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Review

A Critical Assessment of Fasting to Promote Metabolic Health and Longevity

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Abstract

The adaptive starvation response allows us to survive periods of starvation—a characteristic of the environment in which humans evolved. We are now in an evolutionary transition from a global environment that was characterized by periods of famine to a world where obesity and caloric excess have become a new reality, but the mechanisms of fasting physiology remain relevant. First, many parts of the world are still plagued by famine with insufficient food resources and therefore the adaptive mechanisms required for survival during periods of decreased caloric intake are not simply relevant to our evolutionary past. Second, the obesity epidemic provides strong rationale for understanding the biology of fasting, as the same efficiencies that have evolved to allow us to survive periods of starvation also likely drive a genetic predisposition to obesity, and therefore some of the adaptive mechanisms may be maladaptive in the setting of food excess. A third compelling reason to explore the biology of fasting is that in model organisms, caloric restriction, without overt starvation, is an intervention that prolongs lifespan. The purpose of this review is to provide an overview of the biology of fasting. We will highlight potential mechanisms of benefit from fasting as well as examine data from model organisms and humans that indicate potential health risks of fasting, particularly related to bone fragility. Finally, we will review clinical studies to date that have investigated the effects of fasting on metabolic outcomes and suggest signals of benefit.

Key Words: intermittent fasting, alternate day fasting, weight loss, longevity, bone metabolism, caloric restriction, time-restricted eating

Abbreviations: ATGL, adipose triglyceride lipase; BMI, body mass index; CoA, coenzyme A; CREB, cyclic AMP response-element binding protein; FABP, fatty acid-binding protein; FGF, fibroblast growth factor; GLUT4, type 4 glucose transporter; HDL, high-density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; HSL, hormone-sensitive lipase; LDL, low-density lipoprotein; MGL, monoacylglycerol lipase; mTor, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PPARA, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; TCA, tricarboxylic acid.

ESSENTIAL POINTS

- Humans have evolved to survive prolonged periods of starvation through a coordinated set of adaptive responses
- An overarching theme to the adaptive fasting response is the transition from glucose to lipid metabolism
- Hormonal adaptations, mediated in part through a drop in leptin levels, result in decreased energy expenditure during periods of negative energy balance
- Various dietary restriction interventions, including caloric restriction and intermittent fasting, prolong lifespan in model organisms
- Potential benefits of fasting include weight loss and reprogramming of lipid metabolism
- Negative regulation of bone metabolism is an important potential harm from fasting-associated weight loss
- Human studies that examine fasting in a randomized controlled fashion suggest benefit to cardiometabolic metrics, but it remains unclear if signals of benefit or harm are independent of weight loss

Relevance of Fasting Physiology

The adaptive starvation response enables humans to survive for prolonged periods (days to months) without energy intake (1). Although we are in an evolutionary transition from a global environment characterized by cyclic famine to a new reality of food excess and obesity, the mechanisms of fasting physiology remain relevant. First, many parts of the world still have insufficient food resources, and therefore understanding the adaptive response to caloric restriction remains relevant in today's world to understand both survival mechanisms and the pathology associated with prolonged starvation. Second, the obesity epidemic itself has been cited as rationale for understanding the biology of fasting, as the same efficiencies that we evolved to survive periods of starvation may potentially drive a genetic predisposition to obesity, the so-called "thrifty gene hypothesis" (2). Third, and perhaps the more compelling reason to examine the biology of fasting, is that restriction of calories is a conserved behavioral intervention that prolongs lifespan in model organisms (3-7).

The dietary restriction of calories can take different forms. In this review, we will focus solely on total caloric intake

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rather than restriction of specific dietary components, as there is a separate body of literature examining restriction of specific nutrients such as methionine (8). The term "caloric restriction" usually describes a sustained reduction in caloric intake, entailing an approximate 10% to 30% reduction in daily calories. Another approach involves intermittent periods of dramatically reduced calories or complete fasting, alternating with periods of "normal" caloric intake, so-called intermittent fasting. A hybrid of the 2 is time-restricted feeding, where food intake is restricted to a narrow daily time window, thereby incorporating a partial-day fast into each day. The lines between these 3 modes are often blurred and within each category there is substantial variability in approach. Despite the general lack of historical standardization and that the 3 categories share a tendency to achieve net restriction of calories (9), in this review, we will restrict the term caloric restriction to denote protocols of sustained daily calorie reduction and the term intermittent fasting to protocols involving a zero or near-zero calorie fast. Although the primary subject of this article is fasting, we will also include the concept of caloric restriction because there is undoubtedly shared biology between these 2 paths to negative energy balance.

The purpose of this review is to provide an overview of the biology of fasting. The adaptive fasting response has been extensively studied for decades and therefore this review cannot do justice to the complexity and nuance of the operative hormonal and molecular mechanisms. Instead, we focus on central themes and mechanisms that are crucial to fasting and that may be relevant to metabolic health. Indeed, intermittent fasting is increasingly practiced in one form or another by patients, often without prescription or supervision by a medical practitioner. Yet, the burgeoning interest in intermittent fasting is shared by many in the biomedical research community because of theoretical intrinsic salutary properties of fasting and signals of benefit from early translational human studies. As a lead-in to the human clinical studies, we will first provide an overview of the adaptive fasting response. We will highlight potential mechanisms of benefit from fasting; however, we will also examine data from model organisms and humans that indicate potential health risks to fasting, particularly related to bone fragility. Although the science and clinical evidence base is not yet sufficient to widely recommend fasting, the signals of benefit provide rationale for further study, and we will conclude by articulating our view of the key unanswered questions.

The Adaptive Fasting Response (Fig. 1)

Endogenous Glucose Production During Early Fasting

Glycogenolysis

The onset of fasting can be defined as the point at which calories are no longer ingested. However, the physiological adaptation to fasting commences when absorption of digested food is completed. A classical view of the adaptive fasting response describes at least three adaptive post-absorptive phases: (1) release of glucose from glycogen stores; (2) synthesis of glucose by gluconeogenesis; and (3) lipid catabolism and onset of ketogenesis. Progression to the third phase marks the shift from glucose to lipid metabolism, enabling survival during prolonged periods of fasting by preventing preterminal catabolic sacrifice of the crucial protein backbone. In humans, the third phase, during which lipids serve as the principal fuel source,

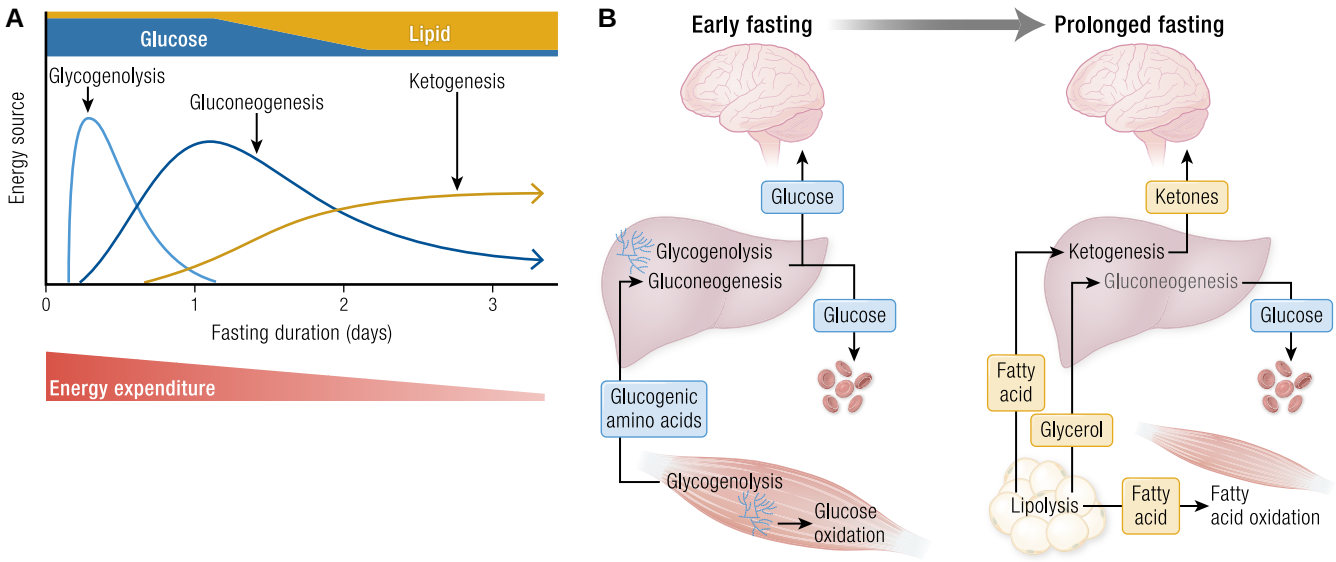

Figure 1. The metabolic adaptation to fasting. (A) The 3 classical phases of the postabsorptive response to fasting overlap in time and include (1) glycogenolysis, which is the release of glucose from glycogen stores; (2) gluconeogenesis; and (3) ketogenesis, which marks the onset of lipid catabolism. Note that time = 0 days marks the onset of fasting. A lag between time = 0 and the onset of glycogenolysis accounts for the absorption of the last meal. The timing of these phases is approximate, as the temporal dynamics may be affected by factors such as basal metabolic rate, composition of the last meal, and degree of glycogen stores. (B) Adaptation to fasting in critical tissues is shown to contrast prolonged from early fasting. Early: glucose metabolism dominates. Glycogen stores in the liver and skeletal muscle supply glucose in the early postabsorptive period. Glucogenic amino acids, for which skeletal muscle is an important source, are used as substrate for gluconeogenesis by the liver and kidney (not shown). Prolonged: lipid stores, predominantly in adipose tissue, are mobilized by lipolysis, releasing fatty acids and glycerol. Glycerol serves as substrate for gluconeogenesis, which must persist to supply minimal glucose needs, especially for glucose obligate red blood cells. Tissues with the machinery to directly oxidize fatty acids (eg, skeletal muscle) do so, whereas the liver uses fatty acids as substrate for ketone production. Ketones serves as a critical lipid-derived fuel source for the central nervous system.

can last for months (10). Although rodent model organisms also exhibit a similar adaptive fasting response, their faster relative metabolic rate compresses the timescale, resulting in death if fasting is extended beyond a few days (11).

