconsecutive days during the week (Fig. 6.1).

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Alternate Day Fasting							
5:2 Diet							

Fig. 6.1 Types of intermittent fasting: ADF and 5:2 diet

6.1.2 Methods: Human Trial Selection

A PubMed search was conducted using the following keywords: “intermittent fasting,” “intermittent energy restriction,” “alternate day fasting,” “5:2 diet,” “intermittent energy restriction,” “fasting,” “clinical trial,” and “human” and MeSH terms “fasting,” “obesity/diet therapy,” “weight loss/physiology,” and “humans.” Inclusion criteria are as follows: (1) adult male and female participants, (2) randomized controlled trials, (3) nonrandomized trials, and (4) outcome measures that included body weight and markers of metabolic disease risk. Exclusion criteria are as follows: (1) cohort studies, (2) observational studies, (3) fasting performed as a religious practice, i.e., Ramadan or Seventh-Day Adventist, (4) trial durations of less than 1 week, (5) trials of time-restricted eating, and (6) animal studies. Our search retrieved 15 human trials of ADF (Table 6.1) and 20 trials of the 5:2 diet (Table 6.2). It should be noted that this is not a formal systematic review or meta-analysis. We were not able to combine the findings from these various studies as their experimental designs, sample compositions, and trial durations were markedly different.

Table 6.1 Human trials of alternate-day fasting (ADF)

References	Subjects	Diet length	Design and intervention groups	Body weight	Body composition			Blood pressure	Plasma lipids			Glucoregulatory factors		Inflamm. markers	Oxidative stress	
					FM	FFM	VF		LDL	HDL	TG	Fasting Glucose	Fasting Insulin			IR/IS/ A1c
Zero-calorie alternate-day fasting (zero calories consumed on the fast day)																
Heilbronn et al. (2005)	n = 16, MF Normal wt No diabetes	3-week	Single arm 1. ADF fast day (0 kcal) Feast day (ad lib)	1. ↓ 3%*	1. ↓ ↓*	–	1. ∅	1. ↓* F only	1. ∅	1. ↓* F only	1. ∅	1. ↓*	–	–	–	
				1. ↓ 4%† 2. ∅	1. ↓† 2. ∅	1. ↓† 2. ∅	1. ↓SBP† ∅ DBP 2. ∅	1. ∅ 2. ∅	1. ↓ † 2. ∅	1. ∅ 2. ∅	1. ↓ † 2. ∅	–	–	–		
Stekovic et al. (2020)	n = 60, MF Normal wt No diabetes	4-week	RCT: parallel arm 1. ADF fast day (0 kcal) Feast day (ad lib) 2. Control (ad lib)	1. ↓ 8%*	1. ↓ ↓*	1. ↓*	–	1. ↓* 2. ↓*	1. ∅	1. ↓* 2. ↓*	1. ∅	1. ∅	–	–	–	
				2. ↓ 6%*	2. ↓ ↓*	2. ↓*	2. ↓*	–	1. ↓* 2. ↓*	1. ∅	1. ↓* 2. ↓*	1. ∅	1. ∅	1. ∅	–	–
Catenacci et al. (2016)	n = 26, MF Obese No diabetes	8-week	RT: parallel arm 1. ADF fast day (0 kcal) Feast day (ad lib) 2. CR (1500 kcal/day)	1. ↓ 8%*	1. ↓ ↓*	1. ↓*	–	1. ↓* 2. ↓*	1. ∅	1. ↓* 2. ↓*	1. ∅	1. ∅	–	–	–	
				2. ↓ 6%*	2. ↓ ↓*	2. ↓*	2. ↓*	–	1. ↓* 2. ↓*	1. ∅	1. ↓* 2. ↓*	1. ∅	1. ∅	1. ∅	–	–

Parvaresh et al. (2019)	<i>n</i> = 69, MF Obese No diabetes	8-week	RT; parallel arm 1. ADF fast day (500 kcal) Feast day (ad lib) 2. CR (1500 kcal/day)	1. ↓ 5%* 2. ↓ 3%*	–	–	1. ↓* 2. ↓*	1. ↓ SBP* ↓ DBP* 2. Ø SBP Ø DBP	1. Ø 2. Ø	1. ↓* 2. ↓*	1. ↓* 2. Ø	1. ↓ IR* 2. Ø IR	–	–
Klempel et al. (2013)	<i>n</i> = 32, MF Obese No diabetes	8-week	RT; parallel arm 1. ADF fast day (500 kcal) Feast day (ad lib) + Low fat diet (25% fat) 2. ADF fast day (500 kcal) Feast day (ad lib) + High fat diet (45% fat)	1. ↓ 5%* 2. ↓ 4%*	1. ↓* ↓* 2. ↓*	1. Ø 2. Ø	1. ↓* 2. ↓*	1. Ø 2. Ø	1. ↓* 2. ↓*	1. Ø 2. Ø	–	–	–	–

(continued)

Table 6.1 (continued)

References	Subjects	Diet length	Design and intervention groups	Body weight	Body composition			Blood pressure	Plasma lipids			Glucoregulatory factors			Inflamm. markers	Oxidative stress
					FM	FFM	VF		LDL	HDL	TG	Fasting Glucose	Fasting Insulin	IR/IS/A1c		
Bhutani et al. (2013b)	n = 83, MF Obese No diabetes	12-week	RCT: parallel arm 1. ADF fast day (500 kcal) Feast day (ad lib) + exercise (aerobic) 2. ADF 3. Exercise 4. Control	1. ↓ 7% ↑ 2. ↓ 4% ↑ 3. ↓ 1% 4. Ø	1. ↓ ↓ 2. ↓ ↓ 3. ↓ Ø 4. Ø	1. Ø 2. Ø 3. Ø 4. Ø	1. ↓ 2. Ø 3. Ø 4. Ø	1. Ø 2. Ø 3. Ø 4. Ø	1. Ø 2. Ø 3. Ø 4. Ø	1. ↑ 2. Ø 3. Ø 4. Ø	1. Ø 2. Ø 3. Ø 4. Ø	1. Ø 2. Ø 3. Ø 4. Ø	1. Ø 2. Ø 3. Ø 4. Ø	IR, A1c: 1. Ø 2. Ø 3. Ø 4. Ø	1. Ø CRP 2. Ø CRP 3. Ø CRP 4. Ø CRP	–
Varady et al. (2013)	n = 32, MF Normal wt No diabetes	12-week	RCT: parallel arm 1. ADF fast day (500 kcal) Feast day (ad lib) 2. Control (ad lib)	1. ↓ 7% ↑ 2. Ø	1. ↓ ↑ 2. ↓ Ø	1. Ø 2. Ø	–	1. Ø 2. Ø	1. Ø 2. Ø	1. Ø 2. Ø	1. Ø 2. Ø	–	–	–	1. ↓ CRP [†] 2. Ø	–

Kalam et al. (2019a)	<i>n</i> = 52, MF Obese No diabetes	24-week	Single arm 1. ADF: fast day (600 kcal) Feast day (ad lib) + Low carb diet (30% carbs)	1. ↓6%*	1. ↓*	1. ∅	1. ∅	1. ∅	1. ∅	1. ∅	1. ∅	1. ∅	1. ∅	1. ∅ IR ∅ A1c	–	–
Trepanowski et al. (2017)	<i>n</i> = 100, MF Overweight obese No diabetes	52-week	RCT: parallel arm 1. ADF fast day (500 kcal) Feast day (ad lib) 2. CR (1500 kcal/day) 3. Control (ad lib)	1. ↓6% [†] 2. ↓5% [†] 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ↓ [†] 2. ↓ [†] 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ↓ [†] 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ↓ [†] 2. ∅ 3. ∅	1. ∅ IR, ∅ A1c 2. ↓IR [†] , ∅ A1c 3. ∅ IR, ∅ A1c	CRP,Hcy: 1. ∅ 2. ∅ 3. ∅	–	

∅: Nonsignificant change

**P* < 0.05, Significantly different from baseline (within group effect)

[†]*P* < 0.05, Significantly different from the control or comparison group (between group effects). When control group is present, only significant changes versus controls were reported

Abbreviations: 8-*iso*, 8-isoprostane; *A1c*, hemoglobin A1c; *Ad lib*, ad libitum energy intake; *ADF*, alternate-day fasting; *CR*, calorie restriction; *CRP*, C-reactive protein; *DBP*, diastolic blood pressure; *FM*, fat mass; *FFM*, fat-free mass; *F*, female; *Hcy*, homocysteine; *HDL*, high-density lipoprotein cholesterol; *IR*, insulin resistance; *IS*, insulin sensitivity; *LDL*, low-density lipoprotein cholesterol; *M*, male; *NT*, nitrotyrosine; *PC*, protein carbonyls; *RT*, randomized trial; *RCT*, randomized controlled trial; *SBP*, systolic blood pressure; *TG*, triglycerides; *VF*, visceral fat mass

Table 6.2 Human trials of the 5:2 diet

References	Subjects	Diet length	Design and intervention groups	Body composition			Blood pressure	Plasma lipids			Glucoregulatory factors			Inflamm. markers	Oxidative stress	
				FM	FFM	VF		LDL	HDL	TG	Fasting Glucose	Fasting Insulin	IR/GT/ A1c			
The 5:2 diet alone or compared to control																
Cook et al. (2022)	n = 52, M/F	4-week	Exploratory analysis 1. 5:2: fast day (500 kcal) Feast day (ad lib)	–	–	–	–	–	–	–	–	–	–	–	–	–
Guo et al. (2021)	n = 39, M/F, metabolic syndrome No diabetes	8-week	RCT: parallel arm 1. 5:2: Fast day (500 kcal) Feast day (ad lib) 2. Control	1. ↓* 2. ∅	1. ↓*† 2. ∅	1. ↓*† 2. ∅	1. ∅ 2. ∅	1. ∅ 2. ↑*	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ↓* 2. ∅	1. ∅ 2. ∅	1. ↓ IR* 2. ∅	1. ↓* sCD40L 2. ∅	1. ↓* MDA, ADMA, ↑ plasma nitrate 2. ∅	
Kord Varkaneh et al. (2022)	n = 44, M/F Overweight Obesity	12-week	RCT: parallel arm 1. 5:2: Fast day (25% TEE) Feast day (ad lib) 2. Control	1. ↓* 2. ↓* 2. ↑*	1. ↓* 2. ↓*	–	–	1. ∅ 2. ↑*	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ↓* 2. ↓*	1. ∅ IR 2. ∅ IR	1. ↓* hsCRP ↓* CK-18 ∅ TAC 2. ↓* hsCRP ↓* CK-18 ∅ TAC	–	–	

Table 6.2 (continued)

References	Subjects	Diet length	Design and intervention groups	Body weight	Body composition			Blood pressure	Plasma lipids			Glucose regulatory factors			Inflamm. markers	Oxidative stress
					FM	FFM	VF		LDL	HDL	TG	Fasting Glucose	Fasting Insulin	IR/GT/ A1c		
Harvie et al. (2011)	<i>n</i> = 89, F Overweight No diabetes	24-week	RT: parallel arm 1. 5:2: fast day (500 kcal) Feast day (ad lib) 2. CR (1500 kcal/day)	1. ↓7%* 2. ↓5%* 2.	1. ↓* ↓* 2.	1. ↓* 2. ↓*	–	1. ↓ SBP* ↓DBP* 2. ↓ SBP* ↓DBP*	1. ↓* 2. ↓* 2.	1. ∅ 2. ↓*	1. ↓* ↓*	1. ↓* 2. ∅	1. ↓*† 2. ↓*	1. ↓IR* 2. ↓IR*	1. ↓CRP* 2. ↓CRP*	1. ↓AOPP* 2. ↓AOPP*
Antoni et al. (2018)	<i>n</i> = 27, M/F, Overweight Obese No diabetes	Achieve 5% target weight loss	RT: parallel arm 1. 5:2: Fast day (20% TEE) Feast day (ad lib) 2. CR (–23% TEE)	1. 5.3% ↓* 2. ↓5%* ↓*	1. ↓* ↓* 2.	1. ↓* 2. ↓*		1. ↓ SBP* 2. ↓ DBP*	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ∅ IR 2. ∅ IR	–	–
Schubel et al. (2018)	<i>n</i> = 150, MF Obese No diabetes	24-week	RCT: parallel arm 1. 5:2: fast day (500 kcal) Feast day (ad lib) 2. CR (1600 kcal/day) 3. Control (ad lib)	1. ↓7%† 2. ↓5%† 3. ∅				1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ IR 2. ∅ IR 3. ∅ IR	CRP, IL-6, TNF-α: 1. ∅ 2. ∅ 3. ∅	–

Sundfor et al. (2018)	<i>n</i> = 112, MF Obese No diabetes	48-week	RT: parallel arm 1. 5:2: fast day (500 kcal) Feast day (ad lib) 2. CR (1500 kcal/day)	1. ↓7%* 2. ↓7%*	–	–	1. ∅ SBP ↓* DBP 2. ↓ SBP* ↓ DBP*	1. ∅ 2. ∅	1. ↓* 2. ↓*	1. ∅ 2. ∅	1. ∅ 2. ∅	1. ↓ A1c* 2. ↓ ↓A1c*	1. ∅ CRP 2. ∅ CRP	–
The 5:2 diet compared to other dietary patterns														
Holmer et al. (2021)	<i>n</i> = 74, M/F, overweight obese NAFLD No diabetes	12-week	RCT: parallel arm 1. 5:2: fast day (F: 500, M:600 kcal) Feast day (ad lib) 2. Low carb high fat (W, 1600 kcal/day; M, 1900 kcal/day) 3. Standard of care (MyPlate)	1. ↓7.4%* 2. ↓7.7%* 3. ↓2.6%*	–	–	1. ∅ 2. ↓ SBP* ↓ DBP* 3. ∅	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ↓* 3. ∅	1. ∅ 2. ↓* 3. ∅	1. ↓ A1c* ↓IR* 2. ↓ A1c* ↓IR* 3. ∅	–	–	–
Headland et al. (2019)	<i>n</i> = 146, M/F Overweight Obese No diabetes	52-week	Parallel arm 1. 5:2: fast day (F: 500, M:600 kcal) Feast day (ad lib) 2. Week-on, week-off (F: 1000, M: 1200 kcal/day) Week-off (ad lib) 3. CR (–30% TEE)	1. ↓5.9%* 2. ↓5.5%* 3. ↓7.6%*	1. ↓* 2. ↓* 3. ↓*	–	–	1. ∅ 2. ∅ 3. ∅	1. ↑* 2. ↑* 3. ↑*	1. ∅ 2. ∅ 3. ∅	1. ∅ 2. ∅ 3. ∅	–	–	–

(continued)

Table 6.2 (continued)

References	Subjects	Diet length	Design and intervention groups	Body weight	Body composition			Blood pressure		Plasma lipids			Glucoregulatory factors			Inflamm. markers	Oxidative stress
					FM	FFM	VF			LDL	HDL	TG	Fasting Glucose	Fasting Insulin	IR/GT/ A1c		
Battucci et al. (2022)	n = 36, F Obesity No diabetes	8-week	RCT: parallel arm 1. 5:2: fast day (600 kcal) Feast day (ad lib) 1. 5:2 + exercise 3. Exercise (HIIT 3x/wk)	1. ↓1.1% 2. ↓1.7%† 3. ↓1.4% ↓*	1. ∅ 2. ↑* 3. ↑*	–	–	–	–	–	–	–	–	–	–	–	–
Kang et al. (2022)	n = 131, M/F, overweight Obese No diabetes	12-week	RT: parallel arm 1. 5:2: fast day (30% TEE) Feast day (70% TEE) 2. CR (70% TEE) 3. High protein meal replacement (70% TEE provided) 150–300 m physical activity	1. ↓9%† 2. ↓5.7% 3. ↓8.6%† ↓*	1. ↑* 2. ↑* 3. ↑*	–	–	–	–	–	–	–	–	–	–	–	–

∅ Nonsignificant change

*P < 0.05, †Significantly different from baseline (within group effect)

‡P < 0.05, §Significantly different from the control or comparison group (between group effect). When control group is present, only significant changes versus controls were reported

Abbreviations: 8-iso, 8-isoprostane; Ad lib, ad libitum energy intake; ADMA, asymmetric dimethylarginine; CK-18, cytokeratin 18; AOPP, advanced oxidation protein products; CR, calorie restriction; CRP, C-reactive protein; DBP, diastolic blood pressure; FM, fat mass; FFM, fat-free mass; F, female; Hcy, homocysteine; HDL, high-density lipoprotein cholesterol; HIIT, high-intensity interval training; hsCRP, high-sensitivity c-reactive protein; IL-6, Interleukin-6; IR, insulin resistance; IS, insulin sensitivity; LDL, low-density lipoprotein cholesterol; M, male; MDA, malondialdehyde; NT, nitrotyrosine; PC, protein carbonyls; RT, randomized trial; RCT, randomized-controlled trial; SBP, systolic blood pressure; sCD40L, soluble CD40-ligand; TAC, T-cell alpha chemoattractant; TEE, total energy expenditure; TG, triglycerides; TNF-α, tumor necrosis factor-alpha; VF, visceral fat mass

6.2 Effects of ADF and the 5:2 Diet on Body Weight, Body Composition, and Energy Intake

6.2.1 *Alternate-Day Fasting (ADF)*

6.2.1.1 ADF: Weight Loss Efficacy

Based on the trials reviewed here, zero-calorie ADF and modified ADF appear to produce similar degrees of weight loss over short trial periods (Table 6.1). For instance, zero-calorie ADF results in 3–8% weight loss over 3–8 weeks, while modified ADF results in 4–8% weight loss over 6–12 weeks. Of note, longer durations of ADF, i.e., 24 weeks, do not produce greater weight loss (6%) (Kalam et al. 2019a; Trepanowski et al. 2017), when compared to shorter trial durations. This would suggest that the weight loss efficacy of ADF may peak at approximately 12 weeks. Both zero-calorie ADF and modified appear to be effective for weight loss in normal weight individuals (Heilbronn et al. 2005; Stekovic et al. 2020; Varady et al. 2013) and those with overweight (Cho et al. 2019) or obesity (Hoddy et al. 2014; Kalam et al. 2019a; Trepanowski et al. 2017; Bhutani et al. 2013a; Catenacci et al. 2016; Eshghinia and Mohammadzadeh 2013; Johnson et al. 2007; Klempel et al. 2013; Parvaresh et al. 2019; Varady et al. 2009). In view of the limited number of studies performed to date, it is difficult to ascertain whether the rate of weight loss is greater in people with obesity versus those with normal weight. This will be an interesting area to explore in future research.

Three human studies (Trepanowski et al. 2017; Catenacci et al. 2016; Parvaresh et al. 2019) have directly compared the effects of ADF versus daily calorie restriction (CR) on body weight. Results from these trials indicate that ADF and CR produce nearly identical degrees of weight loss after 8–24 weeks of treatment. Based on this preliminary evidence, intermittent fasting may be just as effective for reducing body weight as traditional dieting approaches. It will be of interest to compare the weight management efficacy of these two dietary regimens over longer periods of time (2–3 years) in future trials.

6.2.1.2 ADF: Weight Maintenance Efficacy

The ability of ADF to promote weight loss *maintenance* has been examined in three human studies (Kalam et al. 2019a; Trepanowski et al. 2017; Catenacci et al. 2016). The weight maintenance version of the ADF diet differs slightly from the weight loss version. In general, a higher amount of energy consumption is allowed on the fast day, i.e., ~1000 kcal instead of ~500 kcal, during the weight maintenance period. Some trials also allowed participants to participate in fewer fast days per week, i.e., 2–3 fast days, instead of 3–4 fast days per week. Results reveal that the weight maintenance version of ADF was effective at facilitating weight maintenance, as no statistically significant changes in body weight were observed during

follow-up (12–24 weeks). However, subjects did experience small *numerical* (but not significant) increases in body weight, ranging from 1% to 2%, during these follow-up periods. In view of the minor numerical increases in body weight observed, it may be necessary to prescribe a stricter ADF approach, i.e., 3–4 fast days per week with 500 kcal on each fast day, to ensure weight stabilization. Evidently, more long-term (>1 year) human trials of ADF will be required to truly elucidate whether these diets can be used for weight management.

6.2.1.3 ADF: Effect on Body Composition

During traditional weight loss approaches, i.e., daily CR, approximately 75% of the weight lost is fat mass and 25% is lean mass (Willoughby et al. 2018; Heymsfield et al. 2014; Pownall et al. 2015; Ravussin et al. 2015). Based on the evidence in Table 6.1, the majority of ADF trials demonstrate a similar ratio of fat to lean mass loss (75:25), as daily CR (Hoddy et al. 2014; Heilbronn et al. 2005; Stekovic et al. 2020; Catenacci et al. 2016). Therefore, ADF and CR may produce similar body composition changes during weight loss (Trepanowski et al. 2017).

The effects of exercise combined with ADF on body composition have also been examined. When ADF is combined with aerobic exercise, changes in fat mass and lean mass did not differ from that of ADF alone (Bhutani et al. 2013a). No trial to date has combined ADF with resistance training, so the effects of this combined intervention on body composition remain unknown. Changes in visceral fat mass were also examined. In the majority of ADF trials, visceral fat mass was reduced significantly, versus controls, when clinically significant weight loss (>5% from baseline) was observed (Trepanowski et al. 2017; Catenacci et al. 2016; Eshghinia and Mohammadzadeh 2013; Klempel et al. 2013; Parvaresh et al. 2019; Harvie et al. 2011; Schubel et al. 2018; Sundfor et al. 2018; Carter et al. 2018).

6.2.1.4 ADF: Effect on Energy Intake

ADF lowers body weight primarily because subjects consume less energy during these protocols. Findings from clinical trials show that zero-calorie ADF and modified ADF reduce calorie intake by 20–35% (Hoddy et al. 2014; Kalam et al. 2019a; Trepanowski et al. 2017; Stekovic et al. 2020; Varady et al. 2013; Catenacci et al. 2016; Eshghinia and Mohammadzadeh 2013; Klempel et al. 2013; Parvaresh et al. 2019; Varady et al. 2009; Bhutani et al. 2013b). Not surprisingly, the energy restriction achieved with zero-calorie ADF appears to be greater (~30–35%) (Stekovic et al. 2020; Catenacci et al. 2016) than that achieved with modified ADF (~20–25%) (Hoddy et al. 2014; Kalam et al. 2019a; Trepanowski et al. 2017; Varady et al. 2013; Eshghinia and Mohammadzadeh 2013; Klempel et al. 2013; Parvaresh et al. 2019; Varady et al. 2009; Bhutani et al. 2013b). Clinicians are often worried that individuals following ADF will overeat or “binge” on feast days. Contrary to what would be expected, subjects typically only consume an extra 10–15% of energy needs (approximately 200–300 kcal) on feast days, relative to their calculated energy

needs (Trepanowski et al. 2017; Varady et al. 2013; Klempel et al. 2010). Because subjects do not fully compensate for the lack of food consumed on the fast day by eating more on the feast day, mild to moderate energy restriction occurs, which results in body weight reductions.

6.2.2 *The 5:2 Diet*

6.2.2.1 5:2 Diet: Weight Loss Efficacy

Several human trials have examined the effect of the 5:2 diet on body weight (Table 6.2). The 5:2 diet appears to elicit similar weight loss as ADF with only 2 fast days per week rather than 3–4; however, these diets have not been compared directly. Specifically, 5:2 appears to produce significant weight loss of 2–9% in 4–52 weeks independent of comparison group, length of intervention, or diabetes status (Cook et al. 2022; Guo et al. 2021; Kord Varkaneh et al. 2022). Seven studies have examined the 5:2 diet compared to a daily calorie restriction group either with or without a control group (Harvie et al. 2011; Schubel et al. 2018; Sundfor et al. 2018; Pinto et al. 2020; Fitzgerald et al. 2018; Antoni et al. 2018). In all seven of these trials, no differences were reported between the 5:2 and CR groups with both diets producing between 2% and 7% body weight over 4 to 48 weeks (Harvie et al. 2011; Schubel et al. 2018; Sundfor et al. 2018; Pinto et al. 2020; Fitzgerald et al. 2018; Antoni et al. 2018). The 5:2 diet has also been compared to a low-carbohydrate high-fat diet and a week-on/week-off diet with again no difference in body weight outcomes at the end of the respective intervention periods (Holmer et al. 2021; Headland et al. 2019). However, the 5:2 diet did produce significantly more body weight loss than a 6:1 diet (6 days of ad libitum eating and 1 day of fasting) and a high protein diet (Arciero et al. 2022; Cai et al. 2022). In individuals with type 2 diabetes, consecutive and nonconsecutive fasting days and daily CR produced the same weight loss in 12–52 weeks (Carter et al. 2018; Corley et al. 2018). The 5:2 diet and CR combined with resistance training also produce comparable weight loss, yet when combined with 150–300 min of weekly physical activity, the 5:2 and a high protein diet produced significantly more weight loss than when physical activity was combined with CR (Keenan et al. 2022a; Kang et al. 2022). Additionally, 5:2 combined with high intensity interval training produced more weight loss than diet or exercise alone (Batitucci et al. 2022). It will be of great interest in future research to compare ADF and 5:2 to determine if there is a dose response to days of fasting and total weight loss.

6.2.2.2 5:2 Diet: Effect on Body Composition

As discussed earlier, dietary weight loss interventions produce both fat loss and fat free mass loss. As reported in Table 6.2, the 5:2 diet produces similar weight loss distribution of fat mass to fat free mass (75% from fat mass and 25% from fat free mass) as CR and ADF. The 5:2 diet produced significant decreases in fat mass, fat

free mass, and visceral fat mass from baseline, but no group $\times$ time interactions were reported when compared to CR or other dietary modifications. To promote lean mass retention, exercise should be combined with any method of calorie restriction (Keenan et al. 2022a; Batitucci et al. 2022).

6.2.2.3 5:2 Diet: Effect on Energy Intake

The level of energy restriction was significantly greater in the 5:2 diet group than in controls (Cook et al. 2022; Guo et al. 2021; Kord Varkaneh et al. 2022; Tay et al. 2020). However, weekly energy intake decreased comparably between 5:2 and CR. As for self-reported energy intake, the 5:2 diet produced 9–55% energy restriction in 4–48 weeks (Harvie et al. 2011; Schubel et al. 2018; Sundfor et al. 2018; Pinto et al. 2020; Fitzgerald et al. 2018; Antoni et al. 2018; Holmer et al. 2021; Arciero et al. 2022; Corley et al. 2018; Keenan et al. 2022a; Keenan et al. 2022b). This wide range may be attributed to the diet prescribed on the feast days (which occurred 5 days of the week). In the studies that reported the highest overall restriction, the feast day was prescribed with a calorie goal (Arciero et al. 2022) or a Mediterranean-style diet (Pinto et al. 2020) rather than ad libitum intake (Keenan et al. 2022a, b). Interestingly, while energy intake did decrease significantly, distribution of carbohydrate, fat, and protein remained the same (Harvie et al. 2011; Sundfor et al. 2018; Cook et al. 2022; Kord Varkaneh et al. 2022; Antoni et al. 2018; Holmer et al. 2021; Arciero et al. 2022; Corley et al. 2018; Batitucci et al. 2022; Tay et al. 2020; Keenan et al. 2022b).

6.2.2.4 Summary: Weight Loss Efficacy of ADF and 5:2

Zero-calorie and modified ADF result in 3–8% weight loss in 6–12 weeks producing comparable weight loss and body composition changes to CR. The 5:2 diet also results in 2–9% body weight loss in 4–52 weeks. Distribution of weight loss and degree of caloric restriction also seems to mirror CR. The current data indicates that individuals who have not found success with daily calorie restriction may achieve comparable weight loss following these forms of intermittent fasting.

6.3 Effects of ADF and the 5:2 Diet on Metabolic Disease Risk Factors

6.3.1 Alternate Day Fasting (ADF)

6.3.1.1 ADF: Effect on Blood Pressure

Changes in various metabolic disease risk parameters during ADF are reported in Table 6.1. Zero-calorie ADF reduces systolic blood pressure (5 to 10%) (Stekovic et al. 2020) but may have no effect on diastolic blood pressure (Heilbronn et al.

2005; Stekovic et al. 2020). Modified ADF, in contrast, demonstrates rather consistent decreases in both systolic (5 to 10%) and diastolic blood pressure (5 to 10%) (Hoddy et al. 2014; Kalam et al. 2019a; Eshghinia and Mohammadzadeh 2013; Parvaresh et al. 2019; Varady et al. 2009), though findings vary (Trepanowski et al. 2017; Varady et al. 2013; Bhutani et al. 2013a; Klempel et al. 2013). The majority of the studies which report decreases in blood pressure involved participants with elevated blood pressure at baseline (systolic >120 mm Hg, diastolic >80 mm Hg) (Hoddy et al. 2014; Kalam et al. 2019a; Stekovic et al. 2020; Eshghinia and Mohammadzadeh 2013; Parvaresh et al. 2019; Varady et al. 2009). In view of this, it is likely that this intermittent fasting approach may only be effective in patients with hypertension or borderline hypertension.