The first phase, glycogenolysis, involves the release of glucose molecules from glycogen chains, which accumulate in the liver and skeletal muscle in the fed state (12, 13). The breakdown of glycogen is enzymatically driven by glycogen phosphorylase, yielding individual molecules of glucose-1 phosphate, which in turn are converted to glucose-6 phosphate by the action of phosphoglucomutase. In skeletal muscle, where the glycogen stores provide a rapidly accessible energy source during periods of intense muscle contraction, glucose-6 phosphate is directly catabolized by glycolysis. In the liver, by contrast, the phosphate moiety is removed by glucose-6 phosphatase, yielding glucose, which in turn can exit the cell via glucose transporters to maintain systemic glucose levels and supply distant tissues with fuel for glycolytic metabolism. As such, the liver glycogen stores are of greater relevance to the systemic adaptive response to fasting.

Gluconeogenesis

Induction of gluconeogenesis maintains glucose homeostasis with depletion of glycogen stores. The liver and kidney are the principal sites of gluconeogenesis (14). The small intestine also expresses the requisite enzymatic machinery for gluconeogenesis and may also support glucose homeostasis with fasting (15). Gluconeogenesis largely operates as the enzymatic steps of glycolysis in reverse except for the actions of phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase, which, respectively, catalyze conversion of oxaloacetate to phosphoenolpyruvate, conversion of fructose-1,6-bisphosphate to fructose 6-phosphate, and the hydrolysis of glucose 6-phosphate to yield free glucose.

The activity of key enzymatic control nodes in glycogenolysis and gluconeogenesis are regulated at multiple levels, including by the action of canonical hormonal systems, and via transcriptional control. The combination of hypoinsulinemia and glucagon surge, for example, impairs glycolysis and drives glycogen catabolism and gluconeogenesis (16). Signal transduction pathways, including canonical cyclic-AMP signaling, operate dynamically to transmit hormonal signals to phosphorylate key enzymes driving both glycogenolysis and gluconeogenesis (17). Signal transduction cascades may ultimately also end in the nucleus with transcription factor dependent transcriptional programs, which can reinforce transcription of genes encoding the core enzymatic machinery that may already be expressed at some constitutive level or facilitate de novo expression of fasting-specific regulators. One canonical example is the cyclic AMP response-element binding protein (CREB), a transcription factor translocated to the nucleus in response to glucagon and cAMP signaling and which in turn activates genes, including those coding key enzymes such as glycogen phosphorylase (*PYGL*, glycogenolysis) and phosphoenolpyruvate carboxykinase (*PCK1*, gluconeogenesis) (18-21). Although genetic loss of function of CREB results in a phenotype of severe hypoglycemia in mice, consistent with its critical role in fasting glucose metabolism, coactivators such as PGC1α and TORC2 and multiple collaborating transcription factors such as FOXO1, NR4A1, and peroxisome proliferator-activated receptor α (PPARA), amongst others, also play regulatory roles with fasting (22-27).

Gluconeogenesis ultimately also depends on substrate availability. In the fasted state, glycerol released from adipose tissue and glucogenic amino acids derived from skeletal muscle serve as critical substrate for gluconeogenesis (28). Alanine is a particularly important amino acid substrate for gluconeogenesis, the dynamics of which also illustrate the complexity of systemic metabolism during fasting. Studies involving arterial-venous sampling reveal a net release of amino acids, including alanine from skeletal muscle, yet circulating alanine levels drop with fasting because of the greater uptake of alanine by the liver for utilization as gluconeogenic substrate (14). Even though there is net release of amino acids from skeletal muscle in the early phases of fasting, the extent to which this flux is attributable to export of free cytosolic amino acids (29), decreased protein synthesis (30), and/or increased protein catabolism (31) is not definitively resolved. Nonetheless, the high cost of producing gluconeogenic substrate from protein catabolism underscores the importance of the ensuing shift away from glucose-centric metabolism as reflected by a decline in the Respiratory Quotient (an indicator of macronutrient catabolism on indirect calorimetry), and accounting for the relatively short duration of the phase in which glucose is a dominant systemic fuel source (32).

The Shift to Lipids as Dominant Fuel Source

The shift to lipid utilization is the key adaptation that allow humans to survive prolonged periods of fasting. To accomplish this shift, 3 key events must occur: (1) cells containing the requisite mitochondrial machinery must transition to oxidation of fatty acids as their principle energy source; (2) lipid stores, largely contained in adipocytes, must be mobilized into systemic circulation; and (3) the liver must use circulating fatty acids to produce ketone bodies which can cross the blood-brain barrier and supply the sizeable energy needs of the brain and central nervous system (33). Apart from red blood cells, which lack mitochondria, most tissues can transition to use of lipid-derived substrate as fuel, either through the direct oxidation of fatty acids or by the utilization of lipid-derived ketones.

Mobilization of adipocyte lipid stores (Fig. 2)

As the central energy reserve, adipose tissue plays a crucial role during fasting. Energy is stored in adipocytes in the form of neutral lipid droplets—ie, triglycerides. Mobilization of this energy depot requires transfer from intracellular lipid droplets out of the adipocyte and across the capillary endothelial barrier. Triglycerides cannot directly cross the plasma membrane and therefore the canonical model of adipocyte lipid mobilization involves lipolytic release of 3 fatty acid chains capable of crossing the plasma membrane by diffusion or fatty acid transporters. Adipose triglyceride lipase (ATGL/*PNPLA2*), hormone-sensitive lipase (HSL/*LIPE*), and monoacylglycerol lipase (MGL/*MGLL*) catalyze the sequential release of 3 fatty acid chains from each triglyceride molecule (34-38) (Fig. 2A). As such, genetic loss of function of ATGL/*Pnpla2* in mice results in triglyceride accumulation in adipose tissue (39). The high specificity of HSL for diacylglycerols relative to triglycerides accounts for the accumulation of diacylglycerols with targeted disruption of HSL/*Lipe* (35). In turn, monoacylglycerols accumulate with genetic loss of function of *Mgll*, the gene encoding MGL (40).

Lipolysis is generally attributed to hormonal signaling and signal transduction pathways that drive activity of canonical lipases (41). Activation of g-protein coupled β-adrenergic receptors by catecholamines activates adenylate cyclase, which generates cyclic AMP. cAMP-dependent protein kinase A activity phosphorylates—and thus activates—HSL and α/β hydrolase domain-containing protein 5, a key regulator of ATGL. Insulin signaling is also important. In the fed state, insulin engagement of the insulin receptor results in its phosphorylation and activation of IRS1/2, the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, and effectors that dampen cAMP and its stimulatory actions on ATGL and HSL. In the hypoinsulinemic state of fasting, the potent inhibitory effects of insulin on lipase activity are attenuated. Although the exact intracellular signaling path to lipase activation may differ depending on the physiological and hormonal inputs, these signaling pathways converge on lipolytic release of fatty acids at the interface between lipid droplets and the adipocyte cytosol.

Although murine genetic loss of function studies point to important roles for the 3 canonical lipases in adipocyte lipid metabolism, the fasting mediated transcriptional induction of canonical lipase genes is either undetectable or modest relative to any observed changes in lipolytic activity, underscoring the importance of posttranscriptional regulatory functions and/or complementary roles for noncanonical lipases (42-44). Indeed, the canonical view of adipocyte lipolysis has recently been complicated by the identification of alternative paths to adipocyte lipid release. In a recent longitudinal fat transcriptomics study of fasting humans, we found induction of genes associated with noncanonical lysosomal lipolysis (44). Moreover, in a murine model of inducible adipocyte-specific loss of function of lysosomal acid lipase (LAL/*Lipa*), the lipase operative in the low pH state of the lysosome, we discovered that lysosome-mediated lipolysis becomes dominant as fasting is prolonged beyond a few hours (Fig. 2B) (45). Another alternative mechanism of lipid mobilization involves nonlipolytic release of adipocyte triglycerides via exosomes (46). Triglyceride-rich exosomes may then be engulfed by resident macrophages, capable of digesting lipid and releasing the catabolic byproducts, including fatty acids (Fig. 2C). Regardless of the relative contributions from canonical and noncanonical mechanisms, however, the mobilization of lipid stores with a prolonged fast are sufficiently effective that they may even exceed the substrate requirements of the liver and lead to acute steatosis (47).

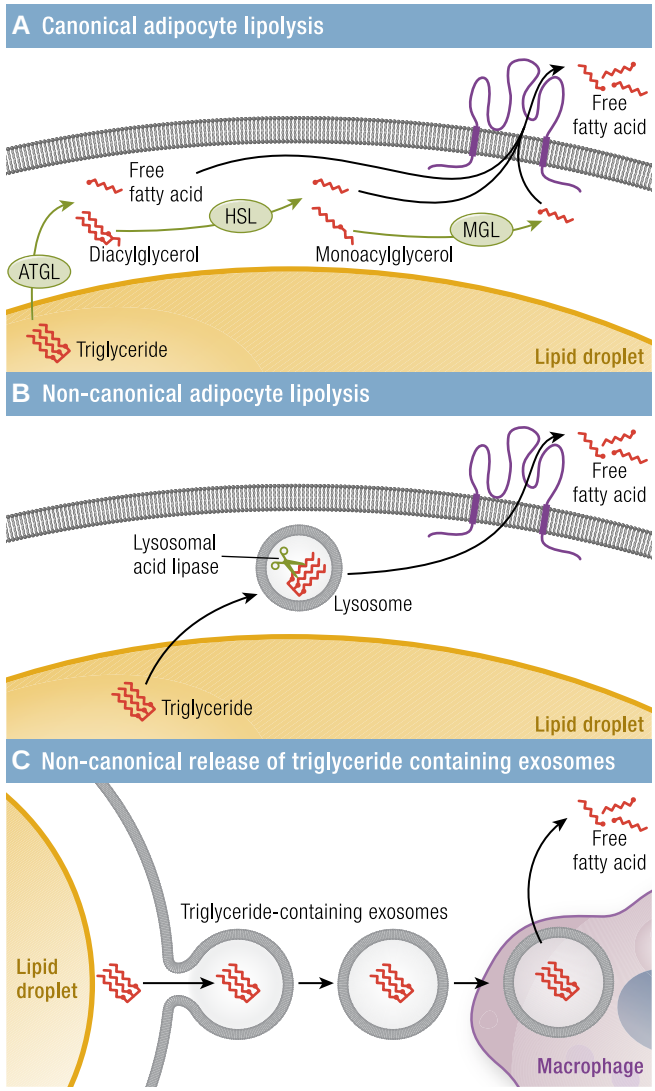

Figure 2. Mechanisms of triglyceride mobilization in adipocytes. (A) Canonical lipolysis pathway operative in adipocytes. ATGL liberates a fatty acid chain from triglycerides contained in adipocyte lipid droplets resulting in a diacylglycerol (DAG). The action of HSL on DAG releases a second fatty acid chain and results in monoacylglycerol. The final fatty acid chain is released by the action of MGL. (B) Recently described mechanism of adipocyte lipolysis with fasting in which lysosomal acid lipase (LAL), the only lipase known to operate in the low pH environment of the lysosome, liberates free fatty acids (45). (C) Recently described mechanism of lipolysis that depends on resident adipose tissue macrophages (46). Triglycerides contained in adipocyte-derived exosomes are released into the extracellular space where they are taken up by macrophages, which have lipolytic capacity.

Abbreviations: ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MGL, monoacylglycerol lipase.

Switch to fatty acid oxidation

Mitochondrial-rich skeletal muscle is 1 of the largest fuel-consuming tissues in the body and therefore is a relevant case study for the fuel switch that occurs with fasting. In the fed state, insulin signaling drives vesicles containing type 4 glucose transporters (GLUT4) to the plasma membrane and subsequent transport of glucose into insulin responsive cells, such as adipocytes and skeletal myocytes (48-51). Like the liver, skeletal muscle stores excess glucose as glycogen (52), whereas adipocytes convert excess glucose into triglycerides by supplying substrate for the glycerol backbone and fatty acids by de novo lipogenesis (53). Glucose is also used to fuel glycolysis, which supplies substrate to the mitochondria for the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. With fasting, these processes are attenuated. Circulating glucose and insulin levels drop, and with prolonged fasting, the effects of hypoinsulinemia may be enhanced further in peripheral tissues by relative insulin resistance (54, 55). The fed-fasting dynamics of GLUT4 levels and activity present an extreme dichotomy in adipocytes, where the insulin deficient state of fasting results in dramatic declines in GLUT4 mRNA, protein, and vesicular trafficking (48-51). Muscle is distinct from fat tissue in that there are insulin-independent mechanisms of GLUT4 vesicular trafficking to meet energy import requirements with exercise (56, 57), which likely accounts for the lack of consistent correlation between insulin levels or activity and GLUT4 mRNA and protein levels (58). Nonetheless, the hypoinsulinemia of fasting reduces GLUT4 in myocyte plasma membranes of resting skeletal

muscle (59), effectively driving a reduction in glucose uptake. Fasting, particularly when prolonged also downregulates expression of the glycolytic gene program (54). Therefore, the collective effects of fasting on resting skeletal muscle reduce glucose uptake and utilization (60).

Concomitant to the reduction in glucose utilization is an increase in fatty acid oxidation. The mechanisms by which fatty acid uptake and utilization are achieved in the fasted state are not as well elucidated relative to regulation of glucose metabolism during fasting. Lipids and fatty acids are a heterogeneous array of molecules. In addition, the immiscibility of neutral lipids in water and the poor solubility of free fatty acids necessitates a variety of specialized protein factors to facilitate trafficking and metabolism, both systemically and at the individual cellular level (61). Indeed, although it was once thought that fatty acids could freely diffuse across plasma membranes—which is supported by flux analyses across synthetic protein-free lipid bilayers—achievement of sufficiently dynamic fatty acid flux to meet the oxidative fuel needs during fasting requires the additional action of protein chaperones (62). Gain and loss of function studies targeting membrane proteins, such as CD36 or members of the fatty acid-binding protein (FABP) family, demonstrate the sensitivity of physiological fatty acid flux to modulation of these critical plasma membrane proteins (63-65). Once fatty acids gain access to the cytosol, they are bound by fatty acid binding proteins (62). Activation of fatty acids by acyl-coenzyme A (CoA) enzymes precedes their transport into mitochondria by the carnitine-palmitoyltransferase system, where fatty acids are catabolized by β-oxidation, producing acetyl-CoA molecules to supply the TCA cycle (66). Analogous to the insulin-stimulated translocation of GLUT4 to the plasma membrane in the fed state, similar mechanisms may be at play in the regulation of CD36 and FABPs, as both have been shown to be dynamically recruited by fasting (67, 68). Mitochondrial fatty acid oxidation gene programs are also induced by fasting (69). These collective actions during fasting result in the augmentation of fatty acid importation and oxidative utilization.

In the preceding 2 paragraphs, we have described the attenuation of glucose utilization and the augmentation of fatty acid utilization as distinct; however, these processes are not independent of one another. Randle first postulated that substrate availability—ie, competition between glucose and fatty acids—is a determinant of fuel utilization (70). Although specific mechanistic details of Randle's hypothesis have been questioned, particularly with respect to the underlying role of lipids in the pathophysiology of insulin resistance and type 2 diabetes (71), human and mouse genetic models support the general concept that fatty acid metabolism can regulate glucose utilization with fasting. For example, homozygous loss of function of many of the genes involved in fatty acid oxidation predisposes to hypoglycemia with fasting (66). In mice, genetic knock out of long-chain acyl-CoA dehydrogenase, a key enzymatic mediator of mitochondrial fatty acid oxidation, phenocopies fasting mediated hypoglycemia in part by augmenting glucose uptake and utilization (72). These genetic models suggest that attenuation of inhibitory mechanisms operative during fasting that are dependent on fatty acid catabolism augments glucose utilization; conversely, intact fatty acid oxidation during fasting reinforces the switch from glucose to lipid metabolism by actively suppressing key nodes in glucose uptake and/or catabolism.

Ketogenesis

Ketones are produced from ketogenic amino acids and from the products of fatty acid catabolism. The production of ketones by the liver requires many of the same steps of fatty acid uptake and oxidation in mitochondria that have already been discussed. The key branch point occurs after generation of acetyl-CoA (73). Instead of supplying the TCA cycle as occurs in skeletal muscle, 3 acetyl-CoA molecules are converted to acetoacetate by the sequential actions of acetyl coenzyme A acetyltransferase, 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase and HMG-CoA lyase. β-hydroxybutyrate is generated from acetoacetate by β-hydroxybutyrate dehydrogenase. Acetoacetate and β-hydroxybutyrate are 2 ketones used by extrahepatic tissues for energy.

The rate-limiting enzyme for ketogenesis is HMG-CoA synthetase. Nutrient sensitive pathways regulate transcription of the HMG-CoA synthetase gene (*HMGCS2*), including by the fasting-mediated induction of the nuclear receptor, PPARα (74). There are PPAR binding motifs at *HMGCS2 cis*-regulatory domains and *HMGCS2* is a PPARα-responsive gene (75); however, there may be additional indirect PPARα-dependent mechanisms that account for its fasting effects. For example, PPARα transcriptionally regulates additional enzymes involved in fatty acid oxidation and the PPARα-regulated secretion of fibroblast growth factor (FGF)21 may in turn regulate lipid metabolism and ketogenesis by indirect autocrine and paracrine mechanisms (76). The role of PPARα as a central node in the regulation of lipid metabolism with fasting is exemplified by the phenotype exhibited by the global genetic loss of function murine model, which has impaired fatty acid oxidation and ketogenesis with fasting (77, 78).

FOXA2 is another transcription factor that binds the promoter for *HMGCS2* and activates its transcription. In the fed state, insulin signaling leads to phosphorylation and nuclear export of FOXA2, thereby inhibiting *HMGCS2* transcription (79, 80). With the reduced insulin action during fasting and the additional counterregulatory action of glucagon signaling, which promotes FOXA2 acetylation and transcriptional regulatory function, transcription of *HMGCS2* is augmented (81). Therefore, FOXA2 represents an important node in the insulin/glucagon regulatory axis that is central to cycles of feeding and fasting.

The result of these fasting-responsive regulatory networks in the liver is that ketones are released into systemic circulation, where they serve as a critical energetic substrate for the central nervous system, which is insulated from circulating lipids because of the blood-brain barrier. In neuronal mitochondria, ketones are converted back to acetyl-CoA to supply the TCA cycle for ATP production. These mechanisms also underscore the central role of the liver in the adaptation to fasting: the liver maintains circulating glucose, first by mobilization of glycogen and then by gluconeogenesis in early fasting; then in the later phase where lipid metabolism is essential to survival, the liver supplies energy in the form of ketones to fulfill the energy demands that cannot be met directly by fatty acids.

Reduction in Energy Expenditure

Superimposed on the 3 classical phases of adaptive starvation is a reduction in energy expenditure. The classic Minnesota 6-month semi-starvation experiments on conscientious objectors during World War II demonstrated sustained reductions in both physical activity and resting energy expenditure (82).