6.3.1.2 ADF: Effect on Plasma Lipids

Zero-calorie ADF appears to consistently reduce LDL cholesterol levels in the range of 10–25% (Heilbronn et al. 2005; Stekovic et al. 2020; Catenacci et al. 2016). In contrast, only a few modified ADF studies demonstrate reductions in LDL cholesterol (Kalam et al. 2019a; Klempel et al. 2013; Varady et al. 2009). Triglyceride levels decreased in all the zero-calorie ADF trials, by 10–20% (Heilbronn et al. 2005; Stekovic et al. 2020; Catenacci et al. 2016). Several modified ADF trials also report reductions in triglycerides ranging from 15% to 40% (Trepanowski et al. 2017; Johnson et al. 2007; Klempel et al. 2013; Parvaresh et al. 2019; Varady et al. 2009). Longer trial durations were not related to greater reductions in LDL cholesterol or triglycerides levels (Trepanowski et al. 2017; Johnson et al. 2007; Klempel et al. 2013; Parvaresh et al. 2019; Varady et al. 2009). Due to the paucity of data available, it is difficult to decipher if these lipid lowering effects vary according to baseline LDL cholesterol level, triglyceride level, or BMI category. The effect of ADF on HDL cholesterol concentrations was also evaluated. Results reveal that neither zero-calorie ADF nor modified ADF have any significant effect on HDL cholesterol (Hoddy et al. 2014; Trepanowski et al. 2017; Heilbronn et al. 2005; Stekovic et al. 2020; Varady et al. 2013; Cho et al. 2019; Eshghinia and Mohammadzadeh 2013; Johnson et al. 2007; Klempel et al. 2013; Parvaresh et al. 2019; Varady et al. 2009). Only one ADF study demonstrated significant increases in HDL cholesterol with ADF (Bhutani et al. 2013a). However, it should be noted that this trial combined ADF with an aerobic exercise intervention. Seeing as aerobic exercise results in fairly consistent increases in HDL cholesterol (Kodama et al. 2007; Leon and Sanchez 2001), it can be speculated that this improvement was due to the exercise intervention rather than ADF.

6.3.1.3 ADF: Effect on Glucoregulatory Factors

Zero-calorie ADF does not appear to alter fasting glucose, fasting insulin, or insulin sensitivity in patients without diabetes (Heilbronn et al. 2005; Stekovic et al. 2020; Catenacci et al. 2016). Modified ADF also does not have any effect on fasting glucose levels in euglycemic subjects (Hoddy et al. 2014; Kalam et al. 2019a;

Trepanowski et al. 2017; Cho et al. 2019; Bhutani et al. 2013a; Eshghinia and Mohammadzadeh 2013; Johnson et al. 2007). This is not surprising, as fasting glucose is generally well controlled in subjects who do not have diabetes (Freckmann et al. 2007). Fasting insulin, on the other hand, was reduced in many trials of modified ADF, by 15–40% (Kalam et al. 2019a; Trepanowski et al. 2017; Parvaresh et al. 2019; Wegman et al. 2015). It was also observed that this effect occurred more regularly in trials where subjects had higher fasting insulin levels at baseline ($>13 \mu\text{IU/mL}$) (Kalam et al. 2019a; Trepanowski et al. 2017; Parvaresh et al. 2019; Wegman et al. 2015). This may suggest that ADF only lowers fasting insulin in subjects with early stages of hyperinsulinemia. As for HbA1c and insulin resistance (measured by Homeostatic Model Assessment for Insulin Resistance, HOMA-IR), no changes were noted by modified ADF in the trials reviewed here (Hoddy et al. 2014; Kalam et al. 2019a; Trepanowski et al. 2017; Cho et al. 2019; Bhutani et al. 2013a).

6.3.1.4 ADF: Effect on Inflammation and Oxidative Stress

In reviewing the evidence presented in Table 6.1, it would appear as though ADF generally has little effect on circulating inflammatory markers, such as TNF-alpha (Johnson et al. 2007), homocysteine (Trepanowski et al. 2017), or C-reactive protein (CRP) (Trepanowski et al. 2017; Cho et al. 2019; Johnson et al. 2007; Bhutani et al. 2013b). On the other hand, reductions in markers of oxidative stress have been demonstrated with ADF. For instance, 8-isoprostane, nitrotyrosine, protein carbonyls, and fast-acting advanced oxidation protein products (AOPP) all decreased after 8 weeks of ADF (Johnson et al. 2007). Whether these improvements in markers of oxidative stress persist in long term with ADF is not yet known but is of great interest.

6.3.2 The 5:2 Diet

6.3.2.1 5:2 Diet: Effect on Blood Pressure

Changes in various metabolic disease risk parameters during the 5:2 diet are reported in Table 6.2. Most studies report an improvement in blood pressure with the 5:2 diet in patients without diabetes (Harvie et al. 2011; Sundfor et al. 2018; Pinto et al. 2020; Antoni et al. 2018; Arciero et al. 2022). However, it is unclear if these improvements are primarily seen in systolic blood pressure (SBP; 3–10%), diastolic blood pressure (DBP; 2–6%), or both. Of the eight studies (Harvie et al. 2011; Schubel et al. 2018; Sundfor et al. 2018; Guo et al. 2021; Pinto et al. 2020; Antoni et al. 2018; Holmer et al. 2021; Arciero et al. 2022; Corley et al. 2018) that examined blood pressure, four report no change (Schubel et al. 2018; Guo et al. 2021; Holmer et al. 2021; Corley et al. 2018), two report improvements in both SBP and DBP (Harvie et al. 2011; Arciero et al. 2022), two report improvements in DBP alone (Sundfor et al. 2018; Pinto et al. 2020), and one reports improvement in SBP

alone (Antoni et al. 2018). When comparing the 5:2 diet with other diet interventions, there were no significant differences between groups. Changes in blood pressure were not dependent on hypertensive status or length of the intervention. Only two studies have examined the effect of 5:2 on blood pressure in patients with diabetes (Carter et al. 2018; Corley et al. 2018). In these trials, no improvements were seen with the 5:2 diet regardless if the fasting days were consecutive or nonconsecutive or the amount of weight loss attained (Carter et al. 2018; Corley et al. 2018).

6.3.2.2 5:2 Diet: Effect on Plasma Lipids

Plasma lipids levels were examined in the majority of the 5:2 trials reviewed here (Table 6.2). Most studies did not report improvements in LDL cholesterol concentrations. However, a few studies observed mild, but significant, decreases in LDL cholesterol of 1–13%, irrespective of diabetes diagnosis (Harvie et al. 2011; Carter et al. 2018; Holmer et al. 2021; Arciero et al. 2022). In trials that did report LDL improvements, these changes were significant from baseline, but not compared to controls or a comparator group (Harvie et al. 2011; Carter et al. 2018; Holmer et al. 2021; Arciero et al. 2022). Interestingly, when the 5:2 diet was combined with resistance training, LDL cholesterol levels decreased significantly more than CR combined with resistance training (Keenan et al. 2022a, 2022b). Triglycerides decreased by 8–25% in over half of the 5:2 trials reviewed here, regardless of the length of the trial, or diabetes diagnosis (Harvie et al. 2011; Sundfor et al. 2018; Carter et al. 2018; Guo et al. 2021; Kord Varkaneh et al. 2022; Pinto et al. 2020; Holmer et al. 2021; Headland et al. 2019; Arciero et al. 2022). Triglycerides were significantly decreased from baseline but time x diet interactions were not reported in any trial (Harvie et al. 2011; Sundfor et al. 2018; Carter et al. 2018; Guo et al. 2021; Kord Varkaneh et al. 2022; Pinto et al. 2020; Holmer et al. 2021; Headland et al. 2019; Arciero et al. 2022). HDL cholesterol, on the other hand, remained unchanged in almost all 5:2 diet studies reviewed here (Harvie et al. 2011; Schubel et al. 2018; Guo et al. 2021; Kord Varkaneh et al. 2022; Fitzgerald et al. 2018; Antoni et al. 2018; Holmer et al. 2021; Cai et al. 2022; Corley et al. 2018; Tay et al. 2020). The one trial that did report a significant 7% increase in HDL cholesterol levels from baseline asked participants to increase their steps per day, which is known to improve HDL cholesterol (Headland et al. 2019). Because this activity was advised in all groups, all comparator groups also increased their HDL cholesterol levels (Headland et al. 2019). Some trials observed reductions in HDL cholesterol levels, which may have been the result of weight loss (Sundfor et al. 2018; Carter et al. 2018; Arciero et al. 2022; Keenan et al. 2022b).

6.3.2.3 5:2 Diet: Effect on Glucoregulatory Parameters

Changes in measures of glycemic control with the 5:2 diet are shown in Table 6.2. There does not appear to be a consistent change in fasting glucose with this fasting protocol (Schubel et al. 2018; Sundfor et al. 2018; Guo et al. 2021; Kord Varkaneh et al. 2022; Pinto et al. 2020; Fitzgerald et al. 2018;

Headland et al. 2019; Tay et al. 2020; Keenan et al. 2022b). Five trials did report a significant decrease from baseline by 2–26%; however, no time x diet interaction was reported in any of these studies (Harvie et al. 2011; Carter et al. 2018; Antoni et al. 2018; Arciero et al. 2022; Corley et al. 2018). As for fasting insulin, reductions of 12–29% were reported in three trials (Harvie et al. 2011; Carter et al. 2018; Guo et al. 2021). Changes in fasting glucose and insulin were not related to diabetes status, baseline fasting glucose or insulin concentrations, or trial duration. Insulin resistance (measured by Homeostatic Model Assessment for Insulin Resistance, HOMA-IR) was reduced by 12–47% in four trials (Harvie et al. 2011; Guo et al. 2021; Pinto et al. 2020; Holmer et al. 2021) and decreased significantly more than CR after 24 weeks (Harvie et al. 2011). HbA1c levels improved in five trials (Sundfor et al. 2018; Carter et al. 2018; Holmer et al. 2021; Corley et al. 2018; Tay et al. 2020). In the trials that enrolled patients with T2DM, HbA1c significantly decreased from baseline (Carter et al. 2018; Corley et al. 2018). It was also noted that HbA1c decreased significantly more by the 5:2 diet, when compared to CR after 52 weeks (Carter et al. 2018).

6.3.2.4 5:2 Diet: Effect on Inflammation and Oxidative Stress

The 5:2 diet appears to improve some markers of inflammation (Table 6.2). For instance, high sensitivity CRP, a marker of chronic inflammation related to obesity, was significantly reduced from baseline in two trials (Harvie et al. 2011; Kord Varkaneh et al. 2022). Additionally, reductions in soluble CD40L (sCD40L) have also been noted. sCD40L is an adipokine belonging to the tumor necrosis factor (TNF) family which promotes insulin resistance (Guo et al. 2021). Another study showed improvements in circulating levels of Cytokeratin 18 (Kord Varkaneh et al. 2022). This cytokine predicts the presence of non-alcoholic steatohepatitis. In contrast, the 5:2 diet had no effect on circulating levels of the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-alpha) or Interleukin-6 (IL-6) (Schubel et al. 2018; Tay et al. 2020). As for markers of oxidative stress, it was shown that malondialdehyde, asymmetric dimethylarginine, and advanced oxidation protein products decreased significantly from baseline (Harvie et al. 2011; Guo et al. 2021).

6.3.2.5 Summary: Changes in Metabolic Disease Risk Factors by ADF and 5:2

The effects of ADF and the 5:2 diet on various metabolic disease risk parameters are summarized in Fig. 6.2. ADF and the 5:2 diet both produced mild decreases in systolic and diastolic blood pressure. As for plasma lipids, LDL cholesterol and triglycerides were reduced in some trials, but findings were highly variable. HDL cholesterol remained unchanged in most studies. Neither diet produced changes in fasting glucose levels. HbA1c remained unchanged by ADF but improved with the 5:2 diet. Reductions in HbA1c by 5:2 were particularly pronounced in those

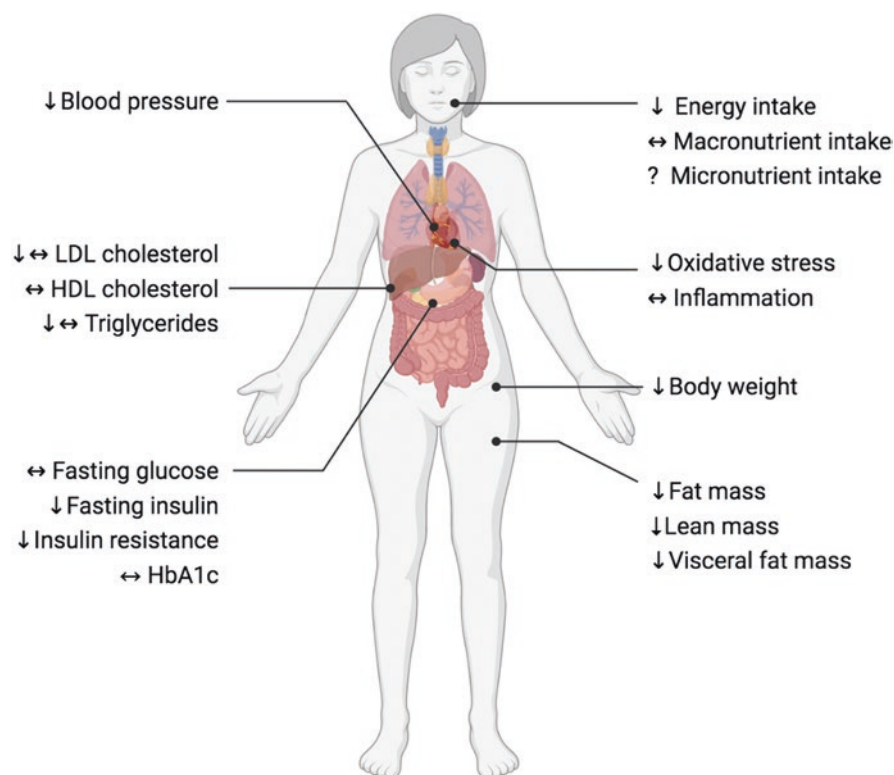


Fig. 6.2 Changes in cardiometabolic risk factors by ADF and 5:2

with T2DM. On the other hand, both regimens showed promise for improving fasting insulin in healthy individuals with obesity and those with prediabetes. Insulin resistance was decreased more consistently with the 5:2 diet. Circulating inflammatory markers, such as CRP, sCD40L, and cytokeratin, decreased only with the 5:2 diet. TNF-alpha and IL-6 were not affected by ADF or 5:2. However, markers of oxidative stress improved consistently with both diets. Taken together, ADF and 5:2 may be effective for reducing the risk of coronary heart disease and diabetes by lowering blood pressure, insulin resistance, and oxidative stress. However, it remains uncertain if either of these fasting regimens can favorably modulate plasma lipids.

ADF and the 5:2 diet produced mild decreases in systolic and diastolic blood pressure. LDL cholesterol and triglycerides were reduced in some trials, but findings were highly variable. HDL cholesterol remained unchanged in most studies. Neither diet produced changes in fasting glucose levels. HbA1c levels remained unchanged by ADF but improved in some 5:2 studies. Both regimens lowered fasting insulin and insulin resistance. Reductions in inflammatory markers were noted with 5:2, but findings were not consistent. Markers of oxidative stress improved consistently with both ADF the 5:2 diet.

6.3.3 Other Potential Benefits

6.3.3.1 Sleep

Obesity is associated with reduced sleep duration (i.e., sleeping less than 7 h of per night), poor sleep quality (Zimberg et al. 2012), and increased sleep disturbances (Dashti et al. 2015). Weight loss can improve sleep in people with obesity (Alfaris et al. 2015). To date, only one study has examined if the 5:2 diet affects sleep. In this trial, the 5:2 diet was combined with calorie restriction, and sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI). By the end of the 8-week study, self-reported sleep quality remained unchanged, relative to controls (Fitzgerald et al. 2018).

6.3.3.2 Gut Microbiota

The link between the gut microbiome and obesity is a growing area of research (Bouter et al. 2017; Castaner et al. 2018; Sweeney and Morton 2013; Tilg and Kaser 2011). Individuals with obesity have a higher proportion of *Firmicutes* and a lower proportion of *Bacteroidetes* (Mariat et al. 2009; Rinninella et al. 2019). *Firmicutes* have been speculated to augment the gut's ability to harvest more calories from each meal, which can contribute to weight gain (Jumpertz et al. 2011; Shortt et al. 2018). In addition, low microbial richness and diversity are associated with obesity (de la Cuesta-Zuluaga et al. 2018; Peters et al. 2018; Stanislowski et al. 2019). Only a few 5:2 trials have evaluated changes in the gut microbiome in human subjects. One trial added a probiotic supplement to the 5:2 diet and compared changes in the microbiome to that of placebo. No changes in microbial diversity or relative abundances were noted, despite significant weight loss achieved by the 5:2 diet/probiotic group (Tay et al. 2020). In contrast, another study conducted in participants with metabolic syndrome showed improved microbial diversity with the 5:2 diet (Guo et al. 2021). It was also noted that these beneficial changes in diversity were related to improvements in cardiometabolic measures (Guo et al. 2021). The production of short-chain fatty acids was also augmented, which may have a downstream effect of decreasing inflammation, modifying satiety, and slowing gastric emptying (Kasubuchi et al. 2015).

6.3.4 Safety of ADF and the 5:2 Diet

6.3.4.1 Reproductive Hormones

Whether intermittent fasting negatively affects reproductive hormones and fertility is of great interest. To date, clinical trial findings suggest that fasting generally does not have any effect on reproductive hormones in healthy men and women. For

instance, Harvie et al. (Harvie et al. 2011) reported no change in testosterone, androstenedione, dehydroepiandrosterone-sulfate (DHEAS), sex hormone binding globulin (SHBG), or prolactin, when premenopausal women fasted for 2 days/week for 24 weeks. As for fertility, no study has examined how intermittent fasting affects the ability of men and women to conceive. Evidently, much more research is needed in this area, but preliminary findings suggest that no major changes in reproductive hormone levels occur with fasting.

6.3.4.2 Resting Metabolic Rate (RMR)

Concerns have been raised regarding the effect fasting on resting metabolic rate (RMR). Despite the widely held belief by the general public that fasting negatively affects metabolic rate, this notion is not supported by the data. Indeed, evidence shows that fasting either has no effect on RMR when weight is maintained (Heilbronn et al. 2005; Moro et al. 2016; Tinsley et al. 2019) or results in minor reductions of 100–200 kcal/day, when weight is reduced by ~5% (Trepanowski et al. 2017; Sundfor et al. 2018; Pinto et al. 2020; Antoni et al. 2018; Batitucci et al. 2022; Tay et al. 2020). It has also been shown that the minor decreases in RMR by intermittent fasting are comparable to that of daily CR (Trepanowski et al. 2017; Sundfor et al. 2018). Further, RMR can be maintained with weight loss if intermittent fasting is combined with exercise (Batitucci et al. 2022).

6.3.4.3 Thyroid Hormones

Changes in thyroid hormones with fasting have only been evaluated in one trial (Akasheh et al. 2020). In the study by Akasheh et al. (2020), thyroid hormones were measured before and after 24 weeks of ADF or CR in patients with obesity and subclinical hypothyroidism (Akasheh et al. 2020). Results show that ADF and CR produced the same degree of weight loss (8% from baseline) after 24 weeks in this population. It was also noted that circulating free thyroxine (T4), T3, and TSH did not change in either the ADF or CR group by the end of the trial. These preliminary results indicate that thyroid hormones levels remain unchanged with fasting in those with obesity and subclinical hypothyroidism.

6.3.4.4 Gastrointestinal and Neurological Adverse Effects

Accumulating evidence suggests that fasting produces little or no gastrointestinal adverse effects, including dry mouth, halitosis, constipation, diarrhea, or nausea (Cienfuegos et al. 2020; Gabel et al. 2019; Wilkinson et al. 2020; Hoddy et al. 2015). As for neurological effects, data indicate that ADF or 5:2 do not result in augmented levels of irritability, fatigue, or dizziness (Sundfor et al. 2018; Fitzgerald et al. 2018; Tay et al. 2020; Cienfuegos et al. 2020; Gabel et al. 2019; Wilkinson

et al. 2020; Hoddy et al. 2015). Thus, intermittent fasting generally produces few gastrointestinal or neurological disturbances in humans.

6.3.4.5 Eating Disorder Symptoms

It has been speculated that fasting regimens may increase one's risk of developing an eating disorder. Studies of ADF report no changes in multiple eating disorder symptoms, including depression, binge eating, purgative behavior, or fear of fatness (Gabel et al. 2019; Hoddy et al. 2015). It should be noted that subjects with a *history of eating disorders* were excluded from these trials (Gabel et al. 2019; Hoddy et al. 2015). Thus, it remains unknown if fasting is safe in those with *diagnosed* eating disorders. These findings for intermittent fasting are comparable to that of daily CR. Data from the CALERIE trial (Redman and Ravussin 2011; Williamson et al. 2008) shows that restricting energy by ~500 kcal per day did not increase eating disorder symptoms and had no other harmful psychological effects. Thus, it is possible that intermittent fasting, like CR, may not increase disordered eating behaviors.

6.3.4.6 Macronutrient, Micronutrient, and Beverage Intake

Changes in macronutrient, micronutrient, and beverage intake have been assessed in several ADF and 5:2 diet trials. In most of these studies, macronutrient composition and diet quality remain unchanged from baseline (Harvie et al. 2011; Sundfor et al. 2018; Cook et al. 2022; Kord Varkaneh et al. 2022; Antoni et al. 2018; Holmer et al. 2021; Arciero et al. 2022; Corley et al. 2018; Batitucci et al. 2022; Tay et al. 2020; Keenan et al. 2022b). Low levels of fiber intake are regularly reported in trials of ADF and 5:2 at baseline and posttreatment (Hoddy et al. 2014; Trepanowski et al. 2017; Bhutani et al. 2013a; Harvie et al. 2011; Antoni et al. 2018; Holmer et al. 2021; Arciero et al. 2022). In view of this, subjects who are fasting should be encouraged to consume plenty of fruits, vegetables, and whole grains to boost their fiber intake. The impact of these diets on vitamin and mineral intake is not yet known, as no study has evaluated changes in micronutrient consumption during ADF or 5:2. It is possible though that prolonged fasting may result in lower intakes of key micronutrients, e.g., vitamin D, vitamin B12, and electrolytes, among others. As such, circulating levels of these vitamins and minerals should be routinely assessed to monitor for deficiencies. Beverage intake (i.e., consumption of alcohol or caffeinated drinks) generally remains unchanged during fasting (Kalam et al. 2019b). Taken together, fasting does not appear to have any beneficial or detrimental effects on macronutrient, micronutrient, or beverage intake. However, vitamin, mineral, and fiber supplements may be recommended to patients to prevent deficiencies while following these protocols.

6.4 Practical Considerations

6.4.1 *Who Should Not Do ADF and the 5:2 Diet?*

Fasting is not recommended for children under the age of 12. Whether intermittent fasting is safe and effective in teenagers is still uncertain. Recent findings show that ADF may be an effective for weight control in adolescents with obesity (Jebeile et al. 2019; Lister et al. 2020; Vidmar et al. 2019), but more data will be required to fully elucidate the safety of these diets in this age group. Women who are pregnant or lactating women should not fast, as no studies have been performed to evaluate the safety of these diets in these women. Elderly individuals should also be cautioned against the use of these diets, as fasting may exacerbate aging-induced sarcopenia, but more research is needed. Individuals with a history of eating disorders and those with a BMI lower than 18.5 kg/m² should also not participate in these regimens.

6.4.2 *Advice on Starting an ADF or 5:2 Diet*

It typically takes 7–10 days to fully adjust to ADF or 5:2 protocols. During this initial adjustment period, headaches are frequently reported (Cienfuegos et al. 2020). Headaches can result from low levels of water consumption, which can lead to dehydration (Benton and Young 2015). Increasing water intake by 1–2 L/day may help individuals to alleviate headaches during fasting (Blau et al. 2004; Spigt et al. 2005). Alcohol intake is allowed during fasting, but not on fast days during ADF. Since energy intake on the fast day is limited to ~500 kcal, it would be better to use those calories to consume healthy foods that will provide nutrients. Caffeinated beverages, such as black coffee, or black tea, are permitted during the fasting window and during periods of eating. Evidence suggest that subjects do not consume higher amounts of caffeinated beverages while partaking in intermittent fasting, compared to baseline (Cienfuegos et al. 2020; Kalam et al. 2019b). However, it is advisable to limit caffeine intake to earlier in the day, so that it does not interfere with one's ability to fall asleep (Clark and Landolt 2017). As for food recommendations, it is important to emphasize a diet high in fruits, vegetables, and whole grains. These foods can help participants boost their fiber and micronutrient intake (Woo et al. 2015; Yang et al. 2012). Clinicians should also recommend that their patients avoid consuming ultra-processed foods. A diet high in processed foods can lead to increased ad libitum energy intake and weight gain, when compared to a diet high unprocessed foods matched for energy (Hall et al. 2019).

6.4.3 Exercise During ADF and the 5:2 Diet

Exercising while fasting should be advised to improve cardiometabolic health and help maintain lean mass (Myers 2003; Nystoriak and Bhatnagar 2018). Many clinical trials have combined ADF or 5:2 with aerobic or resistance exercise (Cho et al. 2019; Bhutani et al. 2013a; Kang et al. 2022; Batitucci et al. 2022; Keenan et al. 2022b; Moro et al. 2016; Tinsley et al. 2017, 2019). In each of these studies, it was noted that participants had no issue performing moderate to high intensity training during 12–36 h periods of food abstention. If an individual wishes to combine ADF with exercise, it is recommended that they hold off on consuming the fast day meal until *after* the exercise session (Bhutani et al. 2013b). A compensatory increase in energy intake can occur 30–60 min after the exercise session is completed (Pomerleau et al. 2004; Westerterp 2018). In view of this, saving the meal for after exercise may help certain participants better adhere to their fast-day calorie goal (Bhutani et al. 2013b).

6.5 Conclusion

In conclusion, ADF and 5:2 are safe diet therapies that can produce clinically significant weight loss in individuals with overweight or obesity. Clinical trial evidence shows that these diets produce reductions in energy intake of approximately 10–30% and mild to moderate weight loss (1–8% from baseline). These regimens may lower coronary heart disease and diabetes risk by decreasing blood pressure, insulin resistance, and oxidative stress. LDL cholesterol and triglyceride levels are also reduced, but improvements in these lipid parameters are not consistent. Favorable changes in the diversity and overall composition of the gut microbiome have also been demonstrated, but more studies will be needed to confirm these findings. These diets do not produce deleterious changes in sleep, reproductive hormones, thyroid hormones, RMR, eating disorder symptoms, or diet quality. While these preliminary data offer promise for the use of ADF and the 5:2 diet as an alternative option to daily calorie restriction for treating obesity and metabolic disturbances, more research will be needed to provide crucial clinical evidence to support the use of fasting in clinical treatment guidelines.

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Chapter 7

Cellular Adaptations to Intermittent Fasting with Emphasis on the Brain



Mark P. Mattson

Abstract Animals living in their natural environments including our human ancestors prior to the agricultural revolution often experience periods of food scarcity during which their fat stores are utilized for production of the ketones that fuel cells in their nervous, musculoskeletal, and cardiovascular systems as they search for food. When initiated in young adulthood, intermittent fasting (IF) can double the life span of rats and mice. When rats or mice are maintained on IF feeding schedules (every other day food deprivation or daily time-restricted feeding), their brain function improves and their neuronal networks become relatively resistant to stress. Indeed, when animals are maintained on IF, fewer neurons degenerate, and functional deficits are reduced in models of Alzheimer's disease, Parkinson's disease, epilepsy, and ischemic stroke. Cellular adaptations of brain cells to IF include enhanced antioxidant defenses, mitochondrial stress resistance mediated by sirtuin 3, mitochondrial biogenesis, DNA repair, synaptic plasticity, and enhanced inhibitory tone. Regarding translatability to modern-day humans, it seems likely that the beneficial effects of IF on the brain will be most profound in overweight sedentary individuals. The latter conclusion is based on the facts that (1) the control animals in animal studies are fed ad libitum, are sedentary, and become obese as they age; (2) human studies have shown that obesity and insulin resistance adversely affect brain structure and function; and (3) recent studies of IF in normal-weight humans reveal significant improvements in health biomarkers. An increasing number of studies have shown that at least some of the beneficial effects of IF can be dissociated from an overall reduction in calories. Evolutionarily conserved adaptations to intermittent food availability explain the evidence that switching back and forth between fasting and fed states can optimize brain function and resilience.

M. P. Mattson (✉)

Department of Neuroscience, Johns Hopkins University School of Medicine,
Baltimore, MD, USA

e-mail: mmattso2@jhmi.edu

7.1 Introduction

Historically there have been widespread albeit anecdotal reports of beneficial effects of IF in humans often in the context of religion (Venagas-Borsellino et al. 2018; Mattson 2022). One of the most fascinating collections of individual experiences with fasting can be found in the book *The Fasting Cure* written by Upton Sinclair and published more than a century ago (Sinclair 1911). In the book he describes numerous examples of people he encountered who had various maladies that were lessened or even eliminated when they fasted. Sinclair arrives at the following conclusion:

The fast is to me the key to eternal youth, the secret of perfect and permanent health. I would not take anything in all the world for my knowledge of it. It is nature's safety valve, an automatic protection against disease.

Intermittent fasting (IF) refers to eating patterns that include frequent periods during which no or very few caloric foods or beverages are consumed. In order to be considered a fast, the period of energy restriction must be sufficient to deplete liver glucose stores, release fatty acids from adipose cells, and elevate circulating levels of the ketones (β -hydroxybutyrate and acetoacetate) produced from the fatty acids (Anton et al. 2018). In humans, this metabolic switch typically occurs between 12 and 14 h after the onset of fasting. In human studies the most commonly used IF regimens are daily time-restricted eating in which the eating window is compressed to 6–10 h (18–14 h with no caloric intake every day); 5:2 IF in which no more than 600 calories are consumed on 2 days every week; alternate-day fasting in which only about 500–700 calories are consumed every other day; and periodic fasting in which ~700 calories are consumed on 5 consecutive days each month. For animal studies the most commonly used IF regimens are every other day fasting; daily time-restricted feeding with caloric restriction; and daily time-restricted feeding without caloric restriction.

As is true for most animals living in the wild today, our human ancestors evolved in environments where food sources were often sparsely distributed and fluctuated with variations in the climate. Presumably, individuals whose brains and bodies functioned very well in a food-deprived state were more likely to acquire food and survive and reproduce compared to individuals who were less capable of acquiring food. Animals and humans, therefore, evolved metabolic, physical, and cognitive adaptations to IF-like eating patterns that enhanced their functional capabilities and stress resilience (Mattson 2022).

Goodrick et al. (1982) reported the results of a study that determined the effects of every other day fasting on the life span of male rats. The IF was initiated when the rats were young adults. Remarkably the mean life span of rats in the IF group was 83 percent greater than rats in the control group fed ad libitum. In a subsequent study, Goodrick et al. (1983) determined the effects of IF, running wheel exercise, or combined IF and exercise on the life span of rats. The IF and exercise were initiated when the rats were either 10.5 or 18 months of age. Compared to the ad libitum-fed control groups, IF rats gained less weight and lived significantly longer with or without exercise. On the other hand, running wheel exercise alone had no significant effect on survival in either age group.

At the time of Goodrick's studies, it had already been established that daily caloric restriction can extend the life span of mice and rats. Indeed, 40 years earlier, Clive McCay had reported that daily caloric restriction can extend the life span of rats (McCay et al. 1943) and dogs (McCay 1947), and Richard Weindruch and colleagues had reproduced and extended the findings of McCay by showing that daily caloric restriction can suppress the development of cancers and other diseases in rats and dogs (Weindruch and Sohal 1997). In such caloric restriction studies, the animals on the restricted diet (typically 30 or 40 percent fewer calories than their *ad libitum* intake) are given their daily food allotment all at one time. More recent studies have shown that animals on such daily caloric restriction protocols consume all their food within 4–6 h of its provision (Duffy et al. 1990; Acosta-Rodríguez et al. 2017). Therefore, it should be recognized that studies of caloric restriction in rodents are also IF studies involving daily 18–20 h fasts. This fact is important from an historical perspective because studies of caloric restriction in the literature were—unbeknownst to the investigators—also studies of daily time-restricted eating IF. But it is also important when considering both qualitative and quantitative aspects of the cellular and molecular mechanisms of action of caloric restriction and IF because an increasing number of studies in laboratory animals and humans are documenting beneficial effects of IF that cannot be accounted for by an overall reduction in calorie intake alone (Anson et al. 2003; Harvie et al. 2011; Mitchell et al. 2019). Indeed, a recent study of mice showed that whereas 30% daily calorie restriction without fasting extended life span by 10%, the same daily caloric restriction with a 2-h feeding window extended life span by 35% (Acosta-Rodríguez et al. 2022). Circadian timing of food restriction to a 12-h time window during the dark period had similar benefits in mice (Hatori et al. 2012; Manooogian and Panda 2017).

Two research groups, one at the National Institute on Aging (NIA) and the other at the University of Wisconsin, evaluated the effects of daily caloric restriction on life span, biomarkers of aging, and disease incidence in rhesus monkeys. Data obtained from both studies found that monkeys on caloric restriction maintain lower body weight, body fat, blood glucose levels, and levels of inflammation as they age (Mattison et al. 2017). Age-related cancers and insulin resistance were also reduced by caloric restriction in both studies. However, whereas life span was significantly increased by caloric restriction in the Wisconsin study, there was no significant increase in life span in the NIA study. This difference can be explained by differences in the caloric intake of animals in the control groups with the Wisconsin control monkeys fed *ad libitum* and the NIA control monkeys already calorie restricted below *ad libitum* intake. This raises the issue that in caloric restriction experiments in mouse and rat studies the control group of animals is fed *ad libitum*. Therefore, while data obtained in such studies may apply to overweight sedentary humans, they may not apply or do so to a lesser extent in normal-weight humans (Martin et al. 2010).

Other chapters in this book describe research into the effects of IF on various organ systems and disease processes. This chapter focuses on studies that have examined the effects of IF and caloric restriction on the brain. The following acronyms are used to distinguish different feeding protocols used in such studies: *ad*

libitum feeding (AL), every other day intermittent fasting (EODIF), daily time-restricted eating without caloric restriction (TRE), and daily time-restricted eating with caloric restriction (TRE/CR).

7.2 Effects of Intermittent Fasting on Brain Function and Structure

In 1900, the physician Edward Dewey published *The No-Breakfast Plan and the Fasting Cure*, a remarkable book in which he describes his observation of the effects of TRE (no calorie intake in the morning) on his patients and himself. He devotes an entire chapter to his observations on the effects of fasting on the cognition and mood of himself and his patients. Here is a quote from that chapter:

The no-breakfast plan with me proved a matter of life unto life. With my morning coffee there were forenoons of the highest physical energy, the clearest condition of mind, and the acutest sense of everything enjoyable. (E. Dewey 1900)

A consistent finding in studies of rats and mice is that IF attenuates age-related cognitive impairment. Idrobo et al. (1987) found that compared to mice fed AL, those on TRE/CR for 12 months performed better on a radial arm maze test of spatial learning and memory. The beneficial effect of TRE/CR on learning and memory was associated with reduced accumulation of lipofuscin (a marker of lipid oxidation) in neurons of the hippocampus and frontal cortex. In another study TRE/CR was initiated in rats in midlife, and their cognition and motor function were evaluated when they were old (Means et al. 1993). The TRE/CR preserved muscle strength, motor coordination, and learning and memory. Eckles-Smith et al. (2000) reported that TRE/CR ameliorates age-related decrements in motor function (rotarod test) and spatial learning and memory (Morris water maze test) in rats. The beneficial effect of TRE/CR on cognition was associated with an enhancement of long-term potentiation of synaptic transmission in the hippocampus and an increase in the amount of the NMDA type of glutamate receptor. Compared to control rats fed AL, rats maintained for 20 months on TRE/CR exhibited greater cerebral blood flow in the hippocampus and frontal cortex, superior learning and memory, and less anxiety-like behaviors (Parikh et al. 2016). In another study rats that had been maintained on TRE for 12 months performed better on a biconditional association task compared to rats fed AL (Hernandez et al. 2022). The latter study further documented favorable effects of TRE on the gut microbiome. A study of rats with diet-induced obesity showed that both TRE/CR and gastric bypass surgery similarly improved hippocampus-dependent learning and memory and reduced inflammation in the hippocampus (Grayson et al. 2014).

IF has also been reported to benefit brain function when initiated in middle-age or old animals. When old rats (21 months of age) were maintained on EODIF for 3 months, their motor and cognitive function was significantly improved compared to old rats fed AL (Singh et al. 2012). These beneficial effects of IF were associated

with reduced levels of oxidative molecular damage and improved mitochondrial function in the hippocampus and hypothalamus. In middle-age female rats with diet-induced obesity, 7 weeks of TRE/CR plus exercise significantly improved learning and memory (Pratchayasakul et al. 2022). In a mouse model in which genetic aberrancies result in accelerated aging, TRE/CR ameliorated deficits in learning and memory (Komatsu et al. 2008).

IF may also enhance brain function in younger animals. When young adult mice were maintained on EODIF for 6–8 months, they performed better on multiple behavioral tests compared to control mice fed AL (Fontán-Lozano et al. 2007). The tests included a rotarod test of motor agility, a Skinner box food reward-based memory test, and a novel object recognition test. Electrophysiological recordings of synaptic function in the hippocampus of the mice demonstrated that EODIF enhances long-term potentiation of synaptic transmission, a cellular correlate of memory consolidation. A recent study compared the effects of 3 months of EODIF and calorie-matched 10% CR on learning and memory in female mice. The mice on EODIF exhibited improved long-term memory, whereas those on 10% CR did not (Dias et al. 2021).

There have been several studies of the effects of long-term IF/CR in nonhuman primates. Dal-Pan et al. (2011) found that lemurs maintained on IF/CR for 18 months performed better on a spontaneous alternation test of working memory compared to lemurs in the control group. In another study, male and female monkeys on long-term CR (more than 10 years) exhibited superior performance in a movement assessment panel of motor function which was associated with reduced accumulation of iron in basal ganglia and temporal cortex (Kastman et al. 2010). In the same cohort of rhesus monkeys, those on caloric restriction exhibited reduced stress reactivity to aversive conditions which was associated with greater sizes of the hippocampus and amygdala (Willette et al. 2012).

Several studies have examined the effects of caloric restriction and IF cognition in humans. In a study of elderly people, 3 months of daily CR resulted in a significant improvement in verbal memory which was associated with decreases in plasma levels of insulin and C-reactive protein (a marker of inflammation) (Witte et al. 2009). In a randomized study of humans with obesity (ages 35–75 years), 4 weeks of caloric restriction improved recognition memory in a mnemonic similarity task and also improved spatial pattern separation, an ability known to be dependent on hippocampal neurogenesis (Kim et al. 2020). In elderly physically active people, 1 month of Ramadan fasting improved executive function, attention, associative memory, and recognition memory (Boujelbane et al. 2022).

A large multicenter trial called CALERIE included 220 healthy volunteers with body mass indexes ranging from 22 to 28 kg/m². Half of the volunteers were maintained for 2 years on ~20% daily calorie restriction which resulted in a mean 17-pound reduction in body weight, and the other half were controls with no caloric restriction and no weight loss during the 2-year period. As part of this study, Leclerc et al. (2020) found that subjects in the caloric restriction group performed significantly better in tests of working memory compared to those in the control group.

In summary, results of numerous animal studies have shown that long-term intermittent fasting results in improvements in learning and memory. Studies of the effects of intermittent fasting on cognition in humans are needed for additional clinical trials.

7.3 The Brain Is Selectively Protected Against Structural Demise During Starvation

I now knew that there could be no death from starvation until the body was reduced to the skeleton condition; that therefore for structural integrity, for functional clearness, the brain has no need of food when disease has abolished the desire for it. Is there any other way to explain the power to make wills with whispering lips in the very hour of death, even in the last moments of life, that the law recognizes as valid? (E. Dewey 1900)

A striking observation from studies of rats and mice on CR (30–50% reduction in daily calorie intake) is that every organ except the brain (and the testes in males) is reduced in size (Weindruch and Sohal 1997). Moreover, the size of some brain regions may increase in response to severe caloric restriction. For instance, a magnetic resonance imaging (MRI) study revealed that the hippocampus is significantly larger in women with anorexia nervosa (who severely restrict their calorie intake and exercise excessively) compared to women with a normal weight and caloric intake (Beadle et al. 2015). After the women recovered from their anorexia, the size of their hippocampus was reduced.

This makes sense from an evolutionary perspective because when food is scarce, the brain must function at a high level in order for the individual to be successful in acquiring food. Presumably, individuals whose brains functioned better than others had behavioral traits (superior cognition, stress resilience, etc.) that facilitated food acquisition and gained a survival advantage. Also of interest from an evolutionary perspective are studies showing that whereas estrus cycles cease, under 40% CR conditions in females, males remain fertile (Martin et al. 2007, 2009a). Females should not become pregnant under conditions of severe food shortages because they would not have sufficient energy to support the development of the offspring. On the other hand, it would be advantageous for a male to remain fertile so that they could inseminate females [before the male starved to death].

Several studies have provided evidence that IF can increase the size of some brain regions and the number of neurons and synaptic connections in those brain regions. Most such studies have focused on the hippocampus because of its fundamental roles in learning and memory and its vulnerability in Alzheimer's disease and epilepsy. Stranahan et al. (2009) performed a study in which wild-type mice and mice with diabetes (leptin receptor mutant mice) were randomly assigned to four different housing conditions: AL feeding, sedentary; AL feeding with running wheels; TRE/CR, sedentary; and TRE/CR running wheels. After 3 months, the mice were euthanized, and the left and right hippocampi were removed. One hippocampus was processed with Golgi stain and used to count the number of synapses on

dentate granule neurons, and the other hippocampus was used for measurement of levels of brain-derived neurotrophic factor (BDNF). BDNF is a neurotrophic factor produced by neurons in response to synaptic excitation and cellular stress. BDNF plays fundamental roles in the formation and maintenance of synapses between neurons, stimulates mitochondrial biogenesis, enhances learning and memory, improves mood, and increases the resistance of neurons to aging and disease (Rothman and Mattson 2013; Marosi and Mattson 2014; Cheng et al. 2012). The results showed that synaptic numbers were reduced in diabetic mice and that TRE/CR and exercise each increased synapse numbers in both wild-type and diabetic mice. The combination of TRE/CR and exercise resulted in greater increases in synapse numbers than did either condition alone. BDNF levels were correlated with synapse numbers consistent with a role for BDNF in synapse formation induced by TRE/CR and exercise.

Throughout the brain, the most abundant neurons (~90%) deploy the excitatory neurotransmitter glutamate (Mattson 2023). Most of the other neurons deploy the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). In the hippocampus, the glutamatergic dentate granule neurons arise from adjacent stem cells. In 1999, van Praag et al. reported that running wheel exercise stimulates hippocampal neurogenesis by stimulating the proliferation of neuronal stem cells and increasing their differentiation into dentate granule neurons. We found that EODIF enhances neurogenesis by increasing the survival of newly generated granule neurons (Lee et al. 2002). Baik et al. (2020) also found that EODIF enhances hippocampal neurogenesis and provided evidence for the involvement of the transcription factor CREB and BDNF. In addition to the hippocampus, neurogenesis occurs in the subventricular zone where stem cells that give rise to olfactory bulb neurons are located. Neurogenesis in the subventricular zone declines during aging, and TRE/CR prevents this age-related reduction in neurogenesis (Apple et al. 2019).

IF/CR may also have beneficial effects on the cellular structure of the human brain. A study of postmenopausal women with obesity showed that 12 weeks of CR resulted in an increase in gray matter volume in the hippocampus and frontal cortex (Prehn et al. 2017). In the same study, the authors performed functional MRI analyses of neuronal network activity and found that CR enhanced the connectivity of the hippocampus and frontal cortex to the parietal cortex. The latter finding is consistent with animal studies demonstrating that TRE/CR increases synapse numbers (Stranahan et al. 2009).

Because it is neurons and their synaptic connectivity that are responsible for all brain functions and behaviors, most of the research on IF and the brain have focused on its effects on neurons. However, the brain contains three different types of glial cells—astrocytes, oligodendrocytes, and microglia. Indeed, the human brain has approximately equal numbers of neurons and astrocytes. Popov et al. (2020) found that TRE/CR stimulates morphological changes in astrocytes such that they increase their interaction with synapses. Oligodendrocytes wrap around the axon of neurons in a process called myelination that functions to insulate the axons in a manner that accelerates the conduction of the electrical impulse along the axon. Brain structures called white matter are where large numbers of myelinated axons reside. A brain

imaging study of mice fed either TRE/CR or AL demonstrated an age-related reduction in brain white matter in the AL control group but not in the TRE/CR group (Guo et al. 2015).

In conclusion, IF enhances synaptic plasticity and neurogenesis in rodents, and recent brain imaging data suggest similar beneficial effects of brain structure in humans.

7.4 Intermittent Fasting and Neurological Disorders

This section describes findings from animal studies showing that IF can protect neurons from damage and improve functional outcomes in animal models of major neurological disorders including Alzheimer's and Parkinson's diseases, epilepsy, stroke, and multiple sclerosis. Also included are findings from epidemiological studies of human populations as well as a small but increasing number of clinical trials.

7.5 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder which most often manifests in the seventh and eighth decades of life (for review see Mattson 2004; Knopman et al. 2021). The first symptom to appear is mild short-term memory loss which progresses to an inability of the affected person to remember any new information. In addition, AD patients typically exhibit disrupted circadian rhythms, agitation, and psychiatric problems. There are currently more than 7 million Americans suffering with AD, and cases will increase during the next several decades in lock step with the increase in the number of elderly people. As AD progresses the brain shrinks as neuronal dendrites atrophy and neurons in the frontal, temporal, and parietal lobes, hippocampus, and entorhinal cortex die. At the histological level, the defining features of AD are the accumulation of extracellular aggregates of the amyloid beta-peptide ($A\beta$ plaques) and of aggregates of hyperphosphorylated Tau protein (pTau) inside neurons (neurofibrillary tangles) (Fig. 7.1).

While in most instances AD is sporadic with no genetic cause, rare mutations in the $A\beta$ precursor protein (APP) or presenilin 1 cause early-onset dominantly inherited familial AD. Mutations in Tau do not cause AD, but instead, cause a neurodegenerative disorder called frontotemporal dementia which manifests neurofibrillary tangles but not $A\beta$ plaques. Transgenic mice expressing a human APP mutation alone or in combination with a presenilin 1 mutation exhibit accumulation of $A\beta$ plaques and modest cognitive impairment but no neuronal death (Borchelt et al. 1997; Sun et al. 2005). Transgenic mice expressing APP, presenilin 1, and Tau mutations (3xTgAD mice) exhibit age-related $A\beta$ plaques and neurofibrillary tangles, synaptic dysfunction and degeneration, cognitive impairment, and anxiety-like behavior (Oddo et al. 2003; España et al. 2010; Rothman et al. 2012).

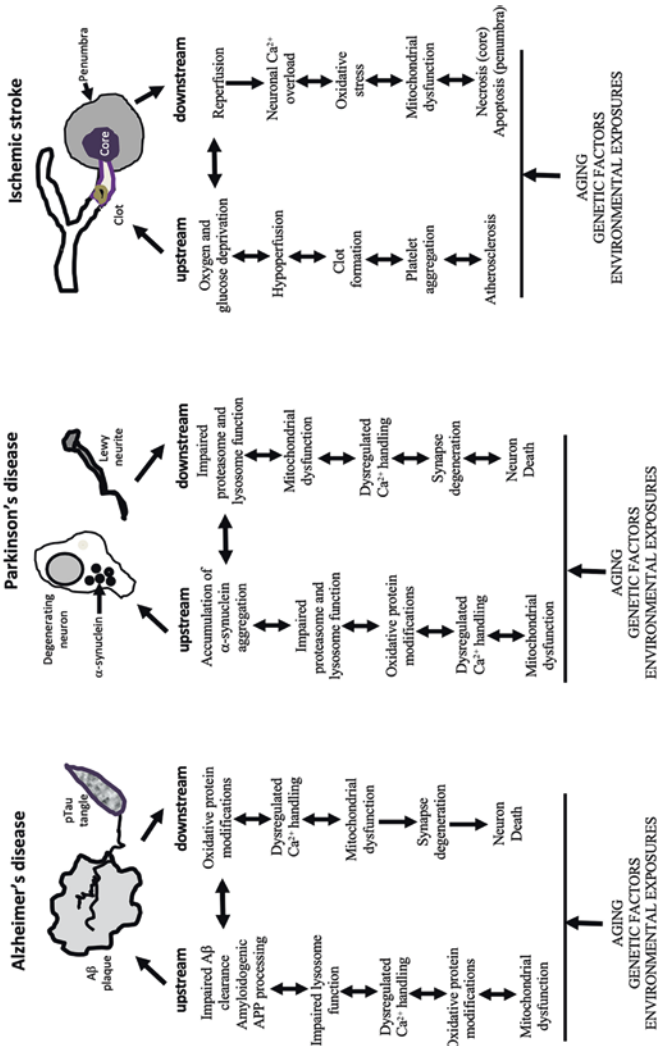


Fig. 7.1 Working models for mechanisms of neuronal dysfunction and degeneration in Alzheimer's disease (AD), Parkinson's disease (PD), and ischemic stroke. AD is defined by the accumulation of extracellular amyloid beta-peptide (Aβ) and intraneuronal hyperphosphorylated Tau protein (pTau). PD pathology is characterized by the intracellular accumulation of α-synuclein protein in neuronal cell bodies and neurites. A stroke occurs when a clot forms in a cerebral artery resulting in severe ischemia and a cerebral infarct characterized by a central core area where neurons rapidly die by necrosis and a surrounding penumbra where some neurons die more slowly by apoptosis. Risk factors AD, PD, and stroke include aging, a sedentary overindulgent lifestyle, and in some cases genetic factors. Preclinical studies in animal models and epidemiological and clinical data in humans have shown that IF counteracts aging processes and can act both upstream and downstream of the disease-defining features of AD, PD, and stroke to protect neurons against dysfunction and damage

Two early studies provided evidence that TRE/CR can reduce the accumulation of A β in the brains of APP mutant mice (Patel et al. 2005; Wang et al. 2005). The same year, we initiated a study in which 3xTgAD mice were randomly assigned to one of three groups: AL feeding, TRE/CR, or EODIF beginning at 5 months of age. Nontransgenic mice on AL feeding were used as controls. Twelve months later, spatial learning and memory were evaluated in water maze tests, and the mice were then euthanized and brain sections were immunostained with antibodies against A β and pTau. Learning and memory were markedly impaired in 3xTgAD mice fed AL compared to nontransgenic control mice (Halagappa et al. 2007). Both TRE/CR and EODIF prevented these learning and memory deficits in the 3xTgAD mice. Another study found that TRE/CR ameliorated cognitive deficits in a transgenic mouse model of frontotemporal dementia (Brownlow et al. 2014).

The role of dysfunction and degeneration of GABAergic interneurons in AD has been reviewed previously (Mattson 2020a). The incidence of epileptic seizures is at least 20-fold higher in persons with AD compared to age-matched neurologically normal people. Electroencephalogram recordings from AD patients and patients with mild cognitive impairment (often a prodrome of AD) revealed abnormal sub-threshold bursts of activity, and similar hyperexcitability occurs in transgenic mouse models of AD (Cheng et al. 2020; Perone et al. 2023). Postmortem examination of the brains of AD patients revealed loss of GABAergic interneurons in the hippocampus and vulnerable regions of the cerebral cortex. We found that there is also early loss of GABAergic neurons that express the Ca²⁺-binding proteins parvalbumin or calretinin in an AD mouse model and that this loss of GABAergic neurons is exacerbated by SIRT3 haploinsufficiency (Cheng et al. 2020). Because GABAergic neurons have a very high firing rate, it is likely that a deficit in mitochondrial function contributes to their early demise in AD.

When cultured human neurons are exposed to subtoxic concentrations of aggregating A β , they become prone to hyperexcitability and can be killed by concentrations of the excitatory neurotransmitter glutamate in a process called “excitotoxicity” (Mattson et al. 1992; Mattson 2023). Studies in animal models of epilepsy and AD have shown that IF can protect neurons against excitotoxicity (Bruce-Keller et al. 1999; Liu et al. 2019). IF may protect neurons against excitotoxicity by several mechanisms. One mechanism demonstrated in experiments with transgenic AD mice that develop severe seizures associated with A β deposition is by upregulating the expression of the mitochondrial protein deacetylase SIRT3 and increasing the activity of neurons that deploy the inhibitory neurotransmitter GABA (Liu et al. 2019).

Epidemiological evidence is consistent with the potential ability of IF to reduce the risk for AD. Studies have shown that midlife obesity and a sedentary lifestyle increase the risk of developing AD (Nianogo et al. 2022). Insulin resistance is strongly associated with AD risk and may adversely affect neurons by impairing glucose transport into neurons and by inducing systemic and brain inflammation (Ferreira et al. 2018). Indeed, glucose uptake into brain cells is impaired in patients with mild cognitive impairment and AD (Ferris et al. 1980), and that exposure of cultured hippocampal neurons to A β impairs glucose transport by a mechanism involving membrane-associated oxidative stress (Mark et al. 1997a). Because IF

enhances insulin sensitivity and elevates ketone levels, it is expected to bolster energy availability to neurons (Mattson 2022). Indeed, treatment of 3xTgAD and APP/PS1 mice with a ketone ester ameliorates neuronal network hyperexcitability and cognitive deficits (Kashiwaya et al. 2013; Cheng et al. 2020). Following up on the latter findings in experimental AD models, clinical studies have shown that dietary ketone precursors (medium chain triglycerides) can ameliorate cognitive deficits in patients with mild cognitive impairment (Fortier et al. 2021) and AD (Ota et al. 2019). Such “brain energy rescue” interventions are increasingly thought applicable not only to AD but also to PD and other neurodegenerative disorders (Cunnane et al. 2020).

7.6 Parkinson’s Disease

Parkinson’s disease (PD) is a fatal neurodegenerative disorder of aging in which there occurs a progressive deterioration of one’s ability to control body movements, often accompanied by cognitive and psychiatric problems (Poewe et al. 2017). PD currently afflicts more than 500,000 Americans. The motor symptoms of PD result from degeneration of dopaminergic neurons in the substantia nigra (Fig. 7.1). In addition to motor and cognitive symptoms, PD patients commonly experience chronic constipation as a result of damage to neurons that control intestinal motility. While in most cases PD does not result from a gene mutation, some cases are familial with mutations in more than a dozen genes being identified as causes of PD (Blauwendraat et al. 2020). Neurons that degenerate in PD exhibit abnormal aggregates of the protein α -synuclein, and both missense α -synuclein mutations and triplication of the gene encoding α -synuclein can cause PD.

Environmental exposures to pesticides that inhibit mitochondrial function are implicated as a risk factor for PD. A chemical called MPTP was discovered as a contaminant of a batch of synthetic heroin that caused PD symptoms, and selective dopaminergic degeneration has been widely used to model PD in rodents and monkeys (see Mattson 2023). In the first experiment aimed at determining whether IF might protect against PD, mice were maintained on EODIF or AL control feeding for 3 months and were then administered MPTP. Mice in the EODIF exhibited less damage to dopaminergic neurons and less motor impairment compared to mice in the control group (Duan and Mattson 1999). In a second experiment, mice were administered 2-deoxyglucose (a non-metabolizable analog of glucose that induces the production of ketones) for several weeks and were then administered MPTP. Similar to IF, mice pretreated with 2-deoxyglucose exhibited less motor impairment and relative preservation of their dopaminergic neurons (Duan and Mattson 1999). Both EODIF and 2-deoxyglucose induced the expression of two neuroprotective protein chaperones (HSP70 and GRP78) in dopaminergic neurons. Fifteen years later, Maswood et al. (2004) reported the results of a study in which rhesus monkeys were maintained on either TRE/CR (30% CR) or AL eating schedules for 6 months and then infused with MPTP intravenously into the right carotid

artery (Maswood et al. 2004). Monkeys in the TRE/CR exhibited fewer motor deficits, significant preservation of dopaminergic neurons, and higher levels of the neurotrophic factors BDNF and glial cell line-derived neurotrophic factor in their caudate nucleus (a brain region innervated by dopaminergic neurons).

Findings from animal studies suggest that IF can ameliorate non-motor functional and pathological abnormalities of PD. Griffioen et al. (2013) found that α -synuclein mutant transgenic mice exhibit α -synuclein accumulation in brainstem neurons and have an elevated heart rate and an impaired cardiovascular stress response. EODIF ameliorated, and a high fat and sugar diet exacerbated the adverse effects of mutant α -synuclein on parasympathetic regulation of heart rate. Other studies have shown that IF (Lee et al. 2002) and exercise (Jachim et al. 2020) induce BDNF expression in the brain and that BDNF enhances the activity of the brainstem cardiovagal neurons that slow heart rate and reduce blood pressure (Wan et al. 2014). Because BDNF can protect neurons against dysfunction and degeneration in experimental PD models (Ji et al. 2019), BDNF likely plays important roles in the neuroprotection conferred by IF.

Braak et al. (2006) reported the surprising results of a study in which the progression of α -synuclein pathology was determined in autopsy samples from PD patients who died at various stages of the disease. Their results suggested that PD pathology begins in enteric (gut) neurons and propagates retrogradely to the brain via the vagus nerve. Their findings are supported by a study in Sweden which showed that people who had undergone truncal vagotomy were at reduced risk for PD (Svensson et al. 2015). Subsequent studies in mouse models of PD showed that truncal vagotomy prevents the spread of α -synuclein pathology from the gut to the brain (Kim et al. 2019). Moreover, chronic mild gut inflammation accelerates the development of α -synuclein pathology in the brain and exacerbates motor deficits (Kishimoto et al. 2019). Interestingly, fecal transplants that improve the gut microbial flora ameliorate motor dysfunction and dopaminergic neuron degeneration in a mouse PD model (Sun et al. 2018), and a pilot trial of fecal transplantation in PD patients generated promising results (Xue et al. 2020). Because IF can improve the gut microbial flora (Liu et al. 2020; Hernandez et al. 2022), this may be one mechanism by which IF ameliorates neurodegeneration and motor impairment in mouse models of PD (Duan and Mattson 1999; Griffioen et al. 2013).

Epidemiological studies are consistent with the possibility that IF might reduce one's risk for PD. For instance, in one study, 6715 Finnish men and women aged 50–79 years who did not have PD at baseline were followed for 22 years during which time 101 were diagnosed with PD (Sääksjärvi et al. 2014). Analyses showed that those who developed PD had significantly a higher body mass index and engaged in less leisure time activities at baseline compared to those who did not develop PD. As of yet, there have been no clinical trials of IF in PD. However, several clinical trials of interventions that mimic features of IF have generated data consistent with the beneficial effect of IF in patients with PD. Based on preclinical findings showing that the GLP-1 analog exenatide is neuroprotective in an animal model of PD (Li et al. 2009), a clinical trial of exenatide in PD patients was conducted. The clinical trial showed that exenatide improved motor function in PD

patients during a 48-week treatment period and that the benefit persisted for at least 12 months after cessation of treatment (Athauda et al. 2017). Exenatide is known to increase insulin sensitivity and suppress appetite, so it is possible that at least some of the benefit of exenatide in PD patients is the result of CR/IF. In addition, a recent study found that when PD patients ingested a ketone (β -hydroxybutyrate) ester prior to exercise, their exercise tolerance was significantly increased (Norwitz et al. 2020).