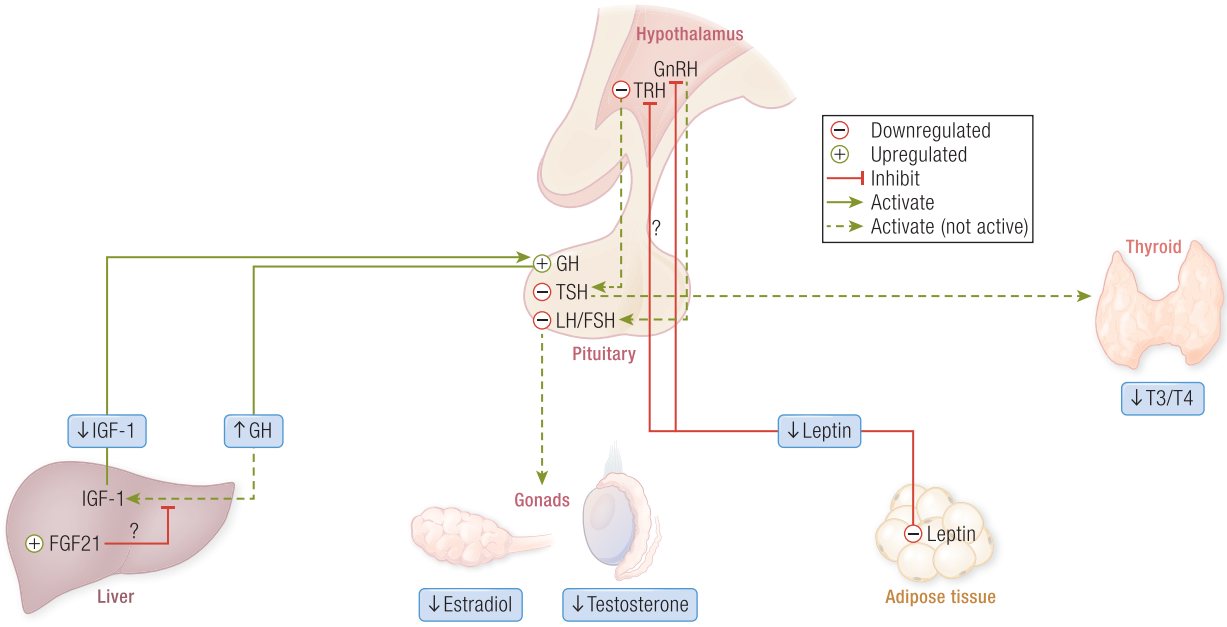

Figure 3. Neuroendocrine adaptations to chronic negative energy balance in humans. Hormonal adaptations to states of chronic caloric deficit include GH resistance, hypogonadotropic hypogonadism, and a decrease in thyroid hormone (T3 and T4) levels that are likely in part due to decreased T4 and T3 secretion as well as decreased peripheral conversion of T4 to T3. In murine models, FGF21 is a mediator of GH resistance (101) and may be in humans as well (102). In animal models, a decrease in leptin secretion may mediate decreased TRH secretion (115).

Abbreviations: FGF, fibroblast growth factor; TRH, thyrotropin-releasing hormone.

Reductions in energy expenditure can also be detected in individuals with anorexia nervosa, a primary psychiatric disorder with inappropriately low caloric intake that serves as a representative cohort for studying chronic caloric deprivation (83), and with less severe forms of long-term caloric restriction or with acute zero-calorie fasting (84, 85).

Neuroendocrine Adaptations to Negative Energy Balance (Fig. 3)

Hypothalamic-pituitary-thyroid axis

The hormonal and adaptive effects of reduced energy intake are well characterized in anorexia nervosa. In this disorder, adaptive hormonal responses result in a reduction in energy expenditure. A critical determinant of basal metabolic rate is the hypothalamic-pituitary-thyroid axis, long recognized to be sensitive to calorie deprivation. A characteristic finding in anorexia nervosa is thyroid laboratory studies consistent with a state of nonthyroidal illness syndrome—depressed T3 and T4 levels coupled with a low or normal TSH level and an increased T4 to T3 ratio suggesting decreased peripheral conversion of T4 to T3 (86-89). Resting energy expenditure is decreased in individuals with anorexia nervosa (83) and this decreased resting energy expenditure is associated with low T3 levels (90). With weight gain, T3 levels rise (88, 89, 91, 92) and, importantly, this increase is associated with an increase in resting energy expenditure (90), suggesting that the low T3 levels in anorexia nervosa, a model of chronic starvation, are an adaptive response that results in decreased energy expenditure.

GH resistance and hypogonadotropic hypogonadism

Two additional neuroendocrine adaptations that help reduce energy expenditure in anorexia nervosa include GH resistance and hypogonadotropic hypogonadism (93). These hormonal adaptations likely decrease overall energy expenditure in states of chronic energy restriction by shunting calories away from processes that are not critical for survival. GH resistance, in states of starvation, allows for the exploitation of the beneficial effects of GH while minimizing energy expenditure on growth. GH is a hormone secreted by the pituitary gland and signals the liver to produce IGF-1. As its name implies, GH is an essential hormone for growth, maintenance of bone density, and muscle mass; these processes are predominantly mediated by IGF-1. Yet GH also has IGF-1-independent lipolytic actions and insulin resistance properties (94). These counterregulatory actions are beneficial or advantageous during periods of caloric deprivation (95). Therefore, a state in which GH levels are normal or elevated but IGF-1 levels are low, to minimize energy expenditure on growth, is adaptive in the short term. This state of GH resistance has been demonstrated with acute fasting (96) and in anorexia nervosa (97-99). Notably, even supraphysiologic recombinant GH supplementation cannot overcome GH resistance in states of chronic starvation (100). What causes GH resistance and therefore the block in GH's ability to signal the liver to produce IGF-1 in states of undernutrition? The exact mechanisms are not known but potential hormonal mediators include FGF-21 (101, 102), low insulin levels (103), low leptin levels (104), and ghrelin (105).

Hypogonadotropic hypogonadism is another key neuroendocrine adaptation to starvation. Hypogonadotropic hypogonadism results in a decrease in expenditure of energy on reproduction and the reproductive axis. Most women with anorexia nervosa, for example, are amenorrheic, the consequence of hypogonadotropic hypogonadism, and males with anorexia nervosa have low testosterone levels compared to normal-weight controls (106). Hypogonadotropic

hypogonadism occurs due to disruption of GnRH pulsatility in the hypothalamus, which results in decreased pulsatility and amplitude of LH secretion from the pituitary (107-111). This disruption is likely mediated by a reduction in leptin—a hormone secreted by adipose tissue—the level of which drops during fasting or when adipose tissue stores are reduced. Indeed, treatment with recombinant human leptin normalizes LH pulse frequency and restores ovulatory cycles in women with hypogondotropic hypogonadism due to a state of negative energy balance (112). Similarly, in men, short-term fasting (72 hours) results in a decrease in LH pulsatility and a decrease in testosterone levels; these changes are prevented with treatment with recombinant human leptin during the fast (113). Therefore, both GH resistance and hypogonadotropic hypogonadism are hormonal responses to decreased caloric intake that minimize energy expenditure on processes that are not acutely critical for survival.

Additional hormonal mediators

Leptin. Soon after its discovery as an adipocyte-derived signal of obesity, leptin was demonstrated to be dynamically repressed during acute fasting and regulate neurohormonal adaptations to fasting in mice (114, 115). Although leptin is secreted by adipose tissue, levels of leptin drop by approximately 50% within 24 hours of a fast and before any significant change in body weight (116). In mice, the drop in leptin levels with fasting has been shown to mediate hypogonadotropic hypogonadism and reduced thyroid hormone (T4) levels (115), thereby reducing energy expenditure. By contrast, treatment of fasted mice with recombinant leptin attenuates the fasting-mediated drop in testosterone in male mice, prevents the estrus delay in female mice, and blunts the reduction in T4 levels (115).

In humans, hypoleptinemia has most clearly been implicated as a mediator of hypogonadotropic hypogonadism. In women in states of negative energy balance with hypogonadotropic hypogonadism from disruption of normal LH pulsatility—so-called functional hypothalamic amenorrhea—treatment with recombinant human leptin improves LH pulsatility and restores ovulatory cycles (112). The improvement in LH pulsatility occurs despite concomitant weight loss, exemplifying that it is not due to an improvement in energy balance (112). Leptin has also been implicated as a mediator of GH resistance in humans, as treatment with recombinant human leptin for 3 months in women with functional hypothalamic amenorrhea and low IGF-1 levels results in a significant rise in circulating IGF-1 (104). Short-term (72 hours) fasting studies in men show leptin to be a possible mediator of decreased TSH secretion (113); therefore, leptin may help mediate the downregulation of the hypothalamic-pituitary-thyroid axis in humans during fasting as well (117).

In rodent models, the acute drop in leptin with fasting has been implicated in the critical shift from carbohydrate to lipid metabolism, mediated by activation of the hypothalamic-pituitary-adrenal axis (118). We have shown that in normal and overweight humans undergoing a 10-day zero-calorie fast, there is also an inverse association between the drop in leptin levels and the rise in cortisol levels during the early fasting response (116). Therefore, the drop in leptin levels with starvation may be an important hormonal mediator of both the acute response to decreased caloric intake as well as an adaptation to chronic states of starvation.

FGF21. FGF21 is a hormone secreted predominantly by the liver with fasting (76, 85, 119). In mice, FGF21 increases within hours of fasting onset and before ketogenesis (76, 119). Experiments with genetic mouse models demonstrate that FGF21 executes PPARα-dependent functions, including promotion of ketone generation by the liver. FGF21 is also attributed roles in fasting-associated hypogonadism (120) and fasting-mediated torpor (119), an extreme manifestation of the drive to reduce energy expenditure. These findings led to the view of FGF21 as the prototypical fasting hormone and generated excitement in the use of FGF21 or FGF21 analogues to pharmacologically drive beneficial metabolic responses. In humans, however, the fasting-mediated FGF21 surge is not seen until 7 to 10 days into a fast (85, 121)—and well beyond the induction of ketogenesis—suggesting that circulating FGF21 levels may play a role in the late adaptive response to fasting, rather than the acute response. FGF21 may play a role in mediating GH-resistance during starvation in both humans (102) and murine models (101). Interspecies differences in FGF21 response and functionality may explain why FGF21 pharmacological efforts have not replicated the full breadth of beneficial metabolic properties observed in mice, although FGF21 effects on lipid metabolism may be sufficient to improve systemic triglyceride levels and metrics of metabolic dysfunction-associated steatotic liver disease (122-128).

Potential Mechanisms of Benefit From Fasting

Many theories have been proposed to explain why we age, often attributing causality to specific processes known to be deranged with aging. Examples include the mitochondrial theory of aging (129), the DNA damage theory of aging (130), and the inflamm-aging theory (131), which assign causal roles in aging biology to mitochondrial dysfunction and reactive oxygen species, cumulative DNA damage, and low-grade inflammation, respectively. A singular unifying theory of aging may not be realistic, however, as there is substantial overlap in pathophenotypes at the cellular, tissue, and organismal levels between these putative processes. Many of the proposed mechanisms of benefit of fasting involve the intersection between the adaptive mechanisms described in prior sections of this manuscript and putative pathological pathways implicated in aging biology. As such, our discussion of the potential clinically measurable benefits of fasting reflects how the molecular and metabolic adaptations to fasting may plausibly modulate aspects of aging and or diseases of aging.