7.7 Stroke

Nearly 1 million Americans will experience a stroke each year, and up to 1 in 7 will die from the stroke. A stroke can occur at any age but is most common in the elderly. There are two types of strokes. The most common is an ischemic stroke which results from the formation of a clot in a cerebral blood vessel (Fig. 7.1). Less common is a hemorrhagic stroke in which a cerebral blood vessel ruptures. Most people who survive a stroke will have a disability which manifests as motor (partial paralysis) and sensory (numbness) on the side of the body opposite the side of the brain in which the stroke occurs. The blood vessel most commonly affected in stroke is the middle cerebral artery where a clot forms resulting in ischemia to the regions of the cerebral cortex and striatum perfused by that vessel. The ischemia is usually followed by reperfusion as the clot resolves over a period of many minutes to hours. Risk factors for stroke are essentially the same as those for cardiovascular disease—obesity, diabetes, hypertension, and hyperlipidemia. Because IF can counteract these risk factors, it can be predicted that IF would reduce one's risk for a stroke. But recent evidence suggests that IF can also improve recovery from a stroke.

The first evidence that IF might protect neurons against a stroke came from a study of rats (Yu and Mattson 1999). Rats were maintained for 3 months on either EODIF or AL feeding, and then an experimental stroke was induced by transient (1 h) occlusion of the middle cerebral artery. This stroke model results in reproducible damage to motor and sensory regions of the cerebral cortex and to the striatum. The damage to and death of neurons in these brain regions results in motor and sensory deficits in the contralateral limbs. The results were clear. Compared to rats in the AL group, rats in the EODIF group suffered less brain damage and fewer functional deficits. In a second experiment, rats that were treated with 2-deoxyglucose daily for 2 weeks prior to experimental stroke exhibited less brain damage and improved functional outcome compared to rats administered saline (Yu and Mattson 1999). Subsequent studies have confirmed and extended the initial report of neuroprotection by IF in experimental stroke (Arumugam et al. 2010; Fann et al. 2014; Kim et al. 2018; Zhang et al. 2019). The latter studies provided evidence that the mechanisms by which IF protects neurons involve the induction of genes encoding antioxidant enzymes, heat-shock proteins, neurotrophic factors, adiponectin, and suppression of inflammation.

The only treatment for ischemic stroke is a clot-disrupting drug, but this is only given to a small percentage of patients whose clot has not already resolved

spontaneously. Physical therapy after a stroke benefits many patients. One preclinical study provided evidence that intermittent fasting initiated soon after a stroke can enhance recovery of function (Hu et al. 2019). However, there are as yet no reports of clinical trials of IF in patients who have suffered a stroke.

7.8 Epilepsy

Epilepsy is a disorder in which there occurs hyperexcitability of neuronal circuits and consequent seizures (Devinsky et al. 2018). The seizures most often occur spontaneously but can be triggered by a brain tumor, a stroke, or a traumatic brain injury. More than 3 million Americans have epilepsy and approximately 500,000 are children. Drugs that block neuronal Na⁺ channels or activate inhibitory GABA receptors are effective in preventing or reducing seizures in many but not all patients. Another epilepsy treatment is a ketogenic diet which keeps blood glucose levels in the low normal range and elevates levels of the ketones BHB and acetoacetate (Rho and Boison 2022).

A commonly used animal model of epilepsy involves administration of the seizure-inducing excitotoxin kainic acid. Rats or mice subjected to kainic acid-induced seizures exhibit degeneration of hippocampal pyramidal neurons and an associated learning and memory deficit. When rats are maintained on EODIF prior to kainic acid administration, their seizures are suppressed, fewer hippocampal neurons degenerate, and their learning and memory are not compromised (Bruce-Keller et al. 1999). The mechanism by which IF protects against seizures involves the enhancement of GABAergic tone (Liu et al. 2019) and the upregulation of BDNF expression (Duan et al. 2001).

From an historical perspective, fasting has been used to treat epilepsy at least from the time of Hippocrates (Bailey et al. 2005). The results of a small retrospective analysis of a study of children with epilepsy suggest that IF can reduce seizure incidence (Hartman et al. 2013).

7.9 Multiple Sclerosis

Multiple sclerosis is an autoimmune disease in which immune cells (T lymphocytes) mistakenly recognize certain molecules on oligodendrocytes (the cells that myelinate axons) as foreign and so initiate a local inflammatory response that demyelinates and damages axons (Filippi et al. 2018). Clinically, the demyelination results in a range of symptoms related to the particular nerves that are affected. Examples include numbness and weakness on one side of the body, lack of coordination, unsteady gait, blurry vision, slurred speech, and impaired cognition. The disease typically manifests in cycles of relapse and remission. Approximately 1 million Americans are currently living with multiple sclerosis.

To model multiple sclerosis in mice, the animals are injected with peptide fragments of certain myelin proteins. This stimulates the immune system to attack the myelin in the spinal cord and brain resulting in motor deficits that can be quantified. In this mouse model, EODIF can lessen motor dysfunction and demyelination of axons in the spinal cord (Razeghi Jahromi et al. 2016). Similar protective effects were seen in mice maintained on a different IF regimen (repeated cycles of 3 days of caloric restriction) (Choi et al. 2016). The latter study further showed that IF stimulates remyelination and that a similar IF regimen is feasible and potentially effective in treating patients with multiple sclerosis. A more recent study further confirmed the beneficial effects of IF in mouse multiple sclerosis and provided evidence that improved gut microbial flora played an important role (Cignarella et al. 2018). Moreover, the latter study demonstrated beneficial effects of IF on the gut microbiome of multiple sclerosis patients.

There have been several clinical trials of IF in multiple sclerosis with promising results. Fitzgerald et al. (2018) found that both daily caloric restriction and 5:2 IF (2 days of modified fasting per week) improved emotional well-being scores relative to controls. A follow-up study showed that IF resulted in a reduction in memory T cell subsets in multiple sclerosis patients demonstrating an attenuation of the autoimmune response (Fitzgerald et al. 2022). Adherence to daily TRE was superior to 5:2 IF or CR without fasting (Roman et al. 2020).

7.10 Traumatic Brain and Spinal Cord Injuries

Each year, approximately a quarter million Americans will suffer a traumatic brain injury (TBI) that requires hospitalization, and more than 100,000 will die from it. A large proportion of TBI patients will experience a long-term disability which may manifest immediately after the injury or may evolve over years in instances of chronic traumatic encephalopathy. Motor vehicle accidents and falls (particularly in the elderly) are the major causes of a TBI. Mechanisms involved in neural damage caused by TBI include mechanical shearing forces, hemorrhage, ion imbalances, excitotoxicity, energy failure, and inflammation (Blennow et al. 2016). Obesity is associated with increased intensive care unit mortality and prolonged dependency on mechanical ventilation (Chabok et al. 2014).

The potential of IF to modify TBI outcomes has been evaluated in mouse models. When initiated immediately after TBI, both EODIF and TRE/CR ameliorated cognitive deficits measured at 30 days post-injury (Rubovitch et al. 2019). The neuroprotection afforded by IF was associated with a preservation of levels of SIRT1, a protein deacetylase involved in cellular stress responses and regulation of the circadian clock. More recent studies have confirmed a neuroprotective effect of EODIF in a mouse TBI model and have provided evidence for mechanisms involving neuropeptide Y and suppression of genes involved in cell death pathways (Cao et al. 2022a; Yang et al. 2023). There have been no trials of IF in human patients with TBI. However, because insulin resistance is associated with poorer outcomes in TBI

patients (Cao et al. 2022b) and IF increases insulin sensitivity (Harvie et al. 2011), it might be expected that IF would be beneficial.

There are approximately 300,000 Americans who are currently living with a disability caused by a spinal cord injury (SCI), and many of these have paralysis. Following the initial mechanical trauma, a secondary cascade results in progressive cell death and spinal cord damage; axons of neurons that survive do not regenerate (Ahuja et al. 2017). Studies in rat models of SCI have shown that EODIF reduces spinal cord damage and improves functional outcome when initiated either prior to (Plunet et al. 2010) or after (Jeong et al. 2011) SCI. There have been no trials of IF in human patients with SCI.

7.11 Anxiety Disorders and Depression

Findings from animal studies and some human studies suggest that IF can reduce anxiety and improve mood. Mice that have been adapted to EODIF exhibit reduced anxiety-like behaviors compared to those fed AL (Liu et al. 2019; Carteri et al. 2021). Electrophysiological recordings from hippocampal neurons showed that IF enhances the inhibition of excitatory glutamatergic neurons by GABAergic interneurons (Liu et al. 2019). Enhancement of inhibitory GABAergic neurotransmission likely explains the anxiolytic effect of IF because drugs such as diazepam that activate GABA receptors have been widely used to treat humans with anxiety disorders. Additional evidence that IF can reduce anxiety comes from a study showing that dietary supplementation with a ketone ester alleviated anxiety in a mouse model of AD (Kashiwaya et al. 2013).

Early evidence that IF might have an antidepressant effect came from a study in which self-reported mood was evaluated in asthma patients during a 2-month period on an alternate-day very low-calorie IF regimen (Johnson et al. 2007). Mood improved during the first month of IF and remained elevated at 2 months. More recent studies have also reported similar beneficial effects of TRE on mood (Hussin et al. 2013; Steger et al. 2023). A meta-analysis of human studies concluded that IF can reduce depression scores while having no significant effect on anxiety (Fernández-Rodríguez et al. 2022). Clinical trials of IF in patients with an anxiety disorder or depression remain to be performed.

7.12 Interactions of Exercise with IF

Imagine that you are an animal living in an environment where food is scarce and competition is intense. Your brain and body must function well in a food-deprived state; otherwise, you will not be able to survive and reproduce. Indeed, the metabolic, musculoskeletal, cardiovascular, and neurobiological systems of humans and other animals have evolved to function optimally in the fasted state (Mattson 2022).

An increasing number of studies have examined the effects of exercise and IF alone or in combination on peripheral organ systems or the brain.

In one study of IF-exercise interactions, mice were divided into four groups: AL feeding and sedentary; AL feeding and daily exercise; EODIF and sedentary; EOD/IF and daily exercise (Marosi et al. 2018). The exercise consisted of running for 45 min on a treadmill every day with weekly increases in running speed and treadmill incline. After 2 months under these conditions, an endurance test was performed in which mice were run to exhaustion. As expected, the endurance of all mice that had previously run every day was vastly superior to those who had remained sedentary. However, the endurance of mice in the EODIF exercise group was superior to the endurance of mice in the AL exercise group. IF elevated plasma ketone levels, and exercise enhanced the ketogenic effect of IF. The respiratory exchange ratio was significantly reduced in mice in IF groups, and this effect was enhanced during running consistent with a metabolic switch from glucose to ketones. Interestingly, maximum oxygen consumption at peak exercise was not affected by IF. The results of metabolomic analyses of blood and gene expression analysis of soleus muscle (an endurance muscle in the leg) suggested that IF stimulates mitochondrial biogenesis and cellular plasticity (Marosi et al. 2018). Altogether, the results of this study show that IF enhances metabolic efficiency and endurance capacity.

The results of human studies support a beneficial effect of IF on physical endurance. Daily TRE (8 h feeding window) elicited fat loss, maintained muscle mass, and increased performance in elite cyclists (Moro et al. 2020). A study of women with obesity showed that 5:2 IF results in fat loss, a gain in lean mass, and increases in strength and aerobic capacity during a 2-month period of high-intensity interval training (Batitucci et al. 2022). Similar findings came from a trial of TRE plus aerobic and resistance training in overweight and obese adults. Compared to those in the control eating pattern plus exercise group, those in the TRE plus exercise group lost more fat and exhibited a lower resting heart rate suggesting a greater improvement in cardiovascular fitness (Kotarsky et al. 2021). In another study trained male endurance runners were randomized to either a normal eating pattern (control) or TRE (8-h eating window) for 4 weeks in a crossover study design (Richardson et al. 2023). Compared to the control condition, TRE resulted in significant fat loss with retention of lean mass. Although endurance performance was not evaluated, the authors concluded that IF could be beneficial by reducing fat mass without adversely affecting muscle mass. In the case of resistance training, numerous studies have shown that IF enables increases in lean and loss of fat mass (see Ashtary-Larky et al. 2021 for a meta-analysis).

TRE/CR and running wheel exercise each stimulate an increase in synapse numbers on the dendrites of hippocampal dentate neurons, and the combination of TRE/CR and exercise results in greater synapse numbers than either TRE/CR or exercise alone (Stranahan et al. 2009). In addition to increasing synapse numbers, IF and exercise may also have additive effects in increasing hippocampal neurogenesis. In rats both running wheel exercise and EODIF increase the number of new hippocampal dentate granule neurons produced from stem cells. Whereas exercise stimulates

both the proliferation of stem cells and their incorporation into neuronal circuits (van Praag et al. 1999; Vivar et al. 2016), EODIF does not stimulate stem cell proliferation but does increase the survival and differentiation of newly generated neurons (Lee et al. 2002). Beneficial effects of IF/CR and exercise on peripheral neurons have also been reported. For instance, long-term TRE/CR reduces age-related abnormalities at neuromuscular synapses, loss of motor neurons, and turnover of muscle fibers, and exercise enhanced the effects of TRE/CR on the neuromuscular synapses (Valdez et al. 2010).

7.13 Cellular and Molecular Mechanisms

Me and my colleagues have previously reviewed studies that have revealed the cellular and molecular mechanisms by which IF affects brain function, neuroplasticity, and disease processes (Mattson 2015, 2022; Camandola and Mattson 2017; Mattson et al. 2018). What has emerged from this research is that IF elicits a complex and highly coordinated signaling pathways and patterns of gene expression that enhance neuronal network function, cellular stress resistance, autophagy, protein quality control, DNA repair, and mitochondrial biogenesis. IF suppresses neuroinflammation and the accumulation of misfolded and oxidatively damaged proteins. Signaling pathways engaged by IF include those that enhance cell survival and promote the outgrowth of axons and dendrites, the strengthening of existing synapses, the formation of new synapses and neurogenesis.

One question that has arisen from studies of the effects of IF on neuronal function, plasticity, and resilience is the relative contributions of signals emanating from the periphery and of brain-intrinsic mechanisms. Regarding signals from the periphery to the brain evidence supports important roles for ketone bodies, hormones, and neurotrophic factors.

Beyond providing an efficient energy source for neurons during the fasting period, ketones are known to affect cellular signaling and gene expression (for review see Newman and Verdin 2017). The vast majority of research on cellular responses to ketones have focused on BHB, the major ketone body in mammals. Studies of peripheral cells have revealed direct effects of BHB on cell surface receptors, enzyme activity, and modulation of ion channel activity. BHB affects gene expression by inhibiting class I histone deacetylases (HDACs). BHB may also increase GABAergic inhibitory synaptic activity by increasing the production of GABA from glutamate via the effects of tricarboxylic acid intermediates (Melo et al. 2006) and/or by increasing the release of GABA onto excitatory glutamatergic neurons (Cheng et al. 2020; Liu et al. 2019). Evidence suggests that BHB stimulates the production of BDNF by at least two mechanisms. Acting in the nucleus inhibition of HDACs induces the gene encoding BDNF (Sleiman et al. 2016). In addition, BHB may elicit changes in the mitochondria that result in the activation of the cytoplasmic transcription factor NF- κ B which then translocates to the nucleus where it activates the gene encoding BDNF (Marosi et al. 2016).

In addition to ketone bodies, several other molecules are released into the blood during fasting and might mediate some of the beneficial effects of IF on the brain. These include ghrelin and adiponectin. Studies have shown that in addition to its influences on hypothalamic neurons that control hunger and satiety, ghrelin can enhance learning and memory and can modulate mood (for review see Serrenho et al. 2019). Moreover, ghrelin has been reported to suppress seizures and protect neurons against excitotoxicity in animal models of epilepsy (Buckinx et al. 2021) and enhance synaptic plasticity and cognition in a mouse model of AD (Santos et al. 2017). Circulating levels of adiponectin increase in response to fasting (Wan et al. 2010), and experiments have shown that adiponectin can protect neurons against degeneration in experimental models of epilepsy (Qiu et al. 2011), Alzheimer's disease (Ng et al. 2021), and Parkinson's disease (Sekiyama et al. 2014).

The repeated cycles of fasting and feeding of IF eating patterns—the switching back-and-forth between ketogenic and non-ketogenic states—can over periods of several weeks or more enhance the sensitivity of cells to insulin and so enhance the ability of cells (including neurons) to take up glucose when food is consumed. Increasing evidence suggests that a state of neuronal resistance to insulin occurs during aging, with obesity, and in AD (Kapogiannis et al. 2015; Kellar and Craft 2020; Stranahan 2022). Insulin and insulin-like growth factor 1 can increase the resistance of neurons to oxidative and metabolic stress (Cheng and Mattson 1992; Mattson and Cheng 1993), and clinical trials of intranasal insulin suggest a potential benefit in patients with AD (Claxton et al. 2015). However, a state of neuronal insulin resistance in AD may mitigate any potential therapeutic benefit of insulin.

In addition to IF and exercise, drugs that enhance insulin sensitivity such as GLP-1 receptor agonists can protect neurons against dysfunction and degeneration in animal models of AD (Li et al. 2010), PD (Li et al. 2009), Huntington's disease (Martin et al. 2009b), and stroke (Li et al. 2009). Moreover, the results of recent clinical trials show that GLP-1 receptor agonists can slow disease progression in PD patients (Aviles-Olmos et al. 2013; Athauda et al. 2017).

Leptin resistance is a well-established consequence of obesity, and similar to insulin sensitivity, the sensitivity of neurons to leptin is enhanced by IF. The findings of several studies in animal models suggest that leptin not only functions as a satiety signal but also plays important roles in learning and memory and neurogenesis (Oomura et al. 2006; Stranahan et al. 2008a). Binding of leptin to its receptor results in the activation of the transcription factor STAT3 and the expression of multiple genes including the gene encoding BDNF (Li et al. 2021).

Much further research will be needed to determine what blood-borne factors are necessary for the beneficial effects of IF on brain function, neuroplasticity, and resilience. However, clues are emerging from studies of circulating proteins that may mediate the “anti-aging” or rejuvenating effects on the brain. Such factors include those produced by muscle cells (GDF11, Irisin, and cathepsin B), immune cells (CSF2 and TIMP2), and gut bacteria (short-chain fatty acids) (for review see Pluvinage and Wyss-Coray 2020).

Peripheral organs may also affect brain and whole-body resilience and disease processes via signals conveyed by the vagus nerve. Some of the axons in the vagus

nerve convey information from neurons in the brainstem to peripheral organs, while other vagal axons convey retrograde signals from sensory neurons located in peripheral tissues. For instance, brainstem parasympathetic neurons slow heart rate, reduce blood pressure, and increase gut motility. Vagal neurons in the nodose ganglion convey sensory signals from abdominal and thoracic organs to brainstem nucleus tractus solitarius. Studies have established the existence of an inflammatory reflex in which peripheral tissue inflammation activates vagal sensory neurons which then trigger the activation of vagal motor neurons resulting in suppression of the immune cells that mediate the tissue inflammation (Pavlov and Tracey 2022). Preclinical studies demonstrated the efficacy of vagal nerve stimulation in suppressing inflammation in several disease models, and clinical trials have shown the efficacy of vagal nerve stimulation in rheumatoid arthritis (Pavlov and Tracey 2022). The inflammatory vagal reflex suppresses inflammation via acetylcholine acting on nicotinic receptors on macrophages and other immune cells. IF activates brainstem vagal cholinergic neurons (Wan et al. 2003) which may in part explain the anti-inflammatory effects of IF.

In addition to signals coming to the brain from the periphery, IF may activate signaling pathways that are intrinsic to brain cells. The most widely studied example of such a brain-intrinsic mechanism is that of neuronal activity-dependent production of BDNF (Rothman and Mattson 2013). During fasting neuronal circuit activity is increased in many brain regions including those involved in motivation, learning and memory, and goal-directed behavior, and these responses to fasting are reflected in food-seeking behaviors. In animals maintained on EODIF, the spontaneous movement of animals within their cages increases toward the end of the fasting period which is consistent with increased activity in brain neuronal networks involved in food-seeking behaviors (Fig. 7.2). Increased activity in neuronal circuits results in increased Ca^{2+} influx into neurons and the activation of the transcription factors CREB (for review see West et al. 2001) and NF- κ B (for review see Mattson and Camandola 2001). Activation of these transcription factors results in the expression of genes that encode a range of proteins involved in cellular stress resistance and neuroplasticity. These include heat-shock proteins such as HSP70 and GRP78, antioxidant enzymes including HO1 and SOD2, the DNA repair enzyme APE1, and the neurotrophic factors BDNF, FGF2, and IGF1 (Lee et al. 2002; Peltier et al. 2007; Arumugam et al. 2010; Yang et al. 2010, 2014).

BDNF, FGF2, and IGF1 may play particularly important roles in the beneficial effects of IF on neuroplasticity and disease resistance. These neurotrophic factors control hippocampal neurogenesis and can protect neurons against oxidative, metabolic, and excitotoxic stress in experimental models of stroke, AD, and PD (Cheng and Mattson 1991; Mattson et al. 1993; 2004; Mark et al. 1997b; Cheng et al. 2003, 2004; Llorens-Martín et al. 2009). Among the genes activated by these neurotrophic factors are those encoding antioxidant enzymes (Mattson et al. 1995) and pro-survival proteins such as Bcl2 (Allsopp et al. 1995). BDNF stimulates mitochondrial biogenesis (an increase in the number of healthy mitochondria) in neurons by a mechanism involving the transcription factor PGC-1 α (Cheng et al. 2012). The latter study provided evidence that the increase in mitochondrial biogenesis is

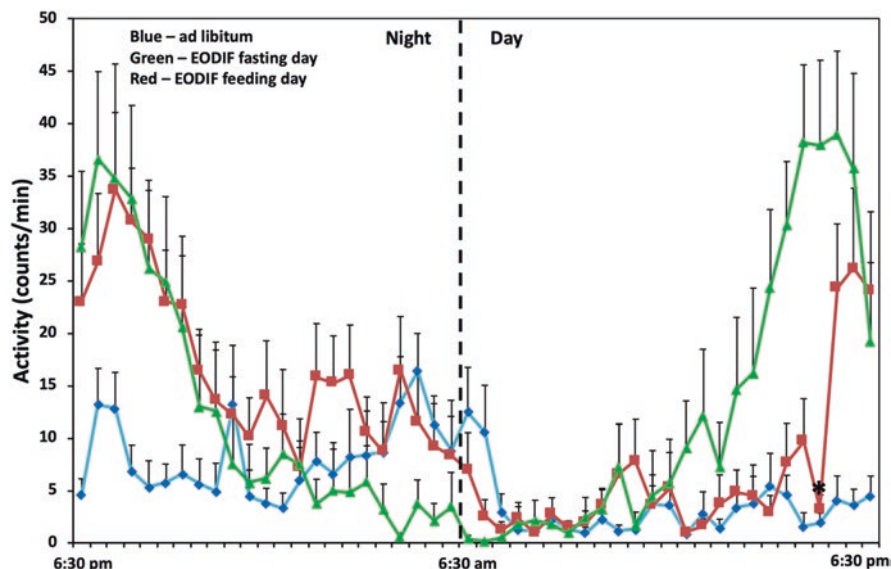


Fig. 7.2 Twenty-four-hour home cage activity levels of mice before (ad libitum) and 3 months after the initiation of EODIF. Data are mean and SD of recordings from six mice. Note the large increase in activity during the last 4 h of the fasting period

necessary for the formation of new synapses in response to BDNF and the maintenance of existing synapses.

Neurotrophic factors may also mediate the beneficial effects of IF and exercise on the autonomic nervous system. In mice adapted to EODIF, there occurs an increase in the activity of cholinergic brainstem vagal neurons which is mediated by BDNF and results in reduced resting heart rate and increased heart-rate variability (Wan et al. 2014) and is associated with enhanced cardiovascular and neuroendocrine responses to stress adaptation (Wan et al. 2003). Similar to daily TRE, daily administration of 2-deoxyglucose improves cardiovascular and neuroendocrine stress adaptation (Wan et al. 2004).

One protein that is increasingly recognized as a mediator of the beneficial effects of IF and exercise on neuronal plasticity and resilience is SIRT3. A seminal study using SIRT3 knockout mice and adeno-associated virus-mediated Sirt3 gene delivery to neurons showed that SIRT3 protects brain neurons against excitotoxic, oxidative, and metabolic stress (Cheng et al. 2016). SIRT3 deficiency results in the hyperacetylation of several mitochondrial proteins including SOD2 and the apoptosis inducing mitochondrial membrane pore protein cyclophilin D. SIRT3 expression is increased in the hippocampus and cerebral cortex in response to running wheel exercise, and activation of NMDA glutamate receptors mediates the upregulation of SIRT3 by exercise (Cheng et al. 2016). Thus, SIRT3 is an activity-dependent mitochondrial enzyme that, by deacetylating certain mitochondrial proteins, protects neurons against stress. SIRT3 levels are also increased in hippocampal neurons in mice maintained on

EODIF, and this increase mediates the effects of the IF on neuronal network excitability, cognition (enhanced), and anxiety (reduced) (Liu et al. 2019). The precise mechanism by which these effects of SIRT3 on synaptic plasticity occur remains to be established. Nevertheless, the evidence that a mitochondrial protein deacetylase is necessary for the enhancement of cognition afforded by IF reveals a previously unrecognized role for mitochondria in the regulation of synaptic plasticity.

Altogether, the evidence indicates that IF engages multiple signaling pathways that act in concert to bolster neuronal stress resistance and enhance neuroplasticity. These mechanisms include those involving metabolic and signaling actions of ketones, the transcription factors CREB and NF- κ B, the neurotrophic factor BDNF, protein chaperones, the mitochondrial protein deacetylase SIRT3, and mitochondrial biogenesis.

7.14 Challenge: Recovery Cycles and Long-Term Adaptations

We previously postulated that general health and brain health are optimized by repeating cycles of metabolic challenge (fasting, exercise, intellectual challenges) and recovery (eating, resting, sleeping) (Mattson et al. 2018; de Cabo and Mattson 2019). While the frequency and duration of the challenge periods may vary, findings from animal and human studies suggest that IF, physical exercise, and intellectual challenges are effective in enhancing brain function, slowing brain aging, and forestalling chronic diseases.

In general, during the challenge period (fasting, physical exercise, mental exercise), cell signaling pathways are engaged that function to conserve resources and bolster stress resistance (Fig. 7.3). These include pathways that reduce overall protein synthesis (inhibition of the mTOR pathway), stimulate autophagy and mitophagy, and upregulate genes encoding antioxidant enzymes and DNA repair proteins (Fig. 7.3). Then during the recovery period, pathways are engaged that shift cells into a growth and plasticity mode (activation of mTOR, mitochondrial biogenesis, and cell growth pathways).

It is instructive to consider the similarities between IF and regular exercise in the context of stress adaptation. Periods of exercise and IF impose stress on cells and organ systems, and periods of rest, eating, and sleeping enable recovery and cellular plasticity. An evolutionary perspective leads to the conclusion that fasting and physical exertion usually occur concurrently. Imagine a fox that has not eaten for several days hunting and then chasing a rabbit, or a herd of antelope searching for a day or more for a new patch of vegetation after having depleted vegetation in another area. These animals' organ systems become strong and efficient having experienced cycles of metabolic switching.

In our first studies of the effects of EODIF on the brain, we found that neuroprotective effects of IF did not become evident until the animals had been maintained

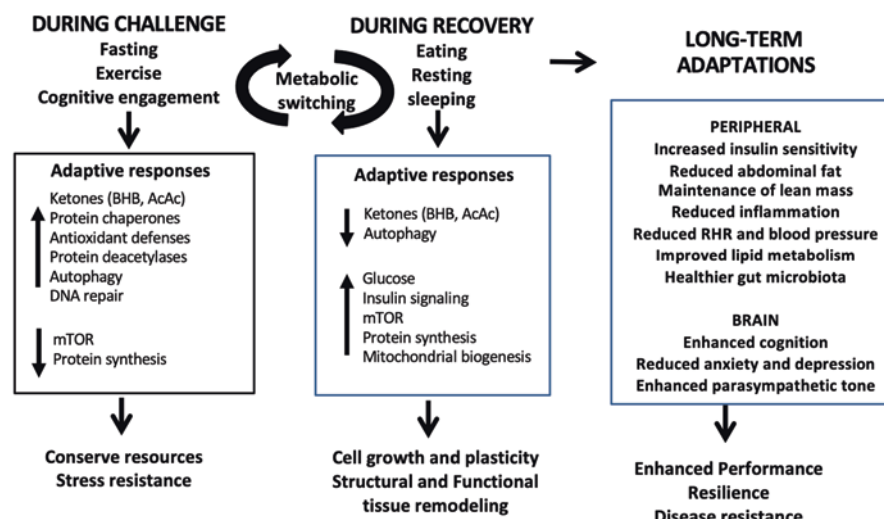


Fig. 7.3 Metabolic challenge—recovery model for the mechanisms by which IF, exercise, and cognitive engagement optimize brain health and resilience. During the challenge period, moderate levels of metabolic, ionic, and oxidative stress are observed resulting in the activation of signaling pathways and genes that function to increase cellular stress resistance (antioxidant enzymes, protein chaperones, DNA repair enzymes, protein deacetylases) and conserve resources (reduced protein synthesis and enhanced autophagy). During the recovery period (eating, resting, sleeping), neurons switch to a growth and plasticity mode in which protein synthesis increases and mitochondrial biogenesis occurs. Over time, intermittent metabolic switching enhances the health and function of peripheral organs and the brain. In the case of the brain, cognitive abilities are enhanced, mood improves, and parasympathetic tone is increased. Together, the effects of intermittent metabolic challenges improve performance and resistance to stress and disease. AcAc, acetoacetate; BHB, beta-hydroxybutyrate; mTOR, mechanistic target of rapamycin; RHR, resting heart rate

on IF for at least 2 weeks (Bruce-Keller et al. 1999). Neuroprotective efficacy continued to increase for another 2 weeks after which the magnitude of the effect was maintained. Similarly, reductions in resting heart rate and blood pressure became significant by 2 weeks of EODIF, continued to decline through 1 month, and thereafter remained reduced. Mitochondrial adaptations to IF (increased SIRT3 expression), increases in GABAergic tone, and reductions in anxiety levels also require at least 2 weeks of IF (Liu et al. 2019). This 2–4-week adaptation period was also clearly seen in our study of asthma patients in which symptoms and circulating proinflammatory cytokines were reduced (Johnson et al. 2007). Interestingly, the time course of adaptation of various organ systems to IF corresponds with the time period required for reductions in hunger and irritability in people when they first change to an IF eating pattern. These behavioral adaptations may be explained by increases in the leptin sensitivity of hypothalamic neurons and enhanced GABAergic tone in the neuronal circuits that mediate anxiety. I surmise that during the “magic month” epigenetic changes occur that result in highly orchestrated changes in gene expression which confers optimal cellular functionality and resilience in all organ

systems. Such patterns of gene expression and epigenetic molecular modifications that sustain those gene expression patterns are being investigated (e.g., see Thompson et al. 2018; Ng et al. 2022).

When laboratory animals or humans are subjected to various stressors, they exhibit acute elevations of adrenal glucocorticoids (corticosterone in rats and mice and cortisol in humans). Chronic elevations of glucocorticoids resulting from prolonged psychosocial stress or diabetes are generally associated with adverse effects on various organ systems including the brain (Stranahan et al. 2008a, b, c). Paradoxically, glucocorticoid levels are also elevated in rodents and humans maintained on CR/TRE or EODIF despite the fact that they live longer and are resistant to a wide range of diseases (Sabatino et al. 1991; Wan et al. 2003; Fontana et al. 2016). We performed several studies aimed at better resolving this glucocorticoid paradox. In one study we maintained rats on either EODIF or AL feeding for 6 months and then subjected them to acute body restraint. Measurements of blood corticosterone levels revealed that prior to restraint stress, basal levels of blood corticosterone were higher in EODIF rats compared to AL rats. However, the elevation of corticosterone levels in response to restraint stress was markedly attenuated in rats in the EODIF group compared to those in the AL group (Wan et al. 2003). We then designed experiments to determine whether neurons in the brains of animals on EODIF respond differently to stress compared to neurons in the brains of animals fed AL. There are two different receptors for corticosterone, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Chronic psychological stress results in a reduction in levels of MR in neurons. In contrast, we found that EODIF results in a reduction in the levels of GR with maintenance of MR levels in hippocampal neurons (Lee et al. 2000). The latter finding suggests that IF alters responses glucocorticoids, presumably in ways that enhance neuroplasticity and resilience. Indeed, in a subsequent study we found that selective activation of MR enhances synaptic plasticity in rats Stranahan et al. 2010). To determine how elevated basal levels of corticosterone might affect neurons in the brain, we performed two studies.

Repeated cycles of challenge (fasting)-recovery (eating) cycles result in adaptive responses of many organ systems that improve their function and resilience. Animal and human studies have consistently shown that such adaptations occur within 2–4 weeks of the onset of IF.

7.15 Overindulgence Results in Cellular Complacency and Vulnerability

The afternoons were always in marked sluggishness by contrast, from the taxing of digestion. Without realizing that the heavy meals of the day were a tax upon the brain, I would scarcely get away from the table before I began to feel more generally tired out than the severest taxing from a long forenoon of general activity ever made me. With the filled stomach, fatigue, general exhaustion, came as a sudden attack rather than as an evolution from labor, and there would be several hours of unfitness for doing any kind of service well. (E. Dewey 1900)

Other chapters in this volume have focused on the effects of IF on peripheral organs and its potential for the prevention and treatment of obesity, diabetes, cardiovascular disease, cancers, and inflammation. And just as overeating and a sedentary lifestyle increase the risk for the latter disorders, so too do such “metabolically morbid” lifestyles adversely affect the brain. Herein, I briefly summarize the major conclusions of research on the impact of obesity and diabetes on the brain. For a recent review on this topic, see Mattson (2019, 2022) and Kendig et al. (2021).

Studies of animal models have shown that obesity and diabetes caused by excessive energy intake result in cognitive impairment and brain atrophy across the life span (Stranahan 2015). For instance, mice that chronically overeat as a result of a mutation in the leptin receptor (leptin is the “satiety hormone”) exhibit impaired hippocampus-dependent learning and memory, impaired long-term potentiation (LTP; an increase in synaptic strength involved in memory encoding), and impaired hippocampal neurogenesis (Stranahan et al. 2008a). When middle-age rats are fed a high-fat, high-sugar (glucose and fructose) diet, they become obese and insulin resistant and perform poorly on tests of cognition compared to control rats on a healthier diet (Stranahan et al. 2008b). The cognitive impairment resulting from a high fat and sugar diet was associated with reduced numbers of synapses on hippocampal neurons and reduced LTP. Studies have shown that providing rats or mice with continuous access to simple sugars (glucose, sucrose, and/or fructose) causes impaired performance on various tests of cognition (spatial learning and memory; object recognition memory; working memory). These adverse effects of high energy diets on brain function are seen not only in adults but also adolescents (Jurdak and Kanarek 2009; Reichelt et al. 2015).

Studies of the effects of obesity and diabetes on brain function and structure in humans are entirely consistent with the results from animal studies described in the preceding paragraph. Adulthood obesity impairs cognition and can cause reductions in gray matter volumes (see Fanelli et al. 2022 for review). Evidence for these adverse effects of obesity and insulin resistance on the brains of children and adolescents are particularly troubling. Indeed, a meta-analysis concluded that there is a negative association between obesity and several domains of brain function including attention, executive functioning, motor skills, and visuo-spatial performance (Liang et al. 2014). Brain imaging studies have provided evidence for neuronal damage occurring in the hippocampus and cerebellum of young adults with obesity (Mueller et al. 2012). It is very concerning that the prevalence of childhood obesity and diabetes has increased dramatically during the past 40 years. Moreover, increasing evidence suggests that children born to parents with obesity or diabetes are at increased risk for autism (Rivell and Mattson 2019). Because IF can prevent and treat obesity, it would also be expected to ameliorate or reverse the adverse effects of obesity and diabetes on brain structure and function in the current and future generations.

In summary, overeating and sedentary lifestyles increase the risk of obesity, diabetes, cardiovascular disease, and AD. These adverse effects of a chronic positive energy balance result in part from a disengagement of adaptive cellular stress responses.

7.16 Implications for Lifestyles and Healthcare Systems

Not being able to give my patients clearly defined reasons for the general and local improvements resulting from a forenoon fast as a method in hygiene, it had to be spread from relieved persons to suffering friends; and according to the need, the sufferers from various ailments would be willing to try anything new where efforts through the family physician or patent medicines had completely failed; it was spread as if by contagion, among the failures of the medical profession. (E. Dewey 1900)

One hundred and twenty-three years ago, Dewey did not know why IF was so beneficial for his patients. Unfortunately, research aimed at understanding the mechanisms of IF's beneficial effects on health and disease treatment was not pursued for more than 80 years since Dewey published his book. Fortunately, as can be appreciated from the information in the chapters of this book, there has since been an exponential increase in IF research in animals and humans, particularly during the past decade.

Despite the now compelling case for the health benefits of IF (de Cabo and Mattson 2019; Mattson 2022), it is unclear whether IF will be incorporated into public health policy and medical practice, particularly in countries with profit-driven healthcare systems and little emphasis on disease prevention. The United States is exemplary of the disconnect between money spent on health care and health outcomes. This disconnect is the result of a capitalist system with major barriers to IF firmly entrenched by the power of large corporations including processed food, monoculture agricultural, pharmaceutical, health care, and technology industries (Fig. 7.4). To put it bluntly, the entire system is geared for letting and even encouraging people to develop a disease and then prescribing drugs and surgeries to manage their disease. Unfortunately, although the general public is mostly aware of this situation, the only incentive for people in positions to make a difference (leaders of the industries contributing to the health crisis) would seem to be a guilty conscience. Government officials are persuaded or even bribed by corporate leaders and their lobbyists so that they make policies that favor the corporate elite. Media outlets make huge profits from advertisements that promulgate unhealthy lifestyles. These include ads for foods and drinks with high amounts of simple sugars, saturated fat-laden meals, and drugs that are touted as answers to the many diseases caused by unhealthy lifestyles.

What can be done to increase the number of people who exercise, eat healthy diets, and adopt an IF eating pattern? Possibilities include taxes on demonstrably unhealthy foods and drinks; abolishing the lobbying of Congress and physicians by representatives of pharmaceutical and processed food industries; increasing government support for preventative health care; government-funded advertisements that highlight the health consequences of “couch potato” lifestyles (heart attack, stroke, cancers, diabetes, Alzheimer's disease); emphasis on preventative medicine in the training of health-care professionals; incentives for companies to encourage healthy lifestyles; and changes in schools so that children have only healthy foods and drinks and exercise daily. As described elsewhere (Mattson 2020b, 2022), physicians can prescribe actionable and effective IF prescriptions, and outpatient and inpatient lifestyle medicine

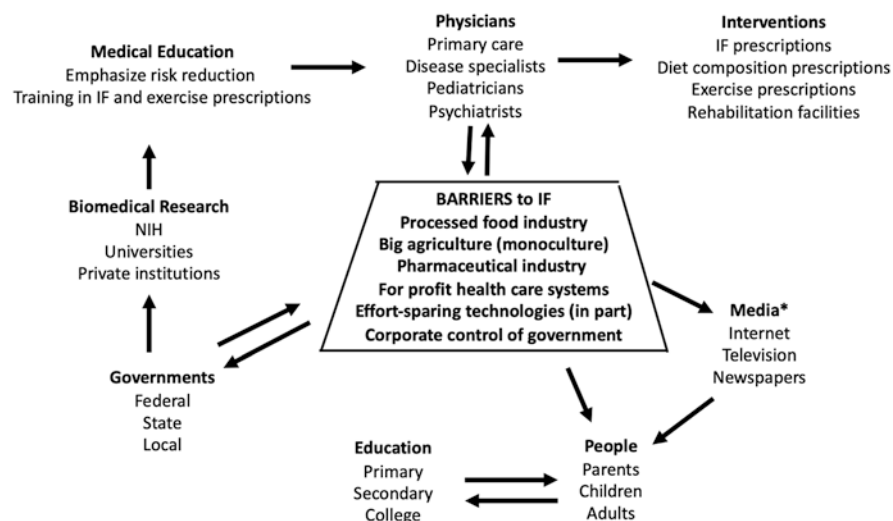


Fig. 7.4 Barriers to and inroads for implementation of IF into lifestyles and health care. Barriers include profit-driven industries that either disregard the adverse effects of their products on their customers (processed food companies and monoculture agriculture), profit only when people become ill (pharmaceutical companies; for profit health care), or sell products that have unintended health consequences (effort-sparing technologies such as automobiles and elevators). Solutions include emphasis on healthy lifestyles and disease prevention in primary and secondary schools and reinforcement by parents; media coverage of health benefits of IF and related practical information; government bulletins and information dissemination; increased support for basic and clinical research on IF; and expanding and emphasizing preventative medicine in medical education. *These can be either a hindrance or solution to the poor health epidemic

centers (covered by insurance) could be established. An important approach to improving brain health for the present and future generations would be to include brain science in curricula for children's primary and secondary education.

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Part V

Periodic Fasting

Chapter 8

Periodic Fasting: Evolutionary Perspectives Explaining the Clinical Benefits



Hanno Pijl

Abstract This chapter delineates the role of nutrition in hominin evolution, in particular with respect to the evolutionary pressure exerted by nutrient deprivation. Our history is characterized by at least three landmark developments. Two million years ago, environmental pressure-driven omnivorous dietary intake allowed for significant growth of our brain. Some 10,000 years back, our corresponding cognitive abilities facilitated the advent of agriculture, which triggered the rapid evolution of social structures and technology. Just recently, the industrial revolution enabled the production of virtually unlimited amounts of food. As a corollary, for the first time in human history, nutrients are available in abundance for almost everyone (in affluent societies). However, critical components of our biostructure have specifically evolved to survive the frequent cycles of food shortage that were an inviolable element of human existence throughout our evolutionary history. Nutrient deprivation drives investment of available resources in cellular maintenance and repair, as well as in plasticity of neural networks involved in cognitive capabilities (required for successful foraging). The absence of episodes of food scarcity in our current society compromises cellular sustenance and neuroplasticity to increase the likelihood of noncommunicable disease and accelerate mortality rates and the aging process.

8.1 Introduction

Today, the majority of the people on this planet eat every day. And not only that, most people consume at least three meals as well as snacks containing lots of calories in between. In fact, many dietitians and doctors advise to eat two “healthy” snacks to facilitate appetite control, and mainstream media often advocate eating six or even more times a day. Moreover, most contemporary (industrial) food products

H. Pijl (✉)

Department of Internal Medicine, Leiden University Medical Center,
Leiden, The Netherlands

e-mail: h.pijl@lumc.nl

are replete with calories. In “Western” countries, this dietary pattern has been the norm for the last 100 years or so (Bailey et al. 2022). But it is not clear how “normal” this is for humans. Relatively, recent history indicates that a couple of thousand years ago in advanced agrarian civilizations like the Roman empire, people usually ate one meal a day (if available), breakfast was introduced in medieval times to “break the night’s fast,” and dinner became mainstream only after the advent of electricity, which facilitated eating after dark (Affinita et al. 2013). Indeed, the industrial revolution has been a prerequisite to provide us with the means to produce enough food for everyone to eat what we eat today. Although it is exceedingly difficult to precisely determine dietary intake in societies even older than those of the Roman empire, it seems likely that hunter-gatherers were faced with food scarcity on a regular basis. Key questions relate to how they survived, how the human physiology adapted to periodic lack of food, and how the body copes with the unprecedented continuous abundance of food. It is also important to address how adaptations to food shortage may help us prevent and overcome diseases.

This chapter will provide a bird’s eye view of the history of dietary intake in humans, focusing on the role of food scarcity in human evolution. It will address the (metabolic) adaptations to food shortage which have evolved to minimize the risk of failure to reproduce and their consequences for our health in a society that offers an excess of food.

8.2 Nutrition and Human Evolution

Our earliest hominin ancestors were African forest dwellers. Their physical environment determined the composition of their diet (as it does for us today): it primarily comprised fruits, leaves, and bark, supplemented by small amounts of insect and meat (Andrews and Martin 1991). In addition, seeds, nuts, roots, and tubers may have been consumed by some (later) hominin species (Luca et al. 2010). During the late Pliocene, approximately two million years ago (Mya), a profound climate shift drove significant ecosystem changes in Africa. Local environments became cooler, drier, and more seasonal, which devastated forests and triggered the emergence of savannas and deserts (Hernandez Fernandez and Vrba 2006). Our ancestors were basically forced out of their wooded habitat to move toward more arid grasslands or waterfronts. These migrations had a profound impact on their diet. They became true omnivores, acquiring significant amounts of food from animal resources, including red meat, organs and fish (Dominguez-Rodrigo et al. 2005; Ferraro et al. 2013).

This ecology-driven dietary shift turned out to be a key event in human evolution: it facilitated the growth of our brain, which tripled in size over the last couple of million years. Since the resting energy demands of neural tissue are extremely high (some 16 times that of muscle), the evolution of the large hominin brain came at a very high metabolic cost (Leonard et al. 2007). Moreover, approximately 60% of human brain tissue is made up of fat, of which at least half is ω -3

polyunsaturated. Although humans can biosynthesize these long-chain ω -3 polyunsaturated fatty acids (ω -3 LCP), their production capacity is too low to accommodate the needs of a fully grown human brain (Eaton et al. 1998). Thus, preformed ω -3 LCPs should be sourced from food to provide enough building blocks. Since animal foods are generally more energy dense and nutrient dense and contain way more preformed ω -3 LCPs than plant foods, the change of our ancestors' subsistence pattern, driven by climate shift some 2–2.5 Mya, was a prerequisite for their brain to grow. Indeed, the fossil record of the first significant surge of human brain growth dates back to around 2 Mya (Leonard et al. 2007). Moreover, the high quality of the new, easy-to-digest (animal) food allowed for the hominin gut to evolve into a smaller, much less energy-intensive organ, which probably helped offset the energy costs of the larger brain (Aiello and Wheeler 1995). Last but not least, a couple of hundred thousand years ago (the exact date is still heavily debated), humans learned how to control fire (Roebroeks and Villa 2011), and from that time onward, heating food probably soon became mainstream (Carmody and Wrangham 2009). Heating enhances dietary quality, as it renders food significantly more digestible and increases its net energy value. Indeed, cooking provided nutritional and energetic benefits beyond those conferred by the consumption of *red* meat and fish (Carmody and Wrangham 2009), thereby reinforcing the impact of animal foods on human evolution.

The advent of agriculture and livestock farming ushered in the next revolution in our nutritional and evolutionary history. The first domestication of plants and animals, approximately 12,000 years ago, was a watershed moment in human history, which profoundly affected the way our ancestors lived and how humans thrive today. Cultivating crops and raising animals for food yields more calories in less time with less effort than searching for edible fruits and hunting for deer. In fact, agriculture enabled a limited number of farmers to produce so much food that a substantial part of farming communities could take on roles as administrators, soldiers, scholars, and artists. Thereby, agriculture spurred advancements of technology (e.g., weapons) and social structures (e.g., “armies”), which is probably why virtually all hunter-gatherer societies were wiped off the planet in no (evolutionary) time (Diamond 1997, 2002). Yet, compelling anthropological evidence indicates that farmers were less healthy than their hunter-gatherer ancestors, probably because agricultural societies were dependent upon one to three crops as nutrient sources as opposed to the highly diverse diet of hunter-gatherers (Armelagos et al. 1991; Latham 2013).

The cultural and technological revolution, driven by the advent of agriculture, ultimately culminated in the industrial revolution, which started off in eighteenth-century Great Britain (Brittanica, The editors of encyclopedia 2023). The process of change from handicraft to mechanical labor fundamentally transformed our society. Over the course of several centuries, the mechanization of agriculture, storage of produce, and transportation made unprecedented amounts of food available to almost everyone (in societies that could take full advantage of the developments) (FAO 2022). The human race never had the luxury of continuous access to unlimited

food sources in its *entire* evolutionary history. In contrast, human physiology has been molded by food scarcity. Moreover, the changes in eating behavior that were enabled by the industrial revolution unfolded much faster than our ability to adapt to novel environmental conditions could handle.

8.3 Food Shortage and Famine in Human History

Although it seems very likely that food insecurity has been an inviolable element of human existence for millions of years, it is impossible to precisely and reliably determine how often and to what extent prehistoric populations were faced with food shortage. Therefore, science relies on observations made in contemporary pre-industrial hunter-gatherer and agricultural societies prior to their disappearance. In general, it seems clear that famine occurred on a regular basis in both hunter-gatherer and agricultural communities (e.g., Benyshek and Watson 2006; Berbesque et al. 2014; Dirks 1993). Interestingly, although the (scientific) literature is replete with (plausible) suggestions that hunter-gatherer subsistence is more susceptible to food shortage than farming, a recent paper denies this notion and indicates that famine occurs even more often in (contemporary) preindustrial horticulturalists than in hunter-gatherers (Berbesque et al. 2014). What were the frequencies and lengths of the famines for the different groups before the last century? Indeed, the frequency and severity of famines fell only over the last century, particularly in industrialized countries (O'Grada 2010), illustrating the extremely brief history of food security in human evolution. As food shortage is an obvious threat to survival and (reproductive) health, and only the fittest tend to survive environmental hazards, food shortage must have molded human physiology. What is it, then, that makes humans survive episodes of food shortage and maintain reproductive capacity over time? And how do these adaptive biological features affect our health in current times of affluence?

8.4 Biology of Food Shortage and Overabundance in Evolutionary Perspective

Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely, the production of the higher animals, directly follows.

Charles Darwin, in: *On The Origin of Species* (last paragraph), 1859.

Charles Darwin was the first to recognize the power of food shortage to affect the evolution of species. Reproductive success is the ultimate goal of any species' bio-structure. Natural selection favors individuals who can outperform their competitors in challenging conditions, particularly with respect to the production of offspring in their lifetime. Balanced resource allocation is a central tenet of physiology. An

organism has to divide available resources over various metabolic tasks, such as maintenance, repair, growth, and reproduction (Kirkwood and Rose 1991). In conditions of food scarcity, growth and reproduction should not be priorities for various reasons (e.g., requirements of energy and building blocks for mother, fetus, and (new born) child). Instead, immediate survival of an episode of food shortage is vital for reproductive success in better conditions. Thus, reduced reproductive capacity, economical use of available fuel, resistance against cellular damage, as well as accurate memory and spatial navigation required for foraging success in complex environments are key features of organismal fitness in periods of food scarcity (Mattson 2019). Therefore, repeated episodes of food deprivation likely drove the evolution of at least four physiological domains that are vital for survival when quality food is available in insufficient quantities: (1) reproductive endocrinology; (2) energy metabolism; (3) stress resistance; and (4) neurophysiology of cognition.

A diverse array of cellular energy and nutrient sensors detects any decline of available fuel and/or tissue building blocks that may occur in times of food scarcity and adapts cell metabolism to increase the likelihood of survival (Mihaylova et al. 2023). In particular, energy expenditure slows down, a process called autophagy serves to recycle and repurpose cell debris for fuel and building blocks, antioxidant and other cell-protective molecular pathways are activated to enhance stress resistance, and protein synthesis and cell division are reduced (Mihaylova et al. 2023; Saxton and Sabatini 2017).

In addition, a coordinated hormonal response to dietary restriction orchestrates whole body physiology to divert resources from growth and reproduction to invest in maintenance and repair. Indeed, calorie restriction effectively suppresses reproductive hormone release to severely compromise fertility and sexual behavior, particularly when body fat is lost (Schneider 2004; Iwasa et al. 2022). In addition, several circulating growth factors are downregulated in response to amino acid and glucose deficits to curtail cellular proliferation and body growth (Green et al. 2022; Longo and Anderson 2022). In concert, these various adaptations to food shortage (1) put reproduction on hold and (2) reduce cellular damage and thereby can extend health span and life span if active on a regular basis (Mihaylova et al. 2023; Saxton and Sabatini 2017; Green et al. 2022; Longo and Anderson 2022). Notably, the cellular and endocrine mechanisms involved in these adaptive processes are evolutionarily conserved across a wide variety of species (Longo and Anderson 2022), testifying to the ubiquitous health threat that food scarcity has been throughout the entire history of life on our planet.

Conversely, in times of affluence, growth and reproduction can and should be prioritized over maintenance and repair. Thus, when nutrients are available in sufficient amounts, all of the above adaptive processes basically go into reverse. This has important consequences for health. Our current eating habits (i.e., multiple meals and intermittent snacks of continuously available sources per day) can promote (the accumulation of) cellular damage, increase the risk of many present day noncommunicable diseases, and accelerate the aging process. In fact, impaired autophagy precludes essential clearance of damaged cell structures and leads to

accumulation of cell debris, which evokes an inflammatory response that plays a crucial role in the pathogenesis of metabolic disease and aging (Kitada and Koya 2021). Moreover, continuously elevated circulating levels of growth factors potentially accelerate aging and promote inflammation, atherosclerosis, and cancer (Witsch et al. 2010; Zhang et al. 2021; Williams and Wu 2016). Clearly, our (evolutionarily driven) urge to eat as much and as often as we can is turning against us since we are now able to produce food in virtually unlimited quantities for the first time in our entire evolutionary history.

Foraging is one of the most primitive and basic behaviors of essentially all primates. As food is perhaps the single most important requirement for life itself, it should come as no surprise that foraging strategies are under strong evolutionary pressure for maximizing return of investment (Charnov 1976). Animals often forage in complex environments where food sources are sparsely distributed. Moreover, the availability of edibles in a particular area fluctuates with season as well as with intensity and success of previous foraging expeditions in that area. Accurate navigation toward patches harboring remaining sources, which thus requires memory for previous foraging trips in addition to spatial orientation and decision-making, is fundamental for success in food acquisition, particularly in nutrient-poor environments (Haydon et al. 2011).

Compelling evidence from experimental studies in rodents shows that dietary restriction enhances hippocampal neuroplasticity as well as learning and memory (Mattson 2019). Moreover, there is experimental evidence to suggest that neural circuits involved in spatial orientation and decision-making have evolved as an adaptation to food shortage in different rodent models and bird species and that similar circuits were probably essential for foraging success in nonhuman primates as well (Mattson 2019). Interestingly, neuroethological studies indicate that such circuitry is also involved in the guidance of a broad variety of social decisions, supporting the notion that sociality may have evolved in parallel with foraging strategies by co-opting neural hardware designed to optimize foraging success for the purpose of interaction with others (Chang 2013). Thus, periodic food shortage may well have played a significant role in the evolution of the human brain.

If food scarcity promotes neuroplasticity, memory, and learning, does the absence of this environmental challenge in our affluent society impair cognitive capacities? Intriguingly, domesticated animals have smaller brains than the wild species from which they originate. Virtually unlimited provision of resources is among the proposed explanations for this remarkable finding, but other features of domestication (e.g., selection for docility and absence of predators) may have been involved as well (Balcarcel et al. 2022; Kruska 1988). Notably, the brain of modern humans is about 5% smaller than that of our late Pleistocene ancestors (Stibel 2021). Can overabundance of available food sources somehow be involved? And does the recent decline of brain size, which is unique in human evolutionary history, affect our cognitive abilities? Obviously, there is no way to directly compare intelligence of Pleistocene hominins with our cognitive capacity today. However, overall brain size predicts cognitive ability across nonhuman primates (Deaner et al. 2007), and brain size explains a modest but robust proportion of variation in intelligence in humans

(Pietschnik et al. 2015; Lee et al. 2019). Moreover, experimental evidence in rodents suggests that (over)consumption of food reduces hippocampal volume, neurogenesis, and synaptic density as well as hippocampus-dependent spatial learning and memory (Stranahan et al. 2008). Analogously, obesity is associated with reduced hippocampal and temporal lobe gray matter volumes and impaired cognitive function in humans (Nguyen et al. 2014; Li et al. 2022; Climie et al. 2015). Conversely, (intermittent) energy restriction enhances neuroplasticity and cognition in laboratory rodents via metabolic signals produced in response to fasting (Mattson et al. 2018). Indeed, mice maintained on a time-restricted feeding schedule do not exhibit the age-related decline of hippocampus-dependent spatial learning and memory of ad libitum fed congeners (Means et al. 1993). Moreover, periodic use of a so called fasting mimicking diet (i.e. 4 consecutive days twice a month) improves a variety of key domains of cognitive function in middle-aged mice (Brandhorst et al. 2015). In concert, the currently available evidence suggests that continuous overconsumption of food without intermittent periods of dietary restriction is deleterious to brain health.

8.5 Conclusions

Cyclic bouts of food scarcity have played a major role in the evolution of our biostructure. In response to nutrient deprivation, cellular and systemic mechanisms prioritize investment of available resources in maintenance and repair to maximize the likelihood of survival and future reproduction. Moreover, neuroplasticity is enhanced to optimize cognitive abilities required for effective foraging strategies. In contrast, when food is available in abundance, our biosystem largely invests resources in growth and reproduction, thereby essentially neglecting cell maintenance. This should not be a problem as long as periods of food abundance are alternated by episodes of food scarcity. Obviously, these latter episodes never occur anymore in most modern societies, which puts cell sustenance at risk and increases the likelihood of noncommunicable disease and accelerates the aging process. Therefore, short-lasting (12–24 h), intermittent (every day or every few days), or long-lasting (4–7 days) periodic (1–2 times a month in mice and 3–12 times a years in humans) fasting may be an effective means to sustain and improve health in affluent societies.

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Chapter 9

Fasting-Mimicking Diets in Longevity and Disease



Frida S. Hovik and Valter D. Longo

Abstract Aging is the major risk factor for many diseases, placing juventology and gerontology research, respectively studying how organisms remain young/are rejuvenated or become older, at the center of disease prevention. Periodic fasting (PF) and fasting-mimicking diets (FMDs) lasting 4–7 days and in most cases applied once a month or less have gained attention as a potential therapeutic approach for promoting longevity and preventing and treating multiple age-related diseases. These periodic interventions represent a departure from the everyday changes required by well-studied anti-aging dietary interventions such as calorie restriction but are also clearly distinct from intermittent fasting methods such as alternate-day fasting or time-restricted eating. Here we discuss the potential effects of PF and FMDs on longevity and diseases including cancer, diabetes, cardiovascular disease, neurodegenerative disease, and autoimmunity, in both rodent models and humans.