Weight Reduction

Epidemiological data demonstrate a range of negative consequences associated with overweight or obesity, including increased risk of diabetes (132), heart disease (133), many cancers (134), and dementia (135), among other diseases. Sustained reduction of caloric intake results in reduced adiposity and weight, even though adaptive reduction in energy expenditure partially attenuates the pace of weight loss (136). In overweight or obese individuals who have already developed impaired glucose homeostasis or frank type 2 diabetes, weight loss improves and, in some cases, reverses diabetes (137). Therefore, patients most likely to derive benefit from fasting-mediated weight loss are those with unambiguously elevated body mass index (BMI) (obese). It is important

to recognize, however, that the use of BMI to define overweight or obese thresholds is crude. Individuals with low muscle mass may have relatively high degrees of adiposity and still fall within the normal BMI range. In addition, genetic differences in body frame influence the relationship between BMI and adiposity. Individuals of Asian descent, for example, may have unhealthy adiposity at the upper limit of "normal" BMI because of a genetic predilection for smaller frame size (138). A more nuanced view of body weight, incorporating ethnicity and clinical observations of adiposity may identify individuals who would benefit from fasting-mediated weight loss even if their BMI measurement does not place them in a high-risk category. Importantly, however, the weight loss associated with fasting and caloric restriction protocols may represent a direct benefit, but also a confounder, as it is difficult to definitively determine the degree to which observed benefits are mediated by changes in weight. Understanding the positive as well as potential negative effects of fasting independent of weight loss is currently an active area of investigation (139, 140).

Insulin Resistance and Glucose Metabolism

Insulin signaling is a critical regulator not just in response to the postprandial carbohydrate surge, but also in the fasted state, where the absence of insulin signaling is a precondition for aspects of the adaptive fasting response as described in this manuscript. A prolonged fast can lead to acute peripheral insulin resistance (55), which may be an adaptive response to limit utilization of glucose by tissues capable of shifting to fatty acid metabolism to support energetic needs. In contrast to this acute response, however, studies in rodents and nonhuman primates demonstrate long-term improvements in glucose homeostasis with caloric restriction or intermittent fasting (141-143). A range of possible cellular mechanisms may account for improved glucose homeostasis with fasting or caloric restriction, including improved β-cell survival or function (144, 145), improved skeletal muscle GLUT4 cell surface occupancy and responsiveness to insulin (55), and augmented mammalian target of rapamycin (mTor) signaling in adipocytes (146). However, improved insulin resistance with caloric restriction or fasting is undoubtedly in part due to reduction in weight, a potential mechanistic confounder that is difficult to fully account for.

Reprogramming of Cellular Metabolism

Energy balance directly impacts cellular metabolism as the requisite shift to lipid metabolism that accompanies caloric restriction or fasting involves activation of catabolic pathways in mitochondria and peroxisomes. Mitochondria catabolize fatty acids to produce acetyl CoA to supply the TCA cycle and produce substrate for ketogenesis. Peroxisomes catabolize long- and very-long-chain fatty acids. To accommodate the shift to fatty acid oxidation, achievement of negative energy balance may promote mitochondrial fission, mitochondrial biogenesis, and improve overall mitochondrial function in part through nutrient-sensitive PGC1α dependent transcriptional programs (147-149). Fasting may also improve coordination of mitochondrial and peroxisome metabolic processes (150). Importantly, augmentation of mitochondrial biogenesis has not been uniformly observed across studies and therefore the degree to which such adaptive mechanisms drive protective effects of dietary restriction are not fully known.

Cellular respiration results in the production of reactive oxygen species (ROS) as an obligate byproduct. The overall reduction in energy expenditure that occurs with fasting reduces energy production and therefore may also reduce cumulative exposure to ROS. Fasting may also directly suppress mitochondrial ROS via induction of transcriptional regulators of the antioxidant response, including Nuclear factor erythroid 2-related factor 2, and downstream antioxidant enzymes including heme oxygenase and glutathione peroxidase 4 (151, 152). Such mechanisms converge to reduce ROS generation as suggested by studies of caloric restriction in aging mice and the demonstration of reduced oxidative damage in the heart of rats subjected to intermittent fasting (153, 154).

One potential mechanism by which metabolic reprogramming effects could be sustained is through remodeling of the epigenome through chemical modifications to either DNA or DNA-associated histones. Indeed, links between aging, exposures that accelerate age-related pathobiology (eg, tobacco use), and epigenomic remodeling provide the conceptual basis for the development of so-called DNA methyl clocks as novel readouts for biological age (155-158). Importantly, some of the same metabolic pathways that provide substrate for epigenetic chemical modifications are also sensitive to energy balance and fasting, including methionine metabolism and the supply of DNA methyl donors, catabolic production of acetyl-CoA from glucose and lipids to supply substrate for histone acetylation, and ketogenesis with ketones such as β-hydroxybutyrate exhibiting direct inhibitory properties on the action of histone deacetylases, while also providing substrate for β-hydroxybutyrylation of histone lysines (159, 160). There are multiple potential points of intersection between fasting-sensitive metabolic processes and epigenomic remodeling that in theory could restore a more youthful epigenomic landscape, with the caveat that genome-scale changes to transcriptional regulation may not be uniformly beneficial. Although fasting mediated changes to the epigenomic landscape provide a potential mechanism for sustained metabolic reprogramming, the exact nature and temporal dynamics of epigenetic remodeling with fasting and refeeding—ie, the durability of any putative reprogramming effect, remains incompletely defined.

Inflammation

Low-grade sterile inflammation is associated with aging and in turn inflammation is a potential driver of aging biology and diseases of aging, including diabetes and cardiovascular disease (131, 161). Chronic caloric restriction in nonhuman primates is associated with reduction in inflammatory biomarkers and intermittent fasting or caloric restriction in rodents reduces inflammatory biomarkers and inflammatory transcriptional programs (153, 162, 163). Humans who engage in religious fasting also demonstrate a reduction in inflammatory biomarkers (164).

Attenuation of leptin and leptin signaling as occurs with acute fasting may directly regulate immunity. Although obesity is generally a pro-inflammatory state, the obesity associated with genetic loss of leptin function is paradoxically associated with immunosuppression and increased risk of severe infection (165). At the other end of the weight spectrum, the leptin deficiency associated with failure of fat development or acquired loss of adipocytes in lipodystrophic syndromes is also associated with increased risk of severe infection (166).

The role of leptin as an immunomodulator is further supported by the expression of the leptin receptor on immune cells and reductionist studies demonstrating that signaling through the leptin receptor in many of these is functionally active (167-169). Collectively, these examples point to a potential intrinsic anti-inflammatory or immunomodulatory effect of caloric restriction or fasting and offer one plausible contributing mechanism of benefit for dietary restriction.

mTor signaling is another example of a nutrient-sensitive pathway that regulates the immune system, including through immune cell proliferation and differentiation, cytokine production, antigen presentation, and immune cell trafficking, which are attributes that form the basis for the use of the mTor inhibitor rapamycin as an immunosuppressant medication (170). It is therefore tempting to speculate that the life-prolonging effect of targeting mTor with rapamycin, as observed in model organisms (171), is attributable at least in part to a reduction in inflammation. Indeed, while targeting mTor signaling may reduce some aspects of sterile inflammation and "inflammaging" in model organisms (172), contradictory evidence suggests that benefits of mTor targeting are uncoupled from the innate inflammatory response (173). Moreover, rapamycin in low doses may paradoxically promote and rejuvenate immune responsiveness (174, 175). The collective data on mTor signaling illustrates how signaling pathways activated by fasting or caloric restriction play complex regulatory roles in immunity and inflammatory responses, while also underscoring the reality of the competing adaptive and maladaptive roles for the immune system in survival. It is also critical to recognize that fasting may have distinct effects on different immune cell types, modulating the immune cell composition of circulating or tissue reservoirs and modulating immune cell phenotypes.

Cancer

Intermittent fasting and caloric restriction attenuate cancer development in preclinical models of diverse cancer types—including melanoma, breast, bladder, colon, and pancreatic cancer—and many of the metabolic and hormonal adaptions involved in the fasting response are relevant to cancer biology (176-180). Fasting mediated changes in hormonal signaling, such as the insulin pathway and the GH/IGF1 axis, and/or fasting-mediated shifts in the metabolic milieu may account for attenuation of malignant transformation and/or cancer progression.

A reduction in calories results in a cumulative decrease in insulin release if carbohydrates are proportionally reduced. Aside from regulating glucose homeostasis, insulin signaling through the PI3K signaling pathway promotes growth (181). The mitogenic effects of insulin-PI3K signaling are well documented in cancer biology, providing the conceptual basis for anticancer drugs targeting the pathway. Therefore, lower cumulative exposure to insulin and in turn insulin stimulated PI3K/AKT signaling over long periods may reduce cancer initiation and/or progression.

IGF-1 can also bind to the insulin receptor, albeit with reduced affinity, and therefore may replicate some aspects of insulin action; however, the more important pro-growth properties are attributable to signaling through the IGF-specific receptor. IGFR1 is a tyrosine kinase receptor that when engaged by IGF-1—or insulin with lesser affinity—leads to signaling cascades that are mitogenic (182, 183). The pro-growth effects of GH are primarily mediated through the actions of IGF-1. Therefore, the GH resistance that is characteristic of the fasting response, which results in reduced circulating IGF-1, may in turn reduce cumulative mitogenic exposure. The potential role of decreased IGF-1 signaling as a mediator of benefit from fasting or caloric restriction is conceptually supported by increased longevity in mice in which the GH/IGF-1 axis is genetically targeted via disruption of the GH receptor and the observation that reduced IGF-1 expression is associated with longevity both within and across diverse species (184, 185).