F. S. Hovik, MS RDN
Department of Nutrition, University of Oslo, Oslo, Norway

Keck Medical Center of USC, University of Southern California, Los Angeles, CA, USA
e-mail: fhovik@usc.edu

V. D. Longo, PhD (✉)
University of Southern California, Los Angeles, CA, USA

Edna M. Jones Professor of Gerontology and Biological Sciences, Longevity Institute,
Leonard Davis School of Gerontology, University of Southern California,
Los Angeles, CA, USA

IFOM, AIRC Institute of Molecular Oncology, Milano, Italy
e-mail: vlongo@usc.edu

9.1 Introduction

9.1.1 *Fasting, Health span, and Longevity*

Fasting refers to the voluntarily abstinence from consuming food or drinks for a specific period. It has been practiced for centuries for various reasons, including religious, spiritual, and cultural purposes. In recent years, fasting has gained attention for its potential health benefits beyond its traditional uses which have not demonstrated health benefits. There are two main types of fasting: intermittent fasting (IF) and periodic fasting (PF). IF involves frequent alternating cycles of fasting and eating. The most common IF methods include the 16/8 method (fasting for 16 h and restricting eating to an 8-h window), alternate-day fasting (consuming regular calories every other day), and the 5:2 method (consuming a normal diet for 5 days and fasting or restricting calorie intake to 500–600 calories for 2 days a week). PF refers to longer periods of fasting, typically lasting 2 days or longer and in most cases 4 days or longer, separated from the next cycle by at least 1 week. PF is generally not applied more than once a month especially if lasting 4 days or longer (Longo and Mattson 2014). Although there are other types of prolonged long-term fasting practices such as therapeutic fasting, these are commonly lasting 1 week or longer, are conducted at specialized clinic in most cases once a year or less, and will not be discussed here. We will focus instead on the periodic use of fasting-mimicking diets (FMDs) as a method to delay aging, reduce biological age but also prevent and treat a wide range of diseases.

9.1.2 *Periodic Fasting (PF) and Fasting-Mimicking Diets (FMDs)*

PF can be carried out with a water-only method; however, this is not only associated with low compliance due to its extreme nature but can also cause potentially severe side effects at least in a portion of the population and especially if conducted outside of a specialized clinic. Side effects include malnourishment, rapid weight loss, hypoglycemia, and hypotension (Goldhamer et al. 2001, 2002). Due to these safety concerns and compliance challenges linked to water only fasting, the FMD was developed, initially for cancer clinical trials. It provides the benefits of fasting while minimizing the side effects and difficulties associated with water-only fasting (Brandhorst et al. 2015). FMDs can provide different levels of calories and display different compositions based on its intended use, which can range from targeting risk factors for aging and diseases to treating cancer, diabetes, Alzheimer's, autoimmune and cardiovascular disease. However, in general, FMDs are formulated to (a) achieve the effects of water-only fasting on markers including IGF-1, IGFBP1, glucose, and ketone bodies, (b) address any requirements specific for a disease or condition (e.g., increased calories in older patients with Alzheimer's), and (c) maximize

compliance. Most FMDs last 4–7 days and are standardized plant-based dietary regimens providing 30–50% of the normal calorie per day with ingredients low in sugars and protein but high in unsaturated plant-derived fats and micronutrients. In humans, each FMD cycle is generally followed by at least 1 month of the normal diet of the subject. The FMD cycles have been applied once every 3–4 weeks for patients with cancer; once a month for normal subjects, patients with diabetes, and patients with Alzheimer's disease; and once every 2 months in its 7-day version for patients with multiple sclerosis. Notably, studies in both animals and humans have shown that many of the improvements in different biomarkers caused by FMD cycles can last for months. Cellular rejuvenation, improved insulin sensitivity, reduced adiposity, reduced inflammatory and aging markers, and reduced disease risk factors are some of the well-documented beneficial effects of FMD cycles in mice, which are beginning to be confirmed in human clinical studies (Longo et al. 2021).

9.2 Fasting Response Mechanisms, Aging, and Age-related Diseases

9.2.1 Physiological Changes During Fasting and FMDs

During fasting or FMDs, several physiological changes occur progressively as the organism adjusts to the absence of food intake. When fasting begins, the body relies on stored carbohydrates in the form of glycogen for energy. Glycogen reserves in the liver and muscles are gradually depleted within the first 24–48 h of fasting. Once glycogen is depleted, the body switches to alternative energy sources including fatty acids and ketone bodies. Ketones are mostly produced by the liver from fatty acids, primarily from stored body fat. This metabolic state, known as ketosis, is characterized by increased levels of circulating ketones in the bloodstream. PF/FMDs normally lead to a decrease in blood glucose levels, reduced insulin, but also enhanced insulin sensitivity. Additionally, FMDs cause a decrease in IGF-1, as well as an increase in ketone bodies and IGFBP-1, which in both mice and humans appears to be generally comparable to the effects observed during water-only fasting (Longo and Panda 2016). For instance, and depending on the calorie content, 5 days of the relatively low-calorie FMD may be equivalent to 4 days of water-only fasting.

Fasting also triggers a process called autophagy, which represent a cellular effort to recycle cellular components. This process helps clear out cellular waste and supports cellular repair and rejuvenation. The length and type of fasting/FMD may cause the organism to either slow or possibly accelerate metabolic rate or at least energy expenditure, thus underlining the need to move away from generic terms like fasting to instead adopt specific terms like FMD5 referring to a 5-day-long specific composition and calorie content while also specifying the frequency (e.g., monthly FMD5). These metabolic adjustments caused by certain types of fasting/FMD

may on one side help the body adapt to the limited or lack of calorie intake to preserve energy but on the other side may burn fat and increase energy expenditure to generate heat as part of a thermogenic mechanism (Cohen and Kajimura 2021).

The specific mechanisms responsible for the effects of FMD cycles have just started to be understood; however, improved insulin sensitivity, reduction in adiposity, and reduced inflammation are among the changes observed consistently (Longo and Anderson 2022).

9.2.1.1 Normal Aging

Changes in metabolism, increased inflammation, decreased cellular function, and a higher risk of age-related diseases are hallmarks of the aging process. PF and FMDs were shown to delay risk factors and diseases associated with the aging process in both mouse models and humans.

– Mice

The FMD induces changes in markers associated with stress resistance and longevity (Brandhorst et al. 2015). Mice undergoing FMD experienced weight loss during each FMD cycle but regained most of the weight upon refeeding. Mice receiving FMD cycles twice a month between 16 and 22 months of age maintained a constant weight but eventually began to lose weight. Despite being calorically restricted during the diet, the FMD group compensated for this restriction by overeating during the ad libitum period, resulting in a cumulative monthly calorie intake equivalent to that of the control group. IGF-1, a growth factor associated with aging and life span, was reduced by 45% by the end of the FMD period but returned to normal levels after the normal diet was resumed. IGFBP-1, which inhibits IGF-1, increased significantly during the FMD but returned to levels similar to the ad libitum group within 1 week of refeeding (Brandhorst et al. 2015). Body composition analysis showed that the FMD group had a trend toward reduced total adipose tissue and significantly reduced visceral fat deposits compared to the control group. However, lean body mass remained similar between the two groups. In another mouse model, FMD cycles administered once a month, prevented the premature mortality but also the obesity, insulin resistance and cardiac dysfunction resulting from a high fat/calorie diet (Mishra et al. 2021).

– Humans

A pilot clinical trial was carried out to evaluate the feasibility and potential impact of FMD cycles in healthy adults. The FMD, adopted by participants for 5 days every month, provided between 34% and 54% of standard caloric intake and was characterized by the macronutrient composition described earlier. Participants were randomized into either the FMD group or the control group. Data from the 19 participants who successfully completed 3 FMD cycles were analyzed. The FMD group showed fasting blood glucose levels reduced by 11.3% at the end of each FMD cycle which remained 5.9% lower than baseline after the normal diet was

resumed. Serum ketone bodies increased significantly during the FMD but returned to baseline levels after refeeding. Circulating IGF-1 levels were reduced by approximately 24% by the end of the FMD period and remained 15% lower after resuming the normal diet. IGFBP-1, which inhibits IGF-1, increased 1.5-fold during the FMD and returned to baseline levels after refeeding. Adverse effects reported by participants following the FMD cycles were mild and generally decreased with subsequent cycles. Compliance to the FMD was high, with participants largely adhering to the provided FMD kits without consuming other foods. A larger randomized cross over clinical trial including 100 patients confirmed and expanded the beneficial effects observed in the pilot trial, showing that 3 FMD cycles can reduce IGF-1 and a range of other markers and risk factors for aging and age-related disease, particularly in subjects with high levels of these markers at baseline (Wei et al. 2017). Notably, the positive impacts of FMD cycles on disease markers and risk factors, such as IGF-1 and leptin, persisted for several weeks and potentially months after returning to a regular diet. While the precise mechanisms underlying the protective and rejuvenating effects of FMD cycles are still being explored, key mechanism may be related to the loss of stored fat and switch to a fat-burning mode avoiding entry into a low-energy expenditure mode (Leibel et al. 1995), but also the atrophy of organs and systems during the FMD, followed by their re-expansion upon mice and human subjects returning to the normal calorie, protein and carb diet. In mice, this atrophy followed by re-expansion has been associated with multisystem activation of stem cells, cellular reprogramming and regeneration (Longo and Anderson 2022). These findings indicate that the FMD is well-tolerated and effectively induces changes in markers associated with stress resistance and longevity, similar to those observed tolerated and effectively in animal studies. They also highlight the importance of PF and FMD in promoting normal healthy aging by positively impacting various physiological processes and reducing the risk of age-related diseases.

9.2.1.2 Pre-diabetes and Diabetes

FMDs improve insulin sensitivity and lead to reduced glucose levels to promote diabetes prevention or regression. In mice, Brandhorst et al. (2015) conducted a study and found that after undergoing the FMD and just before starting refeeding, their blood glucose levels were 40% lower compared to those the control diet group. However, within 7 days of refeeding, their glucose levels returned to normal. The FMD involved feeding the mice starting at 16 months of age for 4 days twice a month, while they were allowed to eat freely during the period between FMD cycles. Mice in the FMD group experienced weight loss, losing about 15% of their weight during each FMD cycle, but they tended to regain most of the lost weight upon refeeding. Interestingly, the FMD group mice maintained a stable weight whereas the mice on the control diet gained weight and reached their maximum weight at 21.5 months of age. In another study using a mouse model for obesity and type 2 diabetes, FMD cycles were able to reverse insulin resistance and hyperglycemia but also promoted the regeneration of functional beta cells in a

mouse model for type 1 diabetes (Cheng et al. 2017). Cycles of a 4-day FMD triggered a sequential expression of specific genes (Sox17 and Pdx-1), resembling the pattern observed during pancreatic development (Cheng et al. 2017). This is followed by the generation of insulin-producing β -cells driven by the gene Ngn3. FMD cycles lead to the restoration of insulin secretion and glucose regulation in mouse models of both type 2 and type 1 diabetes. Moreover, in human type 1 diabetes pancreatic islets, fasting conditions resulted in reduced activity of PKA and mTOR (cellular signaling pathways) and induced the expression of genes Sox2 and Ngn3, leading to insulin production. The effects of the FMD could be reversed by treatment with IGF-1 (insulin-like growth factor 1), and the process could be recapitulated by inhibiting PKA and mTOR. These findings indicate that the FMD has the potential to reprogram pancreatic cells, restoring insulin production in islets from patients with type 1 diabetes and reversing the type 1 diabetes phenotype in mouse models (Cheng et al. 2017). These studies also suggest that FMD cycles enhance insulin sensitivity and reduce the risk of developing type 2 diabetes in part by reducing visceral fat in the mice but also independently of fat loss.

In humans, a study investigating the effects of FMDs examined similar parameters including body weight, abdominal fat, and lean body mass. The FMD led to a 3% decrease in body weight ($3.1\% \pm 0.3\%$; $p < 0.001$), and this reduction was sustained even after the completion of the study ($p < 0.01$). The measurement of trunk fat percentage using dual-energy X-ray absorptiometry showed a trend toward reduction ($p = 0.1$) after three cycles of FMD and 1 week of normal dieting. Additionally, the study indicated that the relative lean body mass, adjusted for body weight, increased after completing three FMD cycles, suggesting that most of the weight loss was due to fat loss (Brandhorst et al. 2015). A study by Sulaj et al. (2022) explored the clinical impact of PF in patients with type 2 diabetes in a randomized controlled trial. It demonstrated that the FMD was safe and well-tolerated when accompanied by intensive diabetes care. No severe hypoglycemic or hypotensive episodes were reported. The study by Sulaj et al. (2022) also observed a significant reduction in albuminuria in the FMD group, comparable to the effects seen with only diabetes medications. The acylcarnitine profile and specific amino acids were found to discriminate between responders and nonresponders to FMD in terms of albuminuria improvement. Notably, the majority of subjects receiving FMD cycles were able to reduce the use of diabetes drugs. Thus, the integration of FMD with intensive diabetes care could be a complementary approach to current guidelines (Sulaj et al. 2022).

9.2.1.3 Cardiovascular Disease (CVD)

FMDs have shown potential benefits also in the prevention and treatment of cardiovascular disease (CVD), including improvements in cholesterol levels and blood pressure. Managing these risk factors is crucial for reducing the risk of heart disease. In mice, FMD decreased inflammatory diseases. In humans, the level of

C-reactive protein (CRP) in the serum serves as a marker of inflammation and is considered a risk factor for CVD. At baseline, the average CRP level for the subjects on the FMD was 1.45 ± 0.4 mg/l, which was similar to that in the control group (1.29 ± 0.5 mg/l), indicating a moderate risk for cardiovascular disease. Three FMD cycles led to a reduction in CRP levels. In the pilot study, among the 19 participants on the FMD, 8 of them had CRP levels in the moderate to high cardiovascular disease risk range (above 1.0 and 3 mg/l, respectively) at the beginning of the study. After completing three FMD cycles, seven of these participants saw their CRP levels return to the normal range (below 1.0 mg/l). For the 11 participants who had CRP levels below 1.0 mg/l at the start of the study, no significant changes were observed upon completion of the trial. These findings indicate that periodic FMD cycles can have anti-inflammatory effects and reduce at least one risk factor associated with CVD (Brandhorst et al. 2015). In the randomized crossover study involving 100 patients, 71 of them underwent three monthly 5-day cycles of FMD. The results showed a decrease in body weight, trunk fat, and total body fat, as well as a reduction in blood pressure and IGF-1 levels (Wei et al. 2017). Additionally, a post hoc analysis of the data revealed that participants with elevated levels of certain risk factors at the beginning of the study experienced improvements. These improvements included reduced levels of fasting glucose, triglycerides, total and low-density lipoprotein cholesterol, and C-reactive protein. Overall, these studies highlight the potential benefits of PF and FMDs in reducing the levels of disease risk factors, both in healthy individuals and those with pathologies suggesting that they can be applied as a therapeutic approach in the prevention and treatment of certain diseases (Wei et al. 2017).

9.2.1.4 Neurodegeneration

FMD cycles have been shown to increase neural stem cells and increase cognitive performance in normal old mice (Brandhorst et al. 2015). A study investigated whether the cognitive improvements observed in the FMD group were associated with neural regeneration. To assess this, the researchers measured the proliferative index of immature neurons in the dentate gyrus of the hippocampal formation. The results showed that the FMD group had increased proliferation of immature neurons compared to the control group. Additionally, when 6-month-old mice (in which cellular proliferation in the dentate gyrus is typically reduced by over 50% compared to 8-week-old mice) were fed a single cycle of the FMD, it resulted in a reduction in circulating and hippocampal IGF-1 levels. However, it also led to an increase in IGF-1 receptor mRNA expression in the dentate gyrus region of the hippocampus. Furthermore, FMD mice displayed a significant reduction in PKA activity and an increase in the expression of NeuroD1, a transcription factor important for neuronal protection and differentiation. In another experiment, a single cycle of the FMD in

CD-1 mice resulted in an increase in various types of neural stem cells, immature neurons, and the area covered by dendrites, further supporting the idea that the FMD promotes neurogenesis in adult mice. Overall, the study's findings indicate that FMD promotes some neurogenesis in adult mice, which represents a potential mechanism underlying the cognitive improvements observed in the FMD group (Brandhorst et al. 2015). Neurodegenerative diseases are characterized by a gradual deterioration of neurons and cognitive function. Studies conducted on animal models have demonstrated that alternate-day fasting can protect neurons and alleviate cognitive decline (Mattson 2012). In a mouse model of Alzheimer's disease (AD) that expresses familial AD mutations in the β -amyloid precursor protein (APP), presenilin 1, and Tau, mice that were subjected to either a 40% calorie restriction (CR) or alternate-day fasting (ADF) regimen for 1 year, starting at the age of 5 months, exhibited reduced cognitive impairment compared to mice that were allowed to eat freely. Remarkably, only the 40% CR approach resulted in reduced levels of β -amyloid (A β) and Tau accumulation in the brains of the AD mice (Halagappa et al. 2007). Parrella, E. et al. demonstrated that restricting essential amino acids intermittently also offers protection against pathology and cognitive decline in mice with triple transgenic mutations for Alzheimer's disease (AD) (Parrella et al. 2013). This indicates that the protein-restriction aspect of fasting plays a significant role in its neuroprotective effects. The process through which fasting protects neurons from degeneration has been associated with an enhanced expression of neurotrophic factors, such as BDNF and FGF2, which play a crucial role in promoting neuronal cell growth and resilience against stress (Arumugam et al. 2010; Cheng et al. 2012). These factors are vital for the development, survival, and protection of neurons. By elevating the levels of neurotrophic factors, FMDs could potentially exert a neuroprotective influence and provide support for neurons affected by these disorders. FMD cycles also have potential as therapy for Alzheimer's disease (AD). In a study by Rangan et al. (2022), FMD cycles were effective in reducing cognitive decline and AD pathology in E4FAD and 3xTg AD mouse models, surpassing the effects of protein restriction cycles. Specifically, in 3xTg mice, which express multiple mutated genes known to accelerate AD development in humans, long-term FMD cycles led to a decrease in hippocampal amyloid-beta (Ab) accumulation and hyperphosphorylated tau, an increase in neural stem cell formation, a reduction in microglia number, and downregulation of neuroinflammatory genes, including the superoxide-generating NADPH oxidase (Nox2) (Rangan et al. 2022). Additional experiments conducted in 3xTg mice lacking Nox2 or treated with the NADPH oxidase inhibitor apocynin and also showed improved cognition and reduced microglia activation compared to control mice. Moreover, clinical data indicated that FMD cycles are feasible and generally safe in a small group of patients with AD patients (Rangan et al. 2022). These findings suggest that FMD cycles can delay cognitive decline in AD mouse models, potentially by reducing neuroinflammation and/or superoxide production in the brain.

9.2.1.5 Autoimmunities and immunosenescence

Aging is linked to progressive immunosenescence, which is partly due to the age-related decline/changes in the function of hematopoietic stem cells (HSCs). This leads to an imbalance in the ratio of myeloid to lymphoid cells, accompanied by a reduction in common lymphoid progenitors and decreased production of T and B cells. Consequently, there is a decline in the immune system's regenerative capacity and an increase in immunosuppression or immunosenescence. Dysfunctional lymphocytes resulting from this process can also contribute to the development of autoimmune disorders such as asthma, systemic lupus erythematosus, multiple sclerosis (MS), and rheumatoid arthritis, although autoimmunities occur at all ages and are not necessarily linked to aging. FMD cycles induce alterations in the immune system of 20.5-month-old mice, making their immune profile more comparable to that of younger mice (4 months old) (Cheng et al. 2014). This aligns with the impact of periodic fasting on the rejuvenation of immune cells through hematopoietic stem cell (HSC)-dependent regeneration (Cheng et al. 2014). In the context of treating established symptoms and pathology of autoimmunity, FMD followed by refeeding cycles has been found to have beneficial effects in a mouse model for multiple sclerosis (MS) (Choi et al. 2017). These cycles were shown to lessen the severity of experimental autoimmune encephalomyelitis (EAE), a model for MS, by influencing immune cells and promoting the regeneration of oligodendrocyte precursor cells. The FMD cycle led to increased apoptosis in autoreactive T cells, and during the refeeding period, with some of T cells replaced by newly generated naive T cells (Choi et al. 2017). Furthermore, in a clinical trial involving MS patients, FMD intervention resulted in a reduction in lymphocytes and an improvement in their quality of life (Choi et al. 2017). These findings suggest that FMD followed by refeeding cycles holds promise as a potential therapeutic approach for alleviating the symptoms and pathology autoimmune diseases. Multiple studies have demonstrated that fasting and CR can lead to the mortality and redistribution of diverse populations of immune cells (Collins et al. 2019; Jordan et al. 2019; Nagai et al. 2019). Correspondingly, a clinical trial involving individuals with MS reported a decrease in lymphocytes following a single 7-day-long PF/FMD intervention, which eventually returned to normal levels, raising the possibility that, as observed in mice, FMD cycles can promote the removal of damaged immune cells followed by stem cell-dependent regeneration (Choi et al. 2017). Rangan et al. (2022) conducted an experiment using a chronic dextran sodium sulfate (DSS)-induced murine model for inflammatory bowel disease (IBD) to test the effects of 4-day FMD cycles, on symptoms and pathology. FMD cycles reduced intestinal inflammation, increased the number of stem cells, promoted beneficial gut microorganisms, and reversed the intestinal pathology caused by DSS. In contrast, water-only fasting increased regenerative markers and decreased inflammatory markers but did not reverse the pathology. Further experiments involved transplanting *Lactobacillus* or fecal microbiota from mice treated with DSS and FMD, which reduced DSS-induced colon shortening, reduced inflammation, and increased colonic stem cells. In summary, these animal studies together with the anti-inflammatory effects observed in a clinical

trial by Wei et al. (2017) in which FMD cycles reduced CRP, indicate that this intervention has the potential to ameliorate autoimmunity-related inflammation in humans (Rangan et al. 2022).

9.2.1.6 Cancer

Studies have indicated that FMDs can lead to improvements in various biomarkers and metabolic pathways linked to cancer development. FMDs have been found to lower insulin-like growth factor 1 (IGF-1) levels, which is a hormone associated with tumor growth and survival. Additionally, FMDs can promote autophagy, a process that helps in clearing damaged cellular components and potentially inhibits cancer initiation and progression. PF and FMD cycles induce system-wide changes that hinder the survival and adaptation of malignant cells, including reductions in IGF-1, insulin, glucose, leptin, and cytokines, as well as alterations in numerous enzymes and pathways (Wei et al. 2016, 2017). Not surprisingly these effects on cancer prevention can also apply to cancer treatment. Combining fasting or FMD with conventional cancer treatments such as chemotherapy, radiotherapy, kinase inhibitors, metabolic drugs, or hormone therapy enhances their effectiveness against cancer cells. These findings highlight that fasting-based interventions may hold promise as complementary strategies to enhance the efficacy of standard cancer therapies (Longo et al. 2021). A series of investigations conducted on both animal and human subjects has indicated that prolonged periods of fasting can be as effective as chemotherapy in slowing down the progression of various cancers. These effects lie in its ability to shield healthy cells from the harmful effects of chemotherapy while simultaneously enhancing the vulnerability of cancer cells to the treatment, which were termed differential stress resistance (DSR) and differential stress sensitization (DSS), respectively (Safdie et al. 2009; Raffaghello et al. 2008; Lee et al. 2012; Buono and Longo 2018). Cycles of PF and FMD can enhance the elimination of cancer cells by inducing the range of systemic/blood changes in a number of factors that hinder the survival and adaptability of malignant cells but that direct normal cells to enter a protected mode. These changes include a decrease in IGF-1, insulin, glucose, leptin, and a range of cytokines. Additionally, numerous enzymes and pathways may undergo modifications during fasting (Wei et al. 2016, 2017). In particular, the effectiveness of PF and FMD in combating cancer cells is maximized when combined with standard of care treatments such as chemotherapy, radiotherapy, kinase inhibitors, metabolic drugs, or hormone therapy (Elgendy et al. 2019; Di Biase et al. 2016; Brandhorst and Longo 2016). Another crucial mechanism by which PF and FMD cycles work is by activating the immune system's ability to recognize and eliminate cancer cells through T cell-mediated mechanisms (Di Biase et al. 2016; Pietrocola et al. 2016). A study investigating the mechanisms linking fasting to the immune-dependent attack of cancer cells in mice confirmed that severe caloric restriction leads to the accumulation of memory T cells in the bone marrow, resulting in enhanced protection against infections and tumors (Collins et al. 2019). Several studies have examined the potential of FMD in cancer

clinical trials. One notable randomized study involving 125 stage II/III patients indicates that FMD enhances the effectiveness of chemotherapy in achieving positive clinical and pathological responses among women with breast cancer. It is worth mentioning that even with most patients completing only two cycles of the dietary intervention, significant clinical and pathological benefits were observed, with a dose response showing that patients completing more cycles of chemotherapy with FMD were much less likely to be resistant to the therapy (de Groot et al. 2020). Furthermore, a feasibility study involving 36 patients explored the combination of FMD with hormone therapy for breast cancer treatment. The study revealed that this approach was safe and resulted in the reduction of markers and risk factors linked to cancer progression. Notably, muscle function and mass were unaffected by the intervention (Caffa et al. 2020). Although research indicates benefits of the FMD, it is important to note that this dietary approach should not be seen as a standard treatment for cancer. It should be viewed as an integrative approach to be considered by oncologists particularly in patients with advanced stage patients for whom standard therapies are not likely to be effective. In summary, PF/FMDs can delay cancer growth but also result in cancer-free survival in mouse models, particularly when combined with the most effective treatments for a particular cancer. The initial human trials are demonstrating the safety and potential efficacy of FMD cycles.

9.2.2 FMD Composition and the Regulation of the Fasting Response

FMD is a dietary regimen designed to mimic the effects of fasting while still providing calories as well as essential nutrients. The FMD tested in clinical trials for normal patients provides approximately 1100 kcal on day 1 and 750–800 kcal on days 2–5, which contributes to only part of the fasting response. The low glucose but relatively high carbohydrate content of the FMD (approximately 40–45% of calories from carbs) was selected in order to allow entry into a ketogenic mode while avoiding very high levels of ketone bodies. Although there is no evidence that alternating a normal diet with fasting periods which cause a strong increase in ketone bodies and of other fasting response factors causes detrimental effects, it is possible that many cycles promoting these major changes could have negative side effects. The other reason for the FMD to be low calorie but relatively high carbohydrate is the putative effect of low protein and low carbohydrate on muscle mass and function. In fact, part of the response to water-only fasting involves the breakdown of muscle and the use of amino acids for gluconeogenesis. Both mouse and clinical studies suggest that this relatively high-carb and low-protein FMD composition protects from lean body mass loss (Wei et al. 2017; Brandhorst et al. 2015).

Notably, FMD is not only low in protein (9–10% of calories) (Brandhorst et al. 2015), but all proteins are from plant-based sources, many of which provide low levels of methionine and other essential amino acids, which play key roles in the

activation of growth and aging pathways. FMD is also high in fat with approximately 50% of calories derived from plant-based fats. Because fatty acids and the ketone bodies derived from them are the major source of energy during fasting periods, these high levels of fats do not interfere with the fasting response, allowing the changes in IGF-1, ketone bodies, etc. Finally, FMDs are supplemented with vitamins and minerals as well as plant-based (algal oil) omega-3 fatty acids to avoid malnourishment, especially in patients who are already malnourished at baseline.

Therefore, the plant-based, high fat, relatively high-carbohydrate, low-protein and severely calorie-restricted but high nourishment composition of the FMD allows it to promote a fasting response while reducing hunger, increasing compliance, and minimizing side effects including hypotension, hypoglycemia, and malnourishment. Because it is provided in a box, with standardized ingredients, it is being tested in many clinical trials for its effects on aging, cancer, and many other age-related diseases.

9.3 Molecular Pathways Involved in PF/FMD-Induced Longevity

Although the precise molecular pathways involved in FMD-induced longevity are not fully understood, several mechanisms have been proposed based on studies in yeast, worms, fruit flies, rodents, and humans. Here we discuss some of the key molecular pathways that contribute to FMD-induced effects on aging, disease, and regeneration. PF and FMD induce a stress response in cells, characterized by resistance to oxidative and other stresses, but also low cell division.

FMD reduces the levels of both insulin and insulin-like growth factor 1 (IGF-1), which regulate key nutrient-sensing pathway involved in growth and metabolism. Reduced signaling in genetic pathways downstream of insulin or IGF-1 has been associated with increased life span and improved health in various organisms, including yeast, worms, flies, and rodents (Longo and Anderson 2022). By lowering glucose as well as insulin and IGF-1 signaling, FMD may promote entry into a low growth and reproduction maintenance mode. The PF/FMD-dependent lowering of insulin and IGF-1 signaling is probably responsible for its effect on reducing TOR-S6K signaling, which is now considered, together with growth hormone-IGF-1 axis, one of the most validated pro-aging pathways. In fact, deletion or inhibition of TOR-S6K increases longevity and health span in yeast, worms, flies, and mice (Longo and Anderson 2022).

PF/FMD has also been shown to regulate other pathways linked to TOR-S6K downregulation: it activates AMP-activated protein kinase (AMPK) (Longo et al. 2021), a master regulator of cellular energy metabolism, and downregulates PKA activity, implicated in glucose signaling and aging in yeast and mammals (Longo

and Anderson 2022). Activation of AMPK has been linked to increased life span in several organisms (Salminen et al. 2016) and is thought to be involved in the beneficial effects of FMD on longevity.