Last, fasting metabolites themselves may play a role in cancer biology. The most notable example may be ketones, which may inhibit cancer cell growth (186). Such observations underpin a current interest in dietary interventions that augment ketone production as an adjunct intervention for cancer therapeutics (187). Even though fasting leads to ketogenesis within approximately 1 day, diets that are ketogenic simply through manipulation of macronutrient composition may be a more efficacious approach to achieve sustained elevation in ketone bodies.

Lipid Metabolism

Circulating bioactive lipids can drive an array of pathophenotypes linked to diseases of aging including atherosclerosis and insulin resistance and therefore any potential reprogramming of lipid metabolism with fasting could be disease modifying. Acute fasting does not have a consistent effect on traditional components of a clinical lipid panel, but when granular metabolomics methods are applied, dramatic changes in triglyceride composition are evident. The evolution of triglycerides with a 10-day fast in humans involves a shift in favor of high carbon content, unsaturated triglycerides and a reduction in low carbon content, saturated triglycerides (116). Importantly, this shift is already evident in the first day of fasting, persists in the day after refeeding, and is a pattern that in epidemiological studies predicts protection from diabetes and age-related frailty (188-193). Transcriptomics data from fasting and refed mouse liver also suggest the possibility that fasting reprograms lipid metabolism, as the upregulation of some genes involved in lipid metabolism persist after refeeding (194). Such data raise the question of whether fasting can reprogram lipid metabolism such that the benefits persist beyond the period of actual fasting.

Catabolic Dissolution of Pathological Biomass

We introduce the term "pathological biomass" to describe the cellular or extracellular molecules that promote disease or aging pathology either because their accumulation in excess drives tissue dysfunction or because the molecules themselves, particularly when damaged, have pathological signaling properties. The term "biomass" is more commonly applied in cancer biology to describe how tumor material expands because of anabolic growth and cellular proliferation (195). Outside of cancer, in states where growth is not unrestrained to the point of tissue invasion and metastasis, less extreme imbalances in the turnover of biomolecules can also lead to pathological accumulation. Certain biomolecular classes turn over at lower rates with aging, including, for example, neutral lipid in adipocytes and specific proteins in the brain (196, 197). In addition, subsets of proteins in a variety of tissues have been shown to exhibit extremely long half-lives, which may therefore render them sensitive to cumulative damage even without

an age-dependent change in turnover rates (198-201). Similarly, exposure of lipids to oxidative stress drives formation of oxidized species including lipid peroxides, which may accumulate in some tissues with aging and have been associated with diseases of aging, in part from damaging effects to cells and proinflammatory properties (202-204). Several diseases of aging are linked to accumulation of specific pathological protein aggregates, such as Alzheimer's disease, where extracellular amyloid plaques and intracellular tau are disease drivers, or senile amyloidosis, where polymerization of transthyretin proteins causes tissue dysfunction in the heart and nervous system among other tissues (197, 205, 206).

Because fasting is an inherently catabolic state, a theoretical mechanism of benefit of fasting is through the catabolic dissolution of pathological biomass. We have advanced the concept that fasting drives a transition from glucose to lipid metabolism and therefore fasting that is of sufficient duration to activate lipid catabolic programs will lead to increased lipid turnover. Indeed, there is precedent for a prolonged acute fast being sufficient to lower circulating biomarkers of lipid peroxidation (207). We have also acknowledged that the shift to lipid metabolism is a survival mechanism to prevent catabolic consumption of the protein backbone of the body. However, even with the reduction in glucose utilization coincident with the adaptive transition to lipid metabolism, there is ongoing gluconeogenesis to support minimal glucose requirements, including those of the pool of red blood cells, for which glucose is the obligate energy source. As such, catabolic breakdown of macromolecules during fasting extends beyond glycogen and lipids to include proteolytic release and utilization of amino acids.

Catabolism of proteins and other biomolecules can proceed via multiple mechanisms. Analogous to the canonical lipolysis pathway, specialized enzymes contained in proteosomes can catabolize intracellular proteins that have been targeted for degradation by ubiquitination (208). Autophagy is a process that can be activated by fasting in which cells consume intracellular components up to entire organelles (209, 210). When mitochondria are damaged, for example, the process of mitophagy entails encompassing of entire mitochondrion by a membrane formed from the endoplasmic reticulum into a vesicle called an autophagosome, which is in turn trafficked to lysosomes, specialized organelles that are acidified and contain degradative enzymes that operate at low pH and serve as the ultimate catabolic effectors (211). Extracellular biomass can be subject to enzymatic degradation, for example by the action of metalloproteinases on extracellular matrix; however, more complex and higher volume states of localized biomass may require the action of professional phagocytic cells. In the case of atherosclerotic vasculopathies, for example, which can be viewed as states of pathological biomass because of the obstruction of blood flow from cholesterol accumulation and the cells and debris that comprise an advanced atherosclerotic lesion, it is increasingly recognized that the failure of professional phagocytic cells to consume debris and dead/dying cells in atherosclerotic plaques is a pathological driver (212) (213). Fasting has been linked to many of the catabolic pathways that could be operative in the dissolution of pathological biomass including autophagy, proteosome activity, and activation of professional phagocytes (44, 214, 215). In mice, the potential benefits of activating catabolic pathways, such as autophagy, are demonstrated by enhanced lifespan when the aging decline in autophagy is rescued by transgenic overexpression of an autophagy program (216). While augmented turnover of biomolecules that are dysfunctional and/or present in excess is a theoretical benefit of fasting, 1 caveat is that catabolic mechanisms may not preferentially target biomolecules that are dysfunctional or present in excess.

Potential Mechanisms of Harm From Fasting

A long-established evidence base suggests life-prolonging benefits of caloric restriction in model organisms, which have been extended to intermittent fasting and time-restricted feeding protocols (217, 218). Yet, the narrow and controlled conditions in which model organisms are studied undoubtedly do not recapitulate the full breadth of stressors that impact human health. Murine studies have historically been biased toward studies in male mice and with much of the evidence base established in a narrow set of inbred mouse strains. In 1 study in which caloric restriction was evaluated across 41 mouse strains, only 5% and 21% of strains in males and females, respectively, exhibited increased lifespan in response to caloric restriction (219). Even if salutary benefits of fasting are conserved in humans, however, it is possible that negative consequences of fasting may be more relevant to free-living human populations (ie, individuals who are exposed to the external stressors of daily life). Epidemiological studies demonstrate a J-shaped curve with respect to the relationship between BMI and mortality, as mortality increases when BMI is low (220, 221). It is also well known that patients with anorexia nervosa have a high mortality rate, that is independent of associated psychiatric disease and suicide (222). These data raise the question of whether achievement of a low BMI by caloric restriction or fasting would be harmful, providing added rationale to consider possible mechanisms of harm.

Inflammation

We discussed improvement in low-grade systemic inflammation as a potential benefit of caloric restriction or intermittent fasting. Surprisingly, when we performed longitudinal transcriptomics analysis of human adipose tissue with prolonged fasting, we discovered an increase in transcripts associated with macrophages and inflammation (44). This translated into a corresponding increase in histological evidence of macrophages and an increase in the circulating inflammatory biomarker, C-reactive protein. The fasting-mediated C-reactive protein surge with prolonged fasting has been confirmed in another human study (223). When the contraction of fat mass and weight loss is protracted over several weeks in a model of chronic caloric restriction in obese mice, an influx of macrophages is detectable (224). Similarly, intermittent fasting in obese mice can drive accumulation of lipid-associated macrophages that may contribute to metabolic inflammation and pathological adipose tissue phenotypes (225). However, even in instances when fasting stimulates inflammatory pathways or inflammation, it may not result in improved immune function. In mice, acute fasting for 24 hours followed by refeeding increases trafficking of old monocytes from the bone marrow to peripheral tissues, such as the lung, and augments pro-inflammatory cytokine responses, but also reduces host defense to clinically relevant bacterial infections of the lung (226). The extreme catabolic state associated with more prolonged fasting periods may partially account for the observed proinflammatory effects; however, these data

suggest that fasting and/or caloric restriction may promote potentially maladaptive inflammation in some contexts.

Loss of Bone Mineral Density

In individuals with anorexia nervosa, a model of chronic starvation, the most common medical complication is low bone mineral density (227). Approximately 85% of women with anorexia nervosa have a bone mineral density value at least 1 SD below the mean of women of similar age and nearly 35% have a bone mineral density value that is at least 2.5 SDs below the mean—the definition of osteoporosis in postmenopausal women (227). Males with anorexia nervosa also have low bone mineral density (228, 229) and, as is seen in other populations with low bone mineral density, individuals with anorexia nervosa also experience a higher rate of fractures (230-234). Similarly, in a randomized controlled trial of 24 months of calorie restriction (CALERIE), significant decreases in bone mineral density at the spine and hip were observed in the calorie restriction group (mean weight loss of 7.5 ± 0.4 kg) compared to the control (ad libitum feeding) group (mean weight change of $+0.1 \pm 0.5$ kg) after both 12 and 24 months of calorie restriction (235). In fasting individuals, bone formation markers decrease within the first 4 days of fasting initiation (236, 237). With recent advances in bone imaging, even short-term fasts result in changes in bone microarchitecture. Trabecular bone parameters including trabecular number and bone volume fraction in the radius were shown to decrease significantly after a 10-day zero-calorie fast (237). Potential mechanisms for this loss of bone mineral density include changes in methionine levels with fasting and hormonal adaptations to fasting including increases in the counter-regulatory hormone cortisol and decreases in IGF-1 levels due to GH resistance (see section Growth Hormone Resistance and Hypogonadotropic Hypogonadism), which have negative effects on bone mass (93).

Translational and Human Fasting Studies With Clinical Endpoints

Data From Model Organisms

In model organisms, the lifespan prolonging effects associated with sustained caloric restriction have also been observed with dietary restriction protocols that enforce periods of zero-calorie fasting (141, 217, 238, 239). In addition, periodic fasting protocols in mice have also improved pathological metrics in experimental models of diseases of aging, such as Alzheimer's disease (240, 241). The timescale of the adaptive fasting response between rodent models and humans challenges easy translation of results from rodent studies to humans. This includes the different timescales of the adaptive fasting response, such that the fasting period in murine "time-restricted feeding" studies (eg, often 18-21 hours) is effectively a much more profound fasting period than a similar fasting duration in humans. The much longer human lifespan sets practical limits on testing the effect of dietary restriction protocols on longevity. Chronic diseases of aging often also transpire over much longer time periods and therefore require long, large-scale studies to assess efficacy of dietary interventions for disease prevention. Consequently, the evidence base supporting fasting interventions to promote metabolic health in humans is limited.

Human Studies

Studies of human populations that engage in fasting for religious or other reasons demonstrate intriguing signals of potential benefit (242, 243). However, the potential benefits of fasting have only recently begun to be explored in a prospective fashion in humans. We undertook a survey of the existing literature and evaluated studies identified via a PubMed search using the following criteria: randomized, clinical trials published in English that included intermittent fasting, time-restricted feeding, time-restricted eating, or alternate-day fasting in the title or abstract through October 14, 2024. We included studies with a control group and did not include studies that were focused on a specific disease population other than metabolic syndrome, fatty liver disease, or diabetes. Therefore, we excluded studies focused on outcomes in patients with rheumatoid arthritis or chronic kidney disease. We also only included studies that were greater than 2 weeks in duration. Our list included 79 publications, with the earliest study being published in 2011 (244).

The review of the literature demonstrates that the studies are predominantly short term. All but 5 studies ranged in length from 3 weeks to 6 months with the remaining studies being 1-year studies. There was great variance and overlap in definitions of time restricted eating and intermittent fasting. With respect to time restricted eating manuscripts ($n = 38$), hours of eating ranged from 4 to 12 hours daily with the majority ($n = 25$) indicating 8-hour eating windows. Similarly, intermittent fasting definitions varied greatly with respect to number of calories that could be consumed during a fasting day and few studies prescribed zero calories during fasting periods. Additionally, 1 study looked at the combination of intermittent fasting with time-restricted eating on metabolic outcomes in individuals at risk for type 2 diabetes mellitus (245), whereas others combined intermittent or alternate-day fasting with exercise, with the majority of these short-term studies showing no added benefit of exercise with respect to cardiometabolic endpoints (246-248).

As changes in dietary composition can also have potential cardiometabolic effects, we looked at whether dietary composition was altered or controlled in any of the reported studies. Nearly half of the 79 studies did not require participants to follow a specific macronutrient composition, although 9 of these studies did provide participants with dietary or nutritional counseling. Eleven additional studies made specific recommendations regarding macronutrient content, with the majority recommending balanced macronutrient intake by either following a Mediterranean diet or a diet of similar macronutrient composition (~25%-30% fat, ~50%-55% carbohydrates, and ~15%-20% protein). An additional 22 studies provided meals to the participants for at least some portion of the study, with the majority providing meals with a balanced macronutrient breakdown. Therefore, while some studies prescribed changes to dietary macronutrient content as an additional and potential confounding factor to any fasting effect on cardiometabolic outcomes, nearly half of the studies did not have participants alter their dietary composition.

In the majority of manuscripts ($n = 69$), mean or median BMI of the participants randomized to time restricted feeding or intermittent fasting was >25 kg/m^2. Overall, studies of intermittent fasting demonstrated metabolic benefits associated with weight loss (Fig. 4). Whether fasting has metabolic benefit independent of weight loss has not been well studied. A 3-week study of lean (BMI < 24.9 kg/m^2) individuals

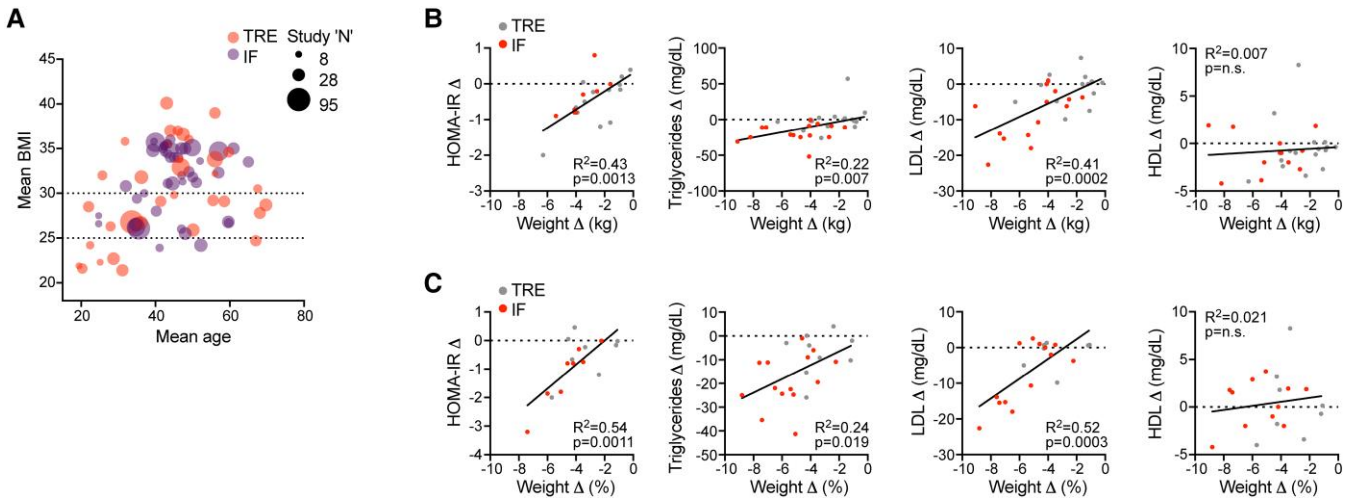

Figure 4. Weight loss and cardiometabolic metrics in human trials of intermittent fasting or time-restricted eating. (A) Time-restricted eating (TRE) and intermittent fasting (IF) studies are plotted as a function of the number of participants in the intervention arm (Study "N"), the mean age, and the mean body mass index (BMI). Dotted lines indicate overweight (BMI of 25 kg/m^2) and obese (BMI of 30 kg/m^2) thresholds, underscoring that the majority of studies have been conducted in participants with excess body weight. Studies conducted in normal weight participants were generally in young, healthy populations, often with sports physiology outcomes rather than cardiometabolic health outcomes. (B) Decrease in absolute weight (kg) is associated with positive changes in insulin resistance (homeostatic model assessment of insulin resistance [HOMA-IR]), triglycerides (mg/dL), and LDL (mg/dL) across studies; change in HDL (mg/dL) was not significantly associated with change in absolute weight (kg) across studies. Mean changes are plotted on both axes. (C) Decrease in weight (% change from baseline) is associated with positive changes in insulin resistance (HOMA-IR), triglycerides (mg/dL), and LDL (mg/dL) across studies; change in HDL (mg/dL) was not significantly associated with percent change in weight across studies. Mean changes are plotted on both axes.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

investigated the effects of alternate day fasting with compensatory increases in caloric intake on nonfasting days as compared to 2 other groups: (1) daily caloric restriction and (2) alternate-day fasting with energy intake matched to the caloric restriction group (139). The calorically neutral alternate day fasting group lost significantly less body mass than the calorically decreased alternate-day fasting group (−0.52 kg vs −1.60 kg; $P = .04$). Despite these differences in weight loss, there were no significant differences in homeostatic model assessment of insulin resistance, total cholesterol, low-density lipoprotein (LDL), or high-density lipoprotein (HDL) between the groups and levels did not significantly change compared to baseline in either of these alternate-day fasting groups (139). Importantly though, this study was of short duration (3 weeks in length), which may in part explain these findings. Collectively, prospective studies of fasting interventions in humans demonstrate signals of metabolic benefit; however, associated weight loss may be an important mediator. Understanding the potential effects of fasting independent of weight loss remains an active area of investigation (140).

Conclusions, Unanswered Questions, and Future Directions

In this manuscript, we have focused on adaptive mechanisms to fasting and their potential relevance to health and disease (Fig. 5). We conclude that there are clear signals of potential health benefits to fasting that are strongest in the context of overweight and obesity, where fasting protocols may promote weight loss and mitigate associated risks of cardiometabolic disease, reproductive dysfunction, and cancer. There may be other patient populations that also derive more pronounced health benefits from fasting interventions, for example, those with autoimmune diseases such as rheumatoid arthritis where fasting has been shown to have at least short-term benefits because of a putative anti-inflammatory effect (249). However, we also acknowledge that there are potential harms from fasting, most notably through effects on bone metabolism. Therefore, it is likely that there are at least some contexts in which the potential harm from fasting exceeds the benefits, for example individuals who are prone to osteoporosis including low-weight women. Despite more than a century of intense scientific study of the biology of fasting, however, much remains unknown. In the ensuing final paragraphs, we contextualize what we view as key unanswered questions.

Can Unbiased Multi-omics Analyses Redefine the Stages of Starvation?