PF/FMD also consistently decreases the levels of leptin, an adipocyte-derived hormone that regulates hunger, in both mice and humans (Mishra et al. 2021; Caffa et al. 2020). Because leptin resistance in obese mice and humans causes constitutively high leptin levels, the reduction of leptin by FMD cycles is likely to be associated with a reduction/reversal of the resistance to this hormone.

Thus, PF/FMD cycles have clear and consistent effects in reducing glucose, insulin, IGF-1, and leptin levels, which, in turn, contribute to the reduction of TOR-S6K and PKA signaling and the activation of AMPK. The mechanisms responsible for the effect of these pathways on aging continue to be investigated but probably involve increased protection against oxidative damage (SODs, catalase, etc.), increased macromolecular and organelle repair (DNA repair enzymes, autophagy), as well as increased stem cell activation and cellular reprogramming leading to rejuvenation and/or delayed aging (Figs. 9.1 and 9.2).

9.4 Safety and Practical Considerations for Fasting-Mimicking Diets

Patient safety and well-being should be a primary concern when adopting any type of nutrition and fasting regimen. It is crucial to consider patient selection when implementing FMDs and follow guidelines to ensure safety and maximize the potential benefits. Patients interested in trying an FMD should consult with a health-care provider familiar with fasting/FMDs, particularly if they have any underlying health conditions or take medications. It is important to conduct a comprehensive evaluation of the patient's overall health status, including the assessment of medical history, current medications, and any existing health conditions. FMDs may not be appropriate for individuals with certain medical conditions, such as eating disorders and pregnancy. After completing an FMD, it is essential to guide patients on how to transition back to a regular diet gradually. Rapidly returning to a normal calorie intake may cause digestive discomfort or other adverse effects.

Fasting can lead to dehydration, so it is crucial to combine fasting with hydration. PF using water only or similar methods should be done under medical supervision and preferably in a specialized clinic to ensure safety. Fasting/FMD should not be combined with insulin or insulin-like drugs as this may cause severe hypoglycemia. Fasting/FMD can have different effects on different individuals, and thus it will be important to personalize fasting/FMD interventions, particularly when deciding the frequency and if the FMD is appropriate or not to help treat a particular condition to achieve a specific goal.

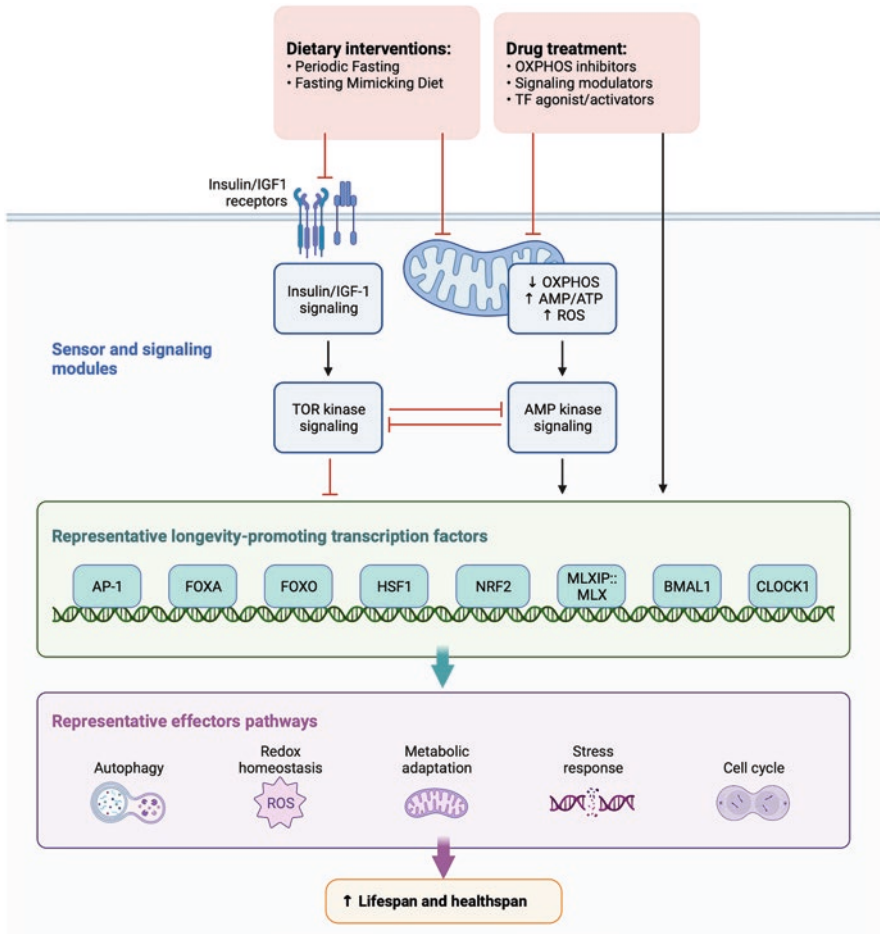


Fig. 9.1 PF and FMD's impact on life span and health span. The response of healthy cells to periodic fasting (PF) or fasting-mimicking diet (FMD) is evolutionarily preserved, offering cellular protection and, at least in model organisms, an extension of both life span and health span (Raffaghello et al. 2008; Brandhorst et al. 2015; Cheng et al. 2014; Fabrizio et al. 2001, 2003; Lee et al. 2010; Levine et al. 2014; Wei et al. 2008). The IGF1 signaling pathway plays a crucial role in mediating the cellular effects of PF or FMD. In normal nutritional conditions, elevated protein intake and increased amino acid levels lead to higher IGF1 levels, activating AKT and mTOR, which in turn enhance protein synthesis. Conversely, PF or FMD lead to a decrease in IGF1 levels and downstream signaling. This reduction diminishes AKT-induced inhibition of mammalian FOXO transcription factors, enabling these factors to activate genes that trigger the production of enzymes like heme oxygenase 1 (HO1), superoxide dismutase (SOD), and catalase, which possess antioxidant properties and protective effects (van der Horst and Burgering 2007; Cheng et al. 2009; Converso et al. 2006). Elevated glucose levels stimulate the protein kinase A (PKA) signaling pathway, negatively affecting the master energy regulator AMP-activated protein kinase (AMPK), consequently preventing the expression of the stress resistance transcription factor early growth response protein 1 (EGR1) (Berasi et al. 2006). PF or FMD and the subsequent limitation of glucose intake inhibit PKA activity enhance AMPK activity and activate EGR1, thereby yielding cellular protective outcomes, including within the heart muscle (Brandhorst et al. 2015; Cheng

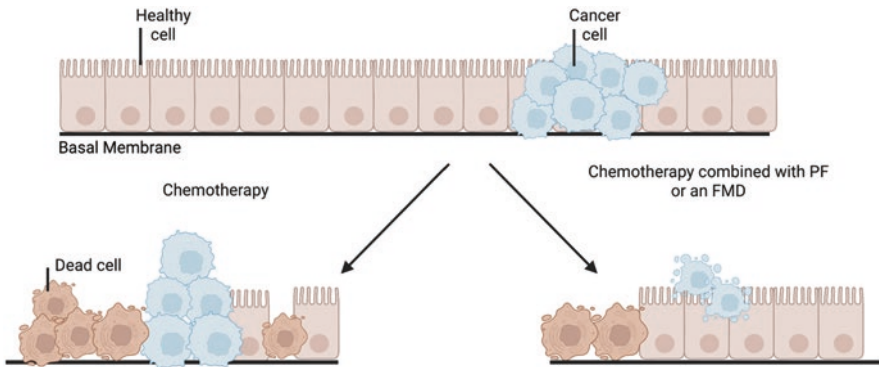


Fig. 9.2 Differential stress resistance and differential stress sensitization in normal and cancer cells. Chemotherapy affects both cancerous and healthy cells, leading to the shrinking of tumors but often resulting in severe or potentially life-threatening side effects due to damage to various types of tissues. Based on existing laboratory data, periodic fasting (PF), or following a fasting-mimicking diet (FMD) could offer a potential strategy to affect the impact of chemotherapy and potentially new cancer medications on regular cells versus cancer cells. Due to the presence of mutations that constantly activate growth-promoting signaling pathways, cancer cells are unable to adapt effectively to conditions of starvation. Consequently, many types of cancer cells, as opposed to normal cells, undergo metabolic changes that make them more susceptible to harmful agents like chemotherapy (termed differential stress sensitization). On the other hand, PF or FMD triggers a molecular response that has been evolutionarily conserved. This response enhances the resistance of normal cells, but not cancer cells, to stressors, which includes chemotherapy and immunotherapy (termed differential stress resistance). The anticipated application of these distinct effects of PF or FMDs on normal versus cancer cells could involve a reduction in the adverse effects of cancer treatments and an improvement in toxicity against the tumor cells, leading to increased progression-free and overall survival

Fig. 9.1 (continued) et al. 2014). PF and FMDs also stimulate regenerative responses through molecular mechanisms, some of which are implicated in cancer. These mechanisms encompass increased autophagy and possibly the induction of sirtuin activity (Brandhorst et al. 2015; Chalkiadaki and Guarente 2015). In cancer cells, PF and FMD can induce an anti-Warburg effect by decreasing the intake of glucose through glucose transporters (GLUTs) and aerobic glycolysis. This compels some cancer cell types to enhance oxidative phosphorylation (OxPhos), leading to a rise in the generation of reactive oxygen species (ROS) within the cell. This "anti-warburg" effect promotes oxidative DNA damage, activation of p53, DNA damage, and ultimately cancer cell death, especially when combined with chemotherapy

9.5 Conclusion and Future Directions

FMDs have shown promise in promoting weight loss, reducing body fat, and reversing insulin resistance while preserving lean body mass. FMDs have also not been associated with reduced energy expenditure, probably because they provide a significant level of calories but also because they only last 4–5 days, in most cases. The initial studies on diabetes indicate that FMD cycles have the potential to reverse diabetes and reduce or even eliminate drug use without imposing a change in the everyday diet of the patient. FMDs are also showing positive effects on CVD risk factors including abdominal fat, CRP, blood pressure and cholesterol, raising the possibility that they could be employed in the prevention or treatment of CVD, particularly in those with diseases in earlier stages. Thus, more research is necessary to determine the specific effects of FMDs cycles on cardiovascular disease prevention and management. FMD cycles also show great potential to make a range of cancer therapies more effective but also less toxic. Finally, FMD cycles have been effective in delaying/reversing autoimmune disease progression in mice, and the initial clinical trials are promising but far from being able to indicate whether they could be effective.

FMDs require individuals to make significant changes to their eating habits and patterns for 5 days a month or less. However, adopting this plant-based dietary program may encourage people to become more mindful of their food choices, portion sizes, and overall dietary habits. These behavioral changes could have broader implications for public health by promoting healthier eating patterns and lifestyle modifications involving higher consumption of healthy and pro-longevity plant-based food.

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Chapter 10

Effectiveness of Prolonged Fasting in Treating Human Chronic Diseases: Clinical Evidence and Empirical Insights from a Specialized University Medical Center



Daniela A. Koppold, Andreas Michalsen, and Etienne Hanslian

Abstract This chapter delves into the historical and medical dimensions of prolonged fasting, tracing its evolution from cultural and religious practices to its contemporary medical applications. It underscores methods and evidence linked to prolonged fasting, particularly within medical contexts, highlighting its potential advantages for prevention and treatment of diverse diseases. Medical professionals play a pivotal role in guiding and overseeing fasting, considering its multifaceted physiological, biochemical, and psychological effects.

Prolonged fasting, spanning at least 4 days, encompasses varied approaches. Traditional European techniques, such as the Buchinger and FX Mayr methods, involve fluid-only or modified fasting, each with distinct caloric intakes and initiation/refeeding protocols, driving metabolic adaptations and potential health-related gains.

Prolonged fasting demonstrates potential in numerous health related facets. It aids in preventing noncommunicable diseases by enhancing insulin sensitivity, curbing inflammation, and bolstering cellular repair. Evidence indicates its poten-

D. A. Koppold

Institute of Social Medicine, Epidemiology and Health Economics, Charité –
Universitätsmedizin Berlin, Berlin, Germany

Department of Internal Medicine and Nature-Based Therapies, Immanuel Hospital Berlin,
Berlin, Germany

Division of Oncology and Hematology, Department of Pediatrics, Charité –
Universitätsmedizin Berlin, Berlin, Germany
e-mail: daniela.koppold@charite.de

A. Michalsen (✉) · E. Hanslian

Institute of Social Medicine, Epidemiology and Health Economics, Charité –
Universitätsmedizin Berlin, Berlin, Germany

Department of Internal Medicine and Nature-Based Therapies, Immanuel Hospital Berlin,
Berlin, Germany

e-mail: andreas.michalsen@immanuelalbertinen.de

tial efficacy in metabolic ailments like type 2 diabetes, ameliorating blood sugar control and corresponding markers. Additionally, fasting presents benefits for essential hypertension by reducing blood pressure and fostering cardiovascular health. Autoimmune diseases could benefit from the anti-inflammatory properties of prolonged fasting, possibly influencing gut microbiota. Conditions like rheumatoid arthritis exhibit improved symptoms and joint function with fasting, especially when followed by plant-based diets. Fasting could improve mental health through mood enhancement and anxiety reduction. In cancer treatment, short-term fasting complementing chemotherapy could potentially mitigate side effects. Fertility implications are being investigated, as fasting induces relevant hormonal adaptations. Despite promising existing data, rigorous research and larger studies are essential for confirming the efficacy of prolonged fasting in diverse health conditions.

Prolonged fasting, though generally safe, entails absolute and relative contraindications necessitating medical attention, including tailored medication adjustments due to altered pharmacokinetics. Minor adverse effects are common, while severe imbalances and dehydration may arise if contraindications are overlooked.

10.1 Introduction

Fasting over longer periods of time has been used in religious, cultural but also traditional medical contexts for thousands of years. Over the last two centuries, medical fasting has gained specific interest in research. In the USA, physicians such as Edward Dewey and Henry Tanner started to use prolonged fasting for a broad array of indications. In Central Europe, physicians like Otto Buchinger, Franz Xaver Mayr, and Johann Schroth shaped distinct forms of prolonged fasting, and subsequently their fasting methods and fasting in general became very popular in countries such as Germany, Austria, Switzerland, and many Eastern European countries.

This chapter provides an outline of the most prominent prolonged fasting methods, reviewing the respective published and empirical evidence. Special emphasis will be given on its use in the medical setting (therapeutic or medical fasting) (Consensus on fasting terminology, to be published).

As a term, prolonged or long-term fasting defines a severe caloric restriction that lasts longer than 4 days (Consensus on fasting terminology, to be published). In some publications the term periodic fasting has also been used to refer to such fasting regimens.

In the academic teaching hospital the group authoring this chapter works (Immanuel Hospital Berlin, teaching hospital of the Charité Universitätsmedizin Berlin), prolonged fasting has been used to treat a variety of diseases for more than 20 years. Hereby, chronic cardiometabolic and inflammatory diseases rank first. Over the years, more than 20,000 inpatients have been treated with a fasting therapy, showing feasibility and safety of the intervention on a large scale. Adding to this, numerous outpatients have applied prolonged fasting as part of their treatment, or

for research purposes, showing feasibility and compliance also in outpatient settings. In the German-speaking European countries, there is a network of physicians, dieticians, and other professionals that accompany fasting as a preventive measure for healthy individuals and increasingly also for therapeutic purposes. When fasting is used as a preventive tool, professionals certified as “fasting guides” can lead and coach groups of fasters, giving them guidance and support throughout the process (Wilhelmi de Toledo et al. 2013). When patients taking regular medication want to fast, consultation of, or accompaniment by, a specifically trained physician is necessary, as medications might need to be adapted, and possible advantages have to be weighed against risks and contraindications.

Throughout the last decades, clinicians involved in medical fasting have given emphasis to the physiological and biochemical mechanisms involved. In line with modern scientific progress, the increasing knowledge about the physiological and molecular pathways of fasting has become part of the education and training of “fasting guides” and specialized physicians.

To better understand the complexity of fasting as an intervention, it might be helpful, among other perspectives, to understand it as a trigger for a hormetic stress response. This makes it an appropriate impulse for metabolic flexibility and organismal adaptability, a basic condition for health and longevity. Various fasting mimicking drugs have been developed over the years, but prolonged fasting itself still appears to remain unique regarding the complexity of its effects, as it impacts multiple biochemical pathways, physiological and microbiotal changes, as well as psychological and behavioral adaptations at a time.

10.2 Methods of Prolonged Fasting

10.2.1 *Prolonged Fasting in Practice*

Prolonged fasting, also called long-term fasting, is defined as a period of fasting that lasts at least 4 days (Consensus on fasting terminology, to be published). The maximum duration of a prolonged fast under medical supervision that has been published by now is up to 41 days (Wilhelmi de Toledo et al. 2019). Methodology can vary between complete fasting (zero caloric intake) and modified fasting (up to a maximum of 25% of daily nutritional energy need) (Consensus on fasting terminology, to be published). Modified fasting regimens have a long-standing tradition in Germany, where different forms coexist since decades. Most famous among these are the Buchinger fasting method, a fluid-only fasting regimen, and the FX Mayr method, where a period of zero fasting is followed by modified fasting with low caloric beverages, soups, and small amounts of fiber-rich solid foods, while an emphasis is set on mindful eating and extensive chewing of the food. For a systematic overview of these fasting methods, see Fig. 10.1.

The method of fasting that is followed in a majority of German fasting clinics belongs to the fluid-only fasting regimens and is similar to the Buchinger fasting

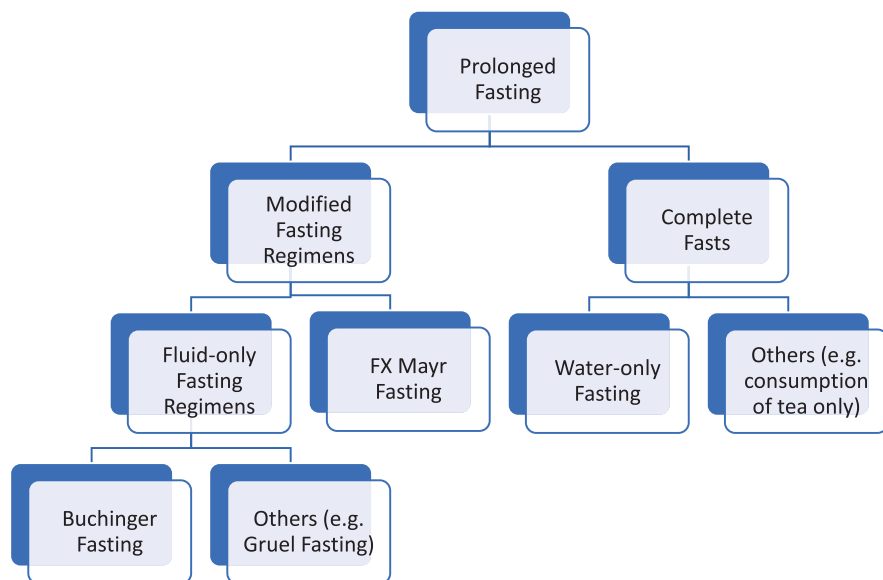


Fig. 10.1 An overview of different prolonged fasting regimens

method. It is modified according to patient needs and can contain vegetable broths, vegetable juices and water boiled flaxseed, oat or rice gruel. Depending on patient preferences, constitution, and therapeutic goal, caloric intake can vary between 150 and 500 kcal daily. In clinical settings with more complex morbidities, fasting regimens higher in calories containing also solid food components may be used and calorie intake may be increased up to 650–700 kcal, approaching the concept of the fasting-mimicking diet. A general timeline is depicted in Figure 10.2

Within the Buchinger method, a prolonged fast is usually introduced by one to three preparatory days. During these, a light plant-based diet of approx. 800–1200 kcal is consumed. In many traditional fasting forms, stimulating substances like coffee, green and black tea, and any caloric and specifically alcoholic beverages are avoided. Moreover, smoking is to be reduced, so that it can be paused during the fasting period.

Fasting itself traditionally begins with the ingestion of an oral laxative, usually Natrium sulfuricum (“Glauber’s salt,” 20–40 g diluted in 500–750 ml of water). There is no clear evidence on the necessity of bowel cleansing during prolonged fasting periods. However, empirically many patients prefer to include bowel cleansing, reporting that feelings of hunger and headaches are reduced, and overall well-being is increased. At the Immanuel Hospital Berlin, bowel cleansing measures are performed optionally upon patient preference.

In our hospital, the highly reduced caloric intake is realized by small amounts of vegetable juice, vegetable broth, and in case of preference, oat gruel or porridge, amounting to a daily total of 250–400 kcal. During fasting, it is recommended to

drink 2–3 l of fluids. They should be free of calories and ultra-processed components, so mainly mineral water, naturally infused/flavored water, or herbal teas are offered. Empirically used supportive measures include warm damp liver packs at noon and moderate exercise. Optionally, an enema or, if this is not wished by the patient, a mild laxative is applied every other day, to support bowel function and general well-being. Other nature-based therapeutic measures used during fasting can include warm- and/or cold-water applications, phytotherapeutics, and elements of mind-body medicine.

The fast is broken with a light meal that is eaten slowly. Traditionally this can be an apple, or a light vegetable soup. During the following days, solid foods are slowly reintroduced into the diet, starting with easily digestible natural foods with little protein and fat content. The refeeding period should last at least for a third of the fasting period, and a focus on mindful eating should be maintained. Protein-rich ingredients, such as legumes or cheese, should be introduced after this refeeding period.

The traditional prolonged fast of FX Mayr uses a slightly different approach. Fasting starts with a few days of water-only fasting, the duration of this period depending on patients' metabolic state and BMI at baseline. Thereafter, daily caloric intake is between 300 and 600 kcal with meals consisting of vegetable broths, gruel and porridge, and small amounts of easy digestible vegetables, yogurt, sour dough bread, and whole meal crackers. Solid food is specifically used to train mindful eating through extensive chewing. To enhance the effects of fasting on the bowels, manual therapies in the abdominal region are applied concomitantly. In the traditions of FX Mayr fasting, a milder laxative (Epsom salt) is applied daily in a low dosage supplemented by enemas only in the case of a history of severe constipation.

Water-only fasting as practiced in the past by Dewey and Tanner and today in a well-known US clinic does not include any bowel cleansing procedures, while allowing only for the intake of distilled water during fasting (Finnell et al. 2018).

In the past years, some hospitals in China started using prolonged fasting in the treatment of the metabolic syndrome and have adapted the fasting method to their traditions. In addition to the Buchinger fasting protocol, they utilize special herbal decoctions of traditional Chinese medicine (TCM) to support patients during the fasting period (Liu et al. 2023) (Fig. 10.2).

The length of the preparatory days can vary according to medical necessities and patient preferences. The reintroduction of solid foods should be scheduled for a third of the length of fasting itself.



Fig. 10.2 Timeline for a prolonged fasting intervention

10.2.2 Physiological Considerations in Prolonged Fasting

Fasting triggers a complex physiological and neuroendocrine adaptation process. During fasting, the body switches from external food sources to endogenous nutrient reserves, using endogenic macro- and micronutrients to supply cells with energy and support their regeneration. In the absence of external food, the body first draws on glycogen storages and then on fats and amino acids as sources of energy. When the duration of fasting is extended, the metabolism adapts further and changes the substrates used for energy production. Thus, after several hours without food, a drop in blood glucose levels leads to the activation of glycogenolysis in the liver (Berg et al. 2013). Once liver and muscle glycogen stores are depleted after about 16–24 h of fasting, energy is obtained primarily from fat storages, e.g., from liver and visceral fat. Ketone bodies, produced by the breakdown of fatty acids, are used by muscles and brain as a compensatory fuel. Ketone bodies compete with uric acid for a common tubular secretory protein, so that uric acid levels may rise during fasting. A small amount of protein is catabolized during fasting days, mainly to provide amino acids for gluconeogenesis, as the brain, erythrocytes, and adrenal medulla need a minimum of glucose supply for their function (Wilhelmi de Toledo 2018; Mathias 2018).

The metabolic switch to a fasting state that is described above is initiated by the release of cortisol and catecholamines (Berg et al. 2013). After about 1–3 days, this initial phase of sympathetic activation is followed by a stepwise increase of parasympathetic activity (Wilhelmi de Toledo 2018). This contributes to a further decrease in blood pressure and heart rate and probably also to the generally calming experience reported by patients in the course of a prolonged fast.

During the last decades, repeatedly a theoretical concern about detrimental effects of prolonged fasting due to protein catabolism has been acknowledged. However, Owen and Cahill, in their seminal works on fasting and starvation, demonstrated that through the intake of a small amount of calories (50–100 kcal/day), protein catabolism can be significantly reduced. Thus, the daily caloric intake of 200–500 calories, as practiced in the European tradition of modified fasting, can largely counteract protein breakdown (Owen et al. 1998). A study in overweight and obese subjects performing prolonged fasting according to the Buchinger method found significant weight loss, mainly due to the loss of fat mass, with only minimal protein loss. Of note, protein degradation during fasting includes insalubrious proteins such as miscarried proteins or autoantibodies (Steiniger et al. 2009). Thus, the loss of muscle protein is less than the loss of lean mass. Furthermore, there is evidence that daily moderate exercise during fasting may also counteract protein breakdown and result in improved fitness and muscle strength.

In summary, the body goes through different phases during fasting: from mobilization of glycogen stores in the liver and muscles to lipolysis of fat reserves. If carried to an extreme, prolonged abstinence from food can of course lead to a starvation response of the organism. Such prolongation of food abnegation or hunger obviously leaves the field of clinical fasting and can lead to muscle wasting and even death, when fat reserves are depleted. This of course is to be strictly avoided during medical fasting (Michalsen and Li 2013).

A helpful concept for comprehending the benefits of fasting in their totality is hormesis. Hormesis describes an adaptive response of the body to moderate challenges, enabling it to respond better to severe ones. Research has shown that biological systems have developed adequate responses to both acute and chronic threats (Calabrese 2016). Fasting, as a moderate stressor, can thus trigger protective responses and improve resilience, promoting optimal health through improving metabolic function, cellular repair mechanisms, and overall resilience (Mattson 2008). These effects may directly and indirectly improve cardiovascular health and brain function and might be relevant for achieving healthy longevity, as has been shown in different animal species (Calabrese and Mattson 2017).

10.2.2.1 Fasting and the Microbiome

The microbiome is a complex ecosystem of microorganisms that live in symbiosis with us on all surfaces communicating with the environment and thus also on the intestinal mucosa. It plays a crucial role in maintaining human health. Fasting has been shown to have an impact on the gut microbiome by promoting the growth of potentially beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and reducing the abundance of potentially harmful bacteria such as *Clostridium* species, thereby leading to an alleviation of dysbiosis (Peltonen et al. 1994; Remely et al. 2015). Additionally, fasting has been shown to increase the production of short-chain fatty acids in the microbiota, which are essential for maintaining gut health and regulating immune function. All these changes have been linked to reduced inflammation, improved insulin sensitivity, and protection against chronic diseases such as colorectal cancer as well as improved gut health and immune function (Mesnage et al. 2019; Tremaroli and Bäckhed 2012; Zheng et al. 2020). The relationship between diet and fasting, gut bacteria, and disease activity requires further study.

10.3 Indications, Clinical Experiences, and Existing Evidence on Prolonged Fasting

10.3.1 Prolonged Fasting in Prevention

Noncommunicable diseases (NCDs) are responsible for over 70% of all deaths worldwide (Bennett et al. 2018). There are several reasons why prolonged fasting may help prevent these diseases. Besides weight loss, fasting has been shown to improve insulin sensitivity, lower blood pressure and lipoprotein levels, and reduce inflammation and oxidative stress (Wilhelmi de Toledo et al. 2019; Michalsen and Li 2013; Goldhamer et al. 2002; Grundler et al. 2021; Wallentin and Sköldstam 1980) – all of which are risk factors for a variety of NCDs. Furthermore, fasting enhances cellular repair systems and stem cell production (Michalsen et al. 2005).

Abundant experimental and increasing clinical research suggests that restricting calories, reducing meal frequency, and implementing prolonged periods of fasting can each delay the onset of age-associated chronic diseases, improve metabolic health, and optimize energy utilization. The physiological processes behind these benefits involve periodic shifts in metabolic fuel sources, the promotion of repair mechanisms, and optimization of energy use for cellular and organismal health. Fasting also seems to enhance self-efficacy and may function as a catalyst for an overall healthier lifestyle (Michalsen et al. 2005). Future research should focus on combining a balanced diet, including controlled meal size and frequency, with periods of fasting to create effective strategies for preventing and treating the chronic diseases associated with aging (Di Francesco et al. 2018).

To date, there are no long-term interventional studies investigating the effects of fasting on disease onset and the course of chronic diseases. As a matter of fact, such studies are very difficult to conduct in practice. However, several uncontrolled and controlled human studies have described beneficial cardiometabolic and anti-inflammatory effects of fasting in the short- and mid-term. One observational study investigated >1400 inpatients practicing prolonged fasting between 5 and 21 days. Fasting led to consistent beneficial effects with significant reductions in weight, abdominal circumference, blood pressure, and blood lipids as well as improved glucose regulation. Participants also reported increased physical and emotional well-being. Surprisingly for many, hunger is not rated as a relevant problem in prolonged fasting by patients, most likely due to the hunger reducing effect of ketosis. These findings point to an overall promising potential of prolonged fasting for cardiometabolic risk reduction (Wilhelmi de Toledo et al. 2019). In addition, a small randomized controlled trial on patients with metabolic syndrome found that fasting improves relevant metabolic parameters, including BMI, waist circumference, glucose, insulin, adipokines, and triglycerides, within just a week (Cramer et al. 2022). Of course, the question of the sustainability of these effects is relevant. Here, an early observational study in inpatients undergoing prolonged fasting according to the Buchinger method for 7 days found that in the 3- and 6-month follow-up, fasters adhered better than controls to a healthy plant-based diet (Michalsen et al. 2005).

10.3.2 Clinical Applications of Prolonged Fasting

The following sections will elaborate on the current state of evidence regarding indications for the therapeutic use of prolonged fasting (Table 10.1).

10.3.2.1 Prolonged Fasting in Metabolic Diseases

Fasting may also engender benefits for metabolic diseases such as hyperlipoproteinemia, arterial hypertension, and type-2 diabetes, and it could reduce the risk of developing coronary artery disease if used as a supportive therapeutic measure. Moreover, when fasting is combined with a transition to a health-promoting

Table 10.1 Indications for prolonged fasting (current state of evidence)

Metabolic and cardiovascular diseases	Pain disorders	Diseases involving the immune system	Others
Prediabetes/type 2 diabetes mellitus Essential arterial hypertension Overweight/Obesity	Fibromyalgia Osteoarthritis	Rheumatoid arthritis Psoriatic arthritis and other forms of spondylarthritis ^a Multiple sclerosis Bronchial asthma ^a Allergies ^a	Support for certain chemotherapeutic regimens in oncological diseases Longevity ^b

^a Large empirical body of evidence but no published human trials

^b Experimental evidence, no human trials

lifestyle, including regular exercise and a balanced diet, the effects may naturally last longer (Wilhelmi de Toledo et al. 2019; Michalsen and Li 2013; Grundler et al. 2021; Göhler et al. 2000; Li et al. 2013; Lithell et al. 1983; Scharf et al. 2022).

In a randomized controlled trial involving participants suffering from metabolic syndrome, fasting followed by a lifestyle modification was compared to lifestyle modification alone. The lifestyle modification included the DASH diet, exercise, and mindfulness. Fasting led to short-term improvements in mood, specifically lower depression and fatigue scores compared to lifestyle modification alone. Both groups experienced positive effects regarding quality of life and psychological parameters, such as self-efficacy and mindfulness, with most benefits persisting at the 24-week follow-up. These findings suggest that lifestyle interventions, including fasting, can have a positive impact on the well-being of individuals with metabolic syndrome. Regarding the cardiometabolic risk scores in this study, fasting led to better blood pressure reduction initially but was not consistently better than the control arm after 12 and 24 weeks. As fasting had a rapid initial effect, it may be specifically used to initiate effective lifestyle modification in patients with metabolic syndrome and cardiovascular risk (Cramer et al. 2022; Jeitler et al. 2022).

10.3.2.2 Type 2 Diabetes Mellitus

Fasting could potentially benefit patients with type 2 diabetes by improving their blood sugar control, as it improves insulin sensitivity, decreases IGF-1 and leptin levels, and increases adiponectin levels (Wilhelmi de Toledo et al. 2019; Lithell et al. 1983; Stange et al. 2013). Prolonged fasting may lead to a normalization of fasting blood glucose levels and a significant decrease in HbA1c, a marker for long-term glucose control (Wilhelmi de Toledo et al. 2019). Furthermore, fasting can be an effective way to rapidly reduce indices which can all contribute to the amelioration of insulin resistance and elevated blood sugar levels (Drinda et al. 2019; Li et al. 2017). Most often, antidiabetic medication can be reduced following prolonged fasting (Li et al. 2017).

For type 2 diabetes, regular periods of prolonged fasting followed by a whole-food plant-based diet may be a supportive treatment option in combination with other lifestyle changes and medication where necessary (Lithell et al. 1983). During prolonged fasting oral medications with metformin, gliflozins, and other medications should be paused, and insulin dosage must be adapted accordingly to avoid hypoglycemia. For type 2 diabetes, regular periods of prolonged fasting followed by a wholefood plant-based diet may be a supportive treatment option in combination with other lifestyle changes and medication where necessary (Li et al. 2013).

So far, no clear indication for fasting exists for type 1 diabetes mellitus. However, one small pilot study showed that, under close medical supervision, adapting insulin dosage and monitoring ketosis, fasting may be safe in patients with type 1 diabetes mellitus and may provide some metabolic benefits and improve well-being (Berger et al. 2021). This clinical study could not confirm improvements of insulin sensitivity and endogenous insulin production as described in a comprehensive experimental study before. In the latter study, *ex vivo* investigations conducted on human type 1 diabetic pancreatic islets demonstrated that fasting increased the expression of Sox2 and Ngn3 and stimulated insulin secretion. Furthermore, signaling pathways such as IGF-1, mTOR, and PKA were identified as potential contributors to cell reprogramming and pancreatic regeneration (Cheng et al. 2017). Further clinical studies are necessary to clarify if there is a role for prolonged fasting in type 1 diabetes.

10.3.2.3 Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is induced by malnutrition with excess intake of calories and obesity and significantly promotes the development of type 2 diabetes. The pathological accumulation of fat in the liver also causes an increase in chronic liver disease worldwide. Among other conditions, NAFLD can cause non-alcoholic steatohepatitis (NASH), increasing the risk of cirrhosis and subsequently also of hepatocellular carcinoma (HCC) (Huang et al. 2021).

The results of a prospective observational study in 2019 show that periodic fasting of 6 days or more reduces liver fat in diabetics and non-diabetics in a disease-preventive manner. It also lowers the fatty liver index (FLI), a risk parameter for non-alcoholic fatty liver disease, significantly, with diabetics benefiting even more.

The Fatty Liver Index (FLI) is a simple and effective method for the evaluation of a fatty liver. The FLI is calculated from the parameters age, body mass index, waist circumference, and the fasting blood levels of triglycerides (TG) and gamma-glutamyl transpeptidase (GGT). Longer fasting durations, greater weight loss, and a higher reduction in abdominal circumference led to a greater improvement of the FLI. In addition, men and individuals who had an elevation of FLI, the liver enzyme GOT and cholesterol levels at baseline benefited particularly from the Buchinger fasting therapy applied. A computational model showed that each additional day of fasting increased the chance of transforming a manifest fatty liver into a lower risk category by 40% (Drinda et al. 2019).

10.3.2.4 Essential Arterial Hypertension

Fasting may be an effective intervention for individuals with hypertension aiming at improving their overall health and reducing uncontrolled high blood pressure or even de-escalating medication. There is extensive evidence from observational and clinical studies showing that prolonged fasting significantly reduces blood pressure levels in patients with hypertension (Wilhelmi de Toledo et al. 2019; Finnell et al. 2018; Goldhamer et al. 2002; Li et al. 2013).

An observational study on 174 individuals suffering from essential hypertension and undergoing a medically monitored water-only fast demonstrated efficacy in reducing blood pressure, with notable improvements especially in severe cases. This method, promoting beneficial lifestyle changes, also led to the successful discontinuation of antihypertensive medication in some patients (Goldhamer et al. 2002).

The largest observational study to date on prolonged fasting corroborates the finding that fasting significantly lowers both systolic and diastolic blood pressure, with the most significant effects seen in non-medicated hypertensive individuals. Some individuals were able to reduce or stop their antihypertensive medication during the fasting period without an immediate rebound in blood pressure, suggesting that fasting may normalize blood pressure. The study also found that the magnitude of the blood-pressure reduction is larger with higher baseline values, while subjects with already very low blood pressure tend to show an increase of their blood pressure during fasting, thus confirming a hormetic type of response. Overall, the existing research findings point to fasting as a promising nonpharmacological adjunctive approach in the treatment of hypertension, although further investigations are needed to understand the long-term effects and the precise mechanism (Wilhelmi de Toledo et al. 2019; Grundler et al. 2020).

The mechanisms contributing to the blood pressure regulating effects of fasting are not completely understood yet. It seems not to be just a result of weight loss and the reduction of salt intake. Clinically, fasting leads to a pronounced initial (days 1–3) natriuresis and diuresis. This natriuresis is promoted by fasting-induced increases of blood levels of aldosterone, glucagon, and natriuretic peptides. Besides the diuretic effect, a decrease in catecholamines after an initial increase may contribute to blood pressure reduction (Göhler et al. 2000). An uncontrolled study involving 16 patients with metabolic syndrome observed that after prolonged fasting, catecholamines decreased both at rest and during exercise. Fasting also triggers other molecular and hormonal signaling pathways that contribute to blood pressure regulation by increasing natriuresis and diuresis (Wilhelmi de Toledo et al. 2019; Heyman et al. 2020; Spark et al. 1975).

Maintaining a healthier lifestyle after fasting, including a fitting dietary approach, can help achieve long-term blood pressure control (Michalsen and Li 2013; Grundler et al. 2020; Maifeld et al. 2021). Among the diets associated with improving blood pressure regulation are the dietary approach to stop hypertension (DASH) diet, the Mediterranean Diet, as well as a wholefood plant-based diet (Lee et al. 2020; Pettersen et al. 2012; Yokoyama et al. 2014).

10.3.3 Prolonged Fasting in Autoimmune Diseases

Autoimmune diseases are a broad group of disorders in which the immune system attacks autologous healthy cells and tissues. Fasting may have potential benefits for individuals with autoimmune diseases by reducing inflammation and oxidative stress and reorganizing immune function (Wilhelmi de Toledo et al. 2019). Moreover, fasting may also influence and beneficially affect the intestinal microbiota, which has recently been linked to the pathogenesis of autoimmune diseases (Peltonen et al. 1994; Remely et al. 2015; Belkaid and Hand 2014; Bischoff et al. 2014).

In this context it should be mentioned that extensive experience exists on the positive effects of prolonged fasting on allergies. This does not only hold true for allergies connected to certain food components but also for skin conditions and allergic asthma. Unfortunately, published data on these indications are lacking.

10.3.3.1 Rheumatoid Arthritis and Other Autoinflammatory Joint Diseases

Rheumatoid arthritis is a chronic autoimmune disease that primarily affects joints, causing pain, inflammation, and subsequent joint destruction. Fasting has long been shown to have potential benefits for individuals with rheumatoid arthritis by reducing inflammation, improving joint function, and providing prompt symptomatic relief even with lasting significant improvements for several months after the fast when followed by a plant-based diet (Hartmann et al. 2022; Kjeldsen-Kragh et al. 1991; Muller et al. 2001; Sköldstam et al. 1979).

A controlled trial demonstrated that fasting, followed by 3–5 months of a vegan, gluten-free diet without refined sugar and then a vegetarian diet, can significantly ameliorate symptoms of rheumatoid arthritis with sustained effects over the full study period of 13 months (Kjeldsen-Kragh et al. 1991). A follow-up of the study corroborated these findings, indicating that specific patient populations could maintain these positive effects for over 2 years (Kjeldsen-Kragh et al. 1994). A recent randomized controlled trial found that both fasting followed by a plant-based diet and an anti-inflammatory diet according to the recommendations of the German Nutrition Society (Deutsche Gesellschaft für Ernährung (DGE)) enhance functionality and decrease disease activity in rheumatoid arthritis patients. Fasting patients showed these improvements after a week of fasting, while the control group had the same effects only after 3 months (Michalsen et al. 2005).

In a small study, the effects of fasting, followed by a lactovegetarian diet, on rheumatoid arthritis were evaluated by assessing intestinal and non-intestinal permeability and clinical symptoms. Results suggested that fasting can alleviate symptoms and reduce both types of permeability, while these benefits were reversed when switching to a lactovegetarian diet (Sundqvist et al. 1982).

It should be mentioned here that although data from randomized clinical studies on other autoinflammatory joint diseases are lacking, there is a large empirical body of evidence that fasting also improves symptomatology in psoriatic arthritis and other forms of spondylarthritis.

To date, the mechanisms behind the anti-inflammatory effects of fasting are not yet fully understood, and it is still unclear whether fasting can halt joint deterioration or disease progression.

10.3.3.2 Multiple Sclerosis (MS)

MS is a chronic autoimmune disease that affects the central nervous system. In MS, the immune system attacks the myelin sheath that surrounds and protects nerve fibers, leading to symptoms such as fatigue, muscle weakness, and difficulties in coordination and balance.

A study conducted on an animal model of MS found that fasting resulted in a reduction of inflammation and oxidative stress markers in the brain, markedly reducing clinical symptomatology as well as overall autoimmunity and even stimulating remyelination of axons by promoting oligodendrocyte regeneration. An additional pilot study on patients with MS found that a prolonged fast of 7 days resulted in a reduction of symptoms and improvements in quality of life (Choi et al. 2016).

An exploratory sub-cohort analysis of a randomized controlled trial showed that prolonged fasting periods combined with a subsequent plant-based diet and time-restricted eating may be a potential method for improving quality of life and function in MS patients, which could complement treatment options for MS. Fasting might further alleviate mild depressive symptoms and improve cognitive performance. In addition, fasting patients reported improved symptoms of physical fatigue (Bahr 2022). However, it is important that fasting interventions be conducted under the supervision of qualified professionals.

10.3.4 Prolonged Fasting in Chronic Pain Syndromes of the Locomotor System

Fasting appears to be a potential supportive therapy for managing chronic pain, including osteoarthritis and possibly fibromyalgia. Pathogenesis and symptomatology of osteoarthritis seem to be partly mediated by low-grade inflammatory processes (Thijssen et al. 2015). As described above, fasting has shown anti-inflammatory properties in animal and human studies (Di Francesco et al. 2018; Hartmann et al. 2022; Kjeldsen-Kragh et al. 1995; Oudmaijer et al. 2022). It is probable that cellular stress-response mechanisms such as autophagy, mitophagy and sirtuin activation as well as systemic organismal hormonal and metabolic responses to nutrient deprivation mediate these effects (Di Francesco et al. 2018; Hofer et al. 2022). Fasting also

contributes to pain reduction at least for the joints of the lower extremities due to weight loss (Di Francesco et al. 2018), lessening mechanical load (Hofer et al. 2022). But even in patients with normal BMI, metabolic factors seem to be associated with disease severity (Andersson et al. 2022). Apart from the abovementioned systemic low-grade inflammation associated with higher visceral fat, it seems that cholesterol metabolism (Papathanasiou et al. 2021; Song et al. 2021) and adipokines (Hofer et al. 2022; Andersson et al. 2022) play a pivotal role themselves regarding disease progression, as they can activate cartilage-degrading mechanisms. Here, the positive influence of fasting on lipid metabolism could play a supportive role (Gabriel et al. 2022). As has already been discussed, fasting has also been shown to possess antioxidative capacities (Di Francesco et al. 2018). The fact that different healthy diets have shown improvements of symptoms in osteoarthritis patients has been attributed to their antioxidative capacities, among others (Wei and Dai 2022). Furthermore, oxidative processes have, *in vitro* and *in vivo*, been found to modulate pathogenesis and disease severity in osteoarthritis (Song et al. 2021; Ertürk et al. 2017). Whether enhancement of serotonin pathways in the central nervous system (Michalsen 2010) and the switch in the microbiota (Maifeld et al. 2021) that have been described in prolonged fasting play an additive role in symptom reduction in osteoarthritis is not yet clear.

A small controlled clinical trial showed that fasting led to pain reduction accompanied by neuroendocrine responses which may regulate stress levels in patients with chronic pain (Michalsen et al. 2003a). In addition, medically supervised fasting for 7–20 days may produce significant mood improvement in addition to pain relief, which is particularly beneficial for chronic pain patients, as they often also suffer from depression and anxiety (Michalsen 2010). A pilot study showed that in the short term, a multidisciplinary integrative medicine (IM) approach involving fasting therapy yielded significant improvements on the Fibromyalgia Impact Questionnaire score as well as pain, depression, and well-being, compared to a conventional rheumatological approach. However, these improvements were not statistically significant after 12 weeks, except for anxiety, indicating that while a multimodal IM treatment with fasting therapy may be superior initially, improving its longer-term effects requires further investigation (Michalsen et al. 2013). A small uncontrolled study of fasting in patients with osteoarthritis of the knee or hip joint found that fasting significantly improved pain, health status, and joint function while also leading to significant weight loss and reductions in BMI and waist circumference (Schmidt et al. 2010). A recent observational study confirmed a significant reduction in pain and improvement in joint function in knee and hip osteoarthritis that lasted up to 1 year after the intervention. In addition, patients reported an improvement in quality of life (Koppold et al. 2023).

Usually, therapeutic fasting approaches entail educational elements on healthy eating to maintain the positive effects. Whole-food plant-based diets have been shown to have systemic anti-inflammatory effects (Wei and Dai 2022). Osteoarthritic symptoms can also be reduced with a Mediterranean diet, which is known to have similar anti-inflammatory properties (Morales-Ivorra et al. 2018).

In conclusion, fasting has the potential to improve pain, functionality, and overall well-being in patients with chronic pain, especially if followed by a healthy diet, but

further research and bigger randomized controlled studies are needed to clarify the exact benefits and mechanisms of fasting in this context.

10.3.5 Prolonged Fasting and Mental Health

Mental well-being is an essential aspect of overall health, and fasting may have potential benefits for supporting mental health by improving mood, reducing anxiety and stress, promoting emotional stability, and possibly easing mild depression (Li et al. 2013; Berthelot et al. 2021; Fond et al. 2013; Longo and Mattson 2014). During fasting the levels of brain-derived neurotrophic factor (BDNF) increase, a protein that promotes the growth and survival of neurons in the brain. Low levels of BDNF have been associated with depression and other mood disorders (Lin and Huang 2020). Fasting has also been shown to increase the production of ketone bodies, which have neuroprotective effects and can improve cognitive function and mood (Gasior et al. 2006). Further, mood-enhancing endogenous substances like serotonin and endorphins and subsequent increases in self-awareness and mindfulness might also play a role in the unique experience of voluntary abstinence (Michalsen 2010; Ring et al. 2022).

A randomized controlled trial on patients with metabolic syndrome found that combining fasting with lifestyle modifications provided short-term mood benefits, particularly decreasing depression and fatigue, compared to those who only made lifestyle changes. Both groups reported positive changes in quality of life and psychological factors like self-efficacy and mindfulness, with many of these benefits lasting until the 24-week follow-up (Jeitler et al. 2022).

A small open pilot study suggests that fasting can potentially have preventive effects on sleep disturbances and daytime fatigue, possibly enhancing brain function and improving daytime performance, with increases in concentration, vigor, and emotional balance (Michalsen et al. 2003b).

Despite the mood-enhancing effects of fasting, it is contraindicated for those with severe depression, as empirically it might, in certain cases, lead to a deterioration of symptoms, especially during the first days of fasting. It is also contraindicated in psychiatric patients, especially in pathologies associated with an overemphasis on control over bodily functions including diet, eating behavior, and weight.

10.3.6 Prolonged Fasting and Cancer

Cancer is a prominent cause of death worldwide, and the last years have seen tremendous progress in the field of oncological treatments. Still, cancer treatments such as chemotherapy, immunotherapy, and radiation can have significant adverse effects, reduce quality of life, and promote fatigue.

There is solid experimental evidence regarding the use of fasting concomitantly to chemotherapy. The term “differential stress response” was first described by Longo and

coworkers. It describes an effect seen in the experimental studies, namely, that fasting reduced adverse effects of chemotherapy on healthy cells while potentially improving therapeutic effectiveness by increasing chemotherapeutic toxicity in tumor cells. The application of fasting during chemotherapy typically requires a fasting period of 48 to 96 h. By definition this is not a prolonged fast, but a short-term fast. In clinical research some studies have used the fasting-mimicking diet, while our research unit has conducted studies that utilized the traditional European fasting approach as described in the section on “Methods of Prolonged Fasting” in a shorter version. Whether fasting reduces treatment-related side effects in different chemotherapeutic schemes and in which tumors it can have direct and indirect anti-cancer effects remains to be studied more extensively. So far, some smaller trials have been published.

These clinical trials on cancer patients found that fasting for up to 24–48 h prior to and 24 h after chemotherapy may reduce side effects such as nausea and vomiting, headaches, stomatitis, fatigue, and weakness as well as hematological toxicity (Bauersfeld et al. 2018; de Groot et al. 2015, 2019; Safdie et al. 2009). This potentially improves patient compliance and minimizes delays in chemotherapy administration due to the reduction of chemotherapy-induced toxicities – an important prognostic factor for the effectiveness of chemotherapy (Zorn et al. 2020).

A small randomized crossover trial investigated the feasibility and impact of short-term Buchinger-type fasting on quality of life and well-being in patients with gynecological cancer undergoing chemotherapy. Short-term fasting seemed to improve quality of life and reduce fatigue during chemotherapy while being well tolerated (Bauersfeld et al. 2018).

Another small pilot study aimed to assess the feasibility and effects of short-term fasting on chemotherapy tolerance in a specific group of patients with early breast cancer. Patients were randomized to either fast for 24 h before and after chemotherapy or follow healthy nutrition guidelines. Fasting was well tolerated, and the fasting group showed significantly higher erythrocyte and thrombocyte counts 1 week after chemotherapy. Additionally, short-term fasting appeared to reduce DNA damage in peripheral blood mononuclear cells after chemotherapy (de Groot et al. 2015).

In summarizing the existing experimental studies as well as the clinical studies, it appears that short-term fasting may offer relevant protective effects for the host, especially in breast cancer, and might potentially harm various tumors. With cancer often depending on good nutrient supply, there seems to be potential in combining dietary interventions and chemotherapy or other cancer therapies, as well as exploring drug targets based on fasting-related systems like IGF-1, which could yield fasting-mimetics providing differential stress resistance (Lee and Longo 2011).

10.3.7 Prolonged Fasting and Fertility

An unfulfilled desire to have children constitutes an increasing problem in Western societies. Older age, unhealthy lifestyle and dietary patterns, as well as increased environmental stresses are known risk factors for infertility. Infertility is estimated to affect one in seven couples and the trend is increasing (Talmor and Dunphy

2015). Often the causes are directly or indirectly related to excessive body weight or metabolic problems in both sexes (Talmor and Dunphy 2015; Kafaie-Atrian et al. 2019; (SGGG) DGfGuGDÖGfGuGOSGfGuG 2019).

Fasting may potentially improve metabolism, induce weight normalization, and improve well-being, thereby impacting fertility (Best et al. 2017). This chapter has already described some mechanisms by which fasting may help in this regard. These include improvement of insulin sensitivity, weight loss, and reduction of inflammation (Wilhelmi de Toledo et al. 2019; Michalsen and Li 2013).

The therapeutic benefits of fasting therapy in infertility have not yet been adequately studied clinically. However, there are studies that indicate links between metabolism and female fertility. The relationship between insulin resistance and hyperinsulinemia in menstrual irregularities is well established and reflected in guideline-based therapy with weight reduction and, if necessary, metformin for menstrual irregularities (Yuan et al. 2021). During fasting, hormones responsible for reproduction are physiologically downregulated (Maeda and Tsukamura 1996). After fasting, hormone regulation is expected to improve. Such effects are already known for other hormonal axes, such as insulin regulation (Stange et al. 2013; Li et al. 2017; Wei et al. 2017; Longo and Panda 2016). Since insulin regulation is closely linked to fertility, fasting could have a similar effect on the menstrual cycle to metformin.

Regarding male fertility, a relationship between diet, weight, and fertility has also been clearly established (Nazni 2014; Salas-Huetos et al. 2019a; b; Gabrielsen and Tanrikut 2016; Nassan et al. 2018; Ricci et al. 2019; El Salam 2018). Male fertility disturbances are estimated to account for up to half of all infertility cases with 25% to 87% of male subfertility considered to be an effect of oxidative stress (Smits et al. 2019). As fasting can positively influence weight and lifestyle and reduce oxidative stress, it seems reasonable to infer a potential benefit for male subfertility. Unfortunately, no studies to date have investigated these issues.

10.4 Safety and Contraindications

10.4.1 Contraindications for Prolonged Fasting

In all studies to date, prolonged fasting with the established techniques and practical considerations appears to be safe and well tolerated. Some absolute and relative contraindications can be found in Table 10.2. When relative contraindications exist, prolonged fasting should only be practiced in case of a strong indication. Special attention should be given to the choice of bowel cleansing interventions in chronic inflammatory bowel diseases, the reintroduction of proteins and fatty acids after fasting in symptomatic gallstone disease, and the general constitution of the patient in cases of exhaustion and fatigue. In symptomatic gout it is recommended to start prophylactic medication 3 weeks before starting the fast. In the central European fasting tradition, modified fasting is used to avoid protein catabolism for basal gluconeogenesis.

Certain medications need to be adapted during fasting. This includes antihypertensive and diuretic medication, as well as antidiabetics and medications with a

Table 10.2 Contraindications for prolonged fasting

Absolute contraindications	Relative contraindications
Cachexia/sarcopenia	BMI < 18.5 kg/m ² and < 45 kg/m ²
Pregnancy and lactation	Consuming diseases
Advanced hepatic or renal insufficiency	Type 1 diabetes mellitus
Decompensated hyperthyroidism	Advanced coronary artery disease
Symptomatic gallstone disease	Retinal detachment
Acute gout attack	Ulcus ventriculi and/or duodeni
Current or former eating disorder (e.g. anorexia nervosa/Bulimia nervosa/Binge eating/ Orthorexia nervosa(?))	Chronic inflammatory bowel diseases
Advanced cerebrovascular insufficiency/dementia	Chronic gout
Addictive disorder	Exhaustion/fatigue
	Manifest anemia
	Severe depression

narrow therapeutic range. For an overview, see Table 10.3. These changes are necessary, as fasting can have effects on pharmacokinetics and pharmacodynamics as well as therapeutic effects synergistic to the effects of certain drugs.

Pharmacokinetics, such as liberation, absorption, distribution, metabolism, and excretion of pharmaceutical substances, depend on variables that change during prolonged fasting. For instance, as solid food intake is reduced or paused, intestinal passage of substances can be accelerated. Similarly, liver metabolism is changed, and protein binding capacities in the serum as well as elimination of active pharmaceutical components through the bile or the kidneys can differ. These factors are especially important when treating patients with medications that have a narrow therapeutic range, as well as oral contraceptives.

Another reason for adapting certain medications during fasting is the fact that the intake of certain food components is reduced during fasting, such as glucose or vitamin K. This makes it necessary to reduce or discontinue antidiabetics and especially insulin during prolonged fasting. The vitamin-K-dependent metabolism of coumarins also needs to be considered, making a close monitoring and adaptation of the medication necessary.

As prolonged fasting has a diuretic effect through an increase in natriuretic peptide, the use of diuretics should be strictly avoided during prolonged fasting, to prevent electrolyte imbalances and, in particular, hyponatremia. Similarly, all antihypertensive drugs should be reduced in their dosage or discontinued, depending on the blood pressure of the patient.

10.4.2 Adverse Effects

In general, prolonged fasting is a safe intervention, when contraindications are observed and qualified medical accompaniment is available. However, there are a number of frequent minor adverse effects.

Table 10.3 Medication interacting with fasting

Antihypertensives	Depending on blood pressure changes, reduction of dosage, or discontinuation during fasting and, if necessary, increase again during refeeding period
Antidiabetic drugs (except insulin), such as biguanides, sulfonylureas, thiazolidinediones, gliflozins, DPP4 inhibitors, GLP-1 receptor agonists	To be paused during fasting. Blood glucose monitoring during fasting as well as the reintroduction of medication depending on glucose levels during refeeding is recommended
Diuretics	Pause during fasting to avoid electrolyte imbalances (especially hyponatremia)
Drugs with a narrow therapeutic range, such as digitalis glycosides, anticonvulsants, and lithium	Serum monitoring necessary due to narrow therapeutic range
insulin	Very close monitoring is obligatory Usually, significant reduction or discontinuation is necessary
Coumarins	Initial dose reduction necessary (less vitamin K intake) and then close monitoring to adapt medication accordingly
Nonsteroidal anti-inflammatory drugs and corticoids	To prevent stomachache and ulcers we suggest the use of rice or oat gruel instead of juices
Oral contraceptives	Contraceptive effect not reliable during fasting and refeeding period

Few studies to date have documented adverse effects of prolonged fasting interventions. One study examined a population of 1422 patients in an inpatient setting of a German sanatorium (Laurens et al. 2021), and one collected the data of 652 patients undergoing water-only fasting (using distilled water) in a US stationary treatment (Finnell et al. 2018). Among the serious complications, severe electrolyte imbalances and dehydration ranked first, leading in the sanatorium to two hospitalizations, one for fluid and the other for natrium replenishment, and in two cases of water-only fasting to ventricular arrhythmia and in one case to hyponatremia. Minor adverse effects reported frequently by patients with both fasting methods include headache and migraine attacks (mainly in the first 2–3 fasting days), palpitations, nausea, dyspepsia, abdominal pain, and sleep disturbances and less frequently diarrhea, flatulence, gastrointestinal pain and bloating, fatigue, back pain, and presyncopal episodes. The rate of reported adverse reactions was slightly higher in the publication on water-only fasting. In situations where contraindications were disregarded, a few cases of gout attacks and biliary colic have been reported empirically.

10.5 Conclusions

Prolonged fasting, particularly the modified fasting regimens of the German-speaking countries like the Buchinger and FX Mayr techniques, presents longstanding clinical applications and some preliminary scientific evidence. It can induce multiple physiological, biochemical, and psychological effects. Their potential benefits include preventing diseases, improving metabolic conditions, ameliorating autoimmune diseases, enhancing mental health, complementing cancer treatment, and influencing fertility. The pivotal role of medical professionals in guiding prolonged fasting, considering contraindications and medication adjustments due to altered pharmacokinetics, is obvious. While promising, further research and controlled studies are necessary to establish its effectiveness across various health conditions.

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