It is impossible in such a review to ignore the centrality of canonical hormonal systems—eg, insulin and glucagon signaling—which have a large evidence base to support their involvement across multiple cell types and tissues. However, when unbiased-omics methods are applied to fasting humans or model organisms, the sheer number of bioactive molecules that are dynamically modulated in circulation or at the tissue level with fasting is substantial. In fasting rats, greater than one third of measured serum metabolites and approximately 5% to 10% of transcripts were statistically modulated in metabolically relevant tissues (liver, muscle, intestine) with fasting (250). We conducted a longitudinal analysis of humans subjected to a 10-day zero calorie fast, with repeated sampling of blood and subcutaneous fat for metabolomics and transcriptomics. Of 544 measured plasma metabolites, 65% changed in a statistically meaningful way (116), whereas 20% of the nearly 25 000 detectable adipose tissue transcripts were significantly modulated (44). The dramatic transcriptional response to fasting is also reflected by the circulating proteome, with a recent study demonstrating significant modulation of approximately one third of ~3000 measured plasma proteins over a 7-day fast (251). Prolonged fasting may also

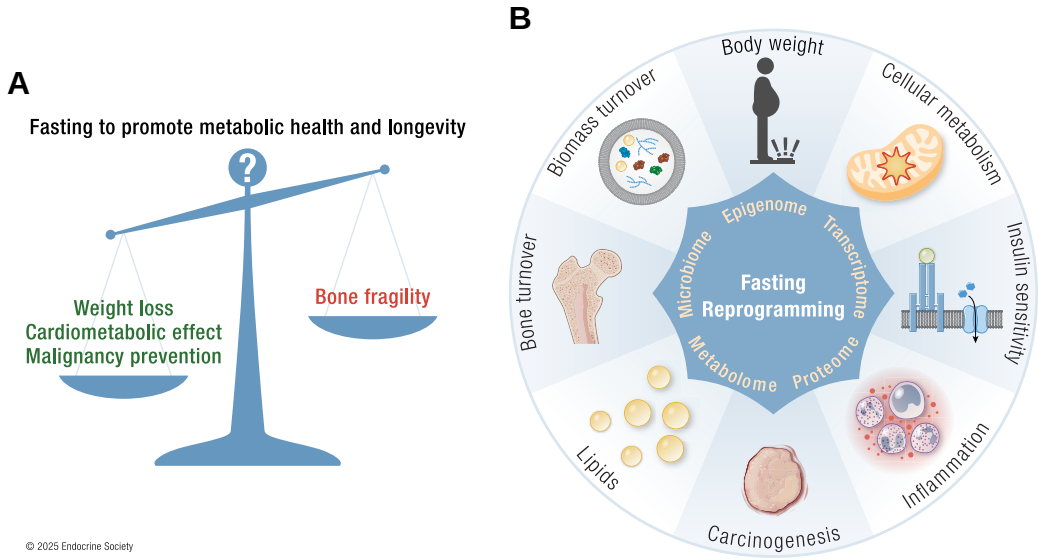

Figure 5. Molecular reprogramming and candidate mechanisms mediating benefits and potential harms of fasting. (A) There are clear signals of potential health benefits from fasting including weight loss, improvement in cardiometabolic metrics, and prevention of malignancy amongst others. The degree to which such benefits of fasting are potentially offset by harm to bone is not known. (B) Multi-omics readouts demonstrate large-scale changes to the microbiome, epigenome, transcriptome, proteome, and metabolome, with functional implications at the cellular, tissue, and systemic levels, including key parameters listed at the perimeter of this wheel and discussed in this manuscript.

remodel the microbiome, changing the composition of microbial populations to which immune cells are exposed. Beyond local effects attributable to the direct interfacing of the microbiome with immune cells, increased microbial diversity is generally viewed as healthy and accounts for some of the observed improvements in systemic metabolic function with fasting protocols (252-256). Additional operative mechanisms of benefit may include modulation of immune cell trafficking and levels of bioactive microbial metabolites in systemic circulation, including short-chained fatty acids (253). These examples demonstrate that there remains much yet to be learned about the bioactivity of many of these molecules in fasting, not to mention how these responses are modulated by disease states. Indeed, other studies involving different -omics time-series analyses challenge the concept of discrete and sequential phases of adaptive fasting, with evidence of transcriptional responses associated with putative late adaptive stages (eg, lipid catabolism) already evident at early timepoints (257, 258), also in line with evidence in mice that nervous system control of adipocyte lipolysis is triggered at an early fasting stage by depletion of liver glycogen (259). This underscores that the current view of adaptive fasting is undoubtedly oversimplified. It is likely that increasingly granular multi-omics time-series analyses integrating all aspects of the regulatory hierarchy including epigenomic, transcriptomic, proteomic, with metabolite fluxes will not only reveal important new regulatory nodes, but also demonstrate that adaptive responses operate at multiple different time scales. The application of single cell methods in human studies will also be critical, in particular to define the nuanced effects of fasting on the immune system, as it is clear from studies in model organisms that fasting not only affects the cellular phenotypes of immune cells in distinct ways (eg, promoting M1 to M2 phenotypic switching of macrophages), but may also modulate cell trafficking and in turn the cellular composition of both circulating and tissue immune cell reservoirs (226, 260, 261). Multi-omics analyses may also reveal the temporal dynamics and durability of putative molecular reprogramming effects after completion of fasting.

How Does Aging Modify the Fasting Response?

Intermittent fasting and caloric restriction are considered potential antidotes to aging, but advancing age may itself modulate the response to these interventions. Epidemiological studies demonstrate that weight loss in the elderly often precedes a functional decline and death. Such associations likely implicate some degree of reverse causation– that the weight loss is a biomarker of illness rather than a causal mediator. Yet, this signal may not be fully accounted for by occult illness. Indeed, recent murine studies in which caloric restriction or intermittent fasting are initiated in older animals in some instances appears to accelerate death (262). It will be important to definitively determine if the weight loss that often accompanies initiation of caloric restriction or intermittent fasting is harmful in the elderly, and if so, what are the underlying mechanisms.

Is There an Optimal Fasting Protocol With Respect to Duration and Frequency?

As described at the outset of this review, intermittent fasting is not well-defined and encompasses protocols with varying durations, frequencies, and degrees of fasting. The duration of each fasting dose may be critical because the adaptive phases to fasting occur at different time scales. If the transition to lipid metabolism is beneficial, for example, then a day or longer of fasting may be required to maximize benefit. It will also be important to determine the optimal frequency of fasting to achieve and maintain any beneficial reprogramming effect. Although it is not unusual for studies to include at least 1 analytical time-point after some period of refeeding, the duration of any putative molecular or metabolic reprogramming effects due to fasting have not been defined to the granular degree applied to the response to fasting itself. Last, it is conceivable that individual factors such as age, sex, and the degree of baseline adiposity may influence the temporal dynamics of both the fasting response and durability of any reprogramming

effect, an understanding of which could inform "precision" fasting prescriptions.

Are There Benefits of Fasting Independent of Weight Loss?

Intermittent fasting (and caloric restriction) are both associated with weight loss, particularly in human studies conducted to date, which have largely been in overweight and obese individuals (see section Human Studies). The potential confounding effects of alleviation of excess adiposity may also be relevant to studies in model organisms. For example, fasting or caloric restriction studies are often conducted in obesogenic mouse strains that may exhibit pathological adiposity even on a standard laboratory "control" diet. It will be important to determine the degree to which benefits of fasting can be extended to normal weight individuals and the degree to which any observed benefits are independent of weight loss.

Are Benefits of Caloric Restriction or Fasting Fully Conserved in Humans?

Definitively testing whether caloric restriction or intermittent fasting extends life in humans is challenging due to the length of the human lifespan relative to model organisms, not to mention the notorious difficulty achieving long-term compliance with any experimental dietary intervention. This reality underscores a more general unmet need in the aging field for more sophisticated aging biomarkers. Major efforts to define and map senescent cells with more sophisticated methods and additional discovery and validation of novel circulating biomarkers such as epigenetic DNA "clocks" could greatly accelerate the testing of dietary—or pharmacological—interventions to promote lifespan.

Are There Sex Differences in the Fasting Response?

Given the differences in body composition and hormonal composition between males and females, it is not surprising that there are differences observed in metabolic responses to various processes including fasting (263). Differences between males and females have been studied predominantly and most rigorously in response to fasting for 72 hours or less. Consistently, glucose levels are significantly lower in females compared to males and plasma free fatty acid levels are higher in response to these shorter term, prolonged fasts (263, 264). These differences in the fasting response may be due in part to estradiol, given estradiol's effects on insulin action. For example, when postmenopausal women with baseline serum estradiol levels of <15 pg/mL are administered intravenous conjugated estrogens, there appears to be improved insulin action in in the setting of a hyperinsulinemic-euglycemic clamp (265). Given differences in response to short-term fasting and hormonal differences, it will be important for future studies investigating the fasting response to adequately delineate outcomes in males vs females and also to differentiate between premenopausal and postmenopausal females.

Can the Beneficial Effects of Fasting be Recapitulated by Pharmacologically Targeting Key Regulatory Nodes?

Even if some version of the dietary interventions described in this article are proven to have robust health benefits in humans, broad implementation may not be practical. In model organisms, caloric restriction or intermittent fasting protocols associated with extension of lifespan are typically implemented early in life. In humans, there is generally poor long-term compliance with dietary prescriptions, even those with proven benefit. Investigation of the cellular and molecular mechanisms underpinning the salutary benefits of fasting could in theory provide a pharmacological target. Canonical signaling pathways that are modulated by fasting are also the targets of drugs that are already in clinical practice. AMP kinase is induced by fasting, is 1 of the pathways targeted by the diabetes drug metformin, and therefore metformin is being repurposed as a potential antiaging therapy (266). mTor is another nutrient sensing regulatory node. Rapamycin increases life and health span in model organisms, which explains ongoing interest in developing the drug for treatment of aging and related diseases (171). The ideal fasting mimetic would activate beneficial fasting pathways in key tissues without recapitulating negative consequences of fasting or producing off target effects, underscoring the importance of incorporating careful consideration of drug toxicity into implementation of mimetic strategies. In the case of rapamycin, for example, potentially harmful metabolic (insulin resistance) and immunological effects may be at least partially mitigated by using lower doses than commonly used for immunosuppression of transplant patients (267). Ultimately, it may be through such repurposing of existing drugs or development of new fasting mimetics that the promise of the health benefits of fasting are realized beyond the rare individuals who have the ability to implement challenging, lifelong dietary prescriptions.

How Can the Potential Life- and Health-span Benefits of Fasting or Fasting Mimetics be Assessed in Human Trials?

The collective clinical trials of fasting interventions to date have generally been modest in size, of duration ranging from weeks to months, and focused on easily quantified cardiometabolic endpoints that tend to be sensitive to weight loss. Because longevity is not a practical endpoint for human trials, short-term randomized studies have looked at potential biomarkers associated with aging (268). An important question, however, is what endpoints would be relevant to assess efficacy of interventions to improve healthspan or augment lifespan in normal weight populations or populations with low cardiometabolic risk? Recognizing that a single biomarker may not capture the aging process, an expert group advanced a multimarker panel inclusive of inflammatory, metabolic, and tissue specific markers of aging phenotypes (269). There is also great interest in validating epigenetic clocks, which quantify patterns of epigenetic modifications purported to indicate biological age, thereby identifying individuals that are aging faster or slower than predicted by chronological age (155). Such ongoing efforts to validate a consensus biomarker predictive of healthspan or lifespan is a critical barrier to advancing antiaging interventions, including fasting or fasting mimetics (270-272).

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