Metabolic Flexibility and Its Impact on Health Outcomes

Biff F. Palmer, MD, and Deborah J. Clegg, PhD

Abstract

A metabolically flexible state exists when there is a rapid switch between glucose and fatty acids during the transition between the fed and fasting state. This flexibility in fuel choice serves to prevent hyperglycemia following a meal and simultaneously ensures an adequate amount of blood glucose is available for delivery to the brain and exclusively glycolytic tissues during fasting. The modern era is characterized by chronic overnutrition in which a mixture of fuels is delivered to the mitochondria in an unabated manner thereby uncoupling the feast and famine situation. The continuous influx of fuel leads to accumulation of reducing equivalents in the mitochondria and an increase in the mitochondrial membrane potential. These changes create a microenvironment fostering the generation of reactive oxygen species and other metabolites leading to deleterious protein modification, cell injury, and ultimately clinical disease. Insulin resistance may also play a primary role in this deleterious effect. The imbalance between mitochondrial energy delivery and use is made worse with a sedentary lifestyle. Maneuvers that restore energy balance across the mitochondria activate pathways that remove or repair damaged molecules and restore the plasticity characteristic of normal energy metabolism. Readily available strategies to maintain energy balance across the mitochondria include exercise, various forms of caloric restriction, administration of sodium-glucose cotransporter-2 inhibitors, cold exposure, and hypobaric hypoxia.

During the course of evolution, human survival was shaped by food scarcity in which there were periods of feast or famine. Daily living was characterized by abundant exercise, often under conditions of fasting, due to foraging behavior required to survive in a setting of unpredictable food supply. Human metabolism required great flexibility in the use of metabolic substrates depending on their availability to meet energy demands. The ability to store fat in anticipation of food shortage was advantageous for survival. With development of agriculture and later the industrial revolution, a mismatch was created between the evolutionary design characteristic of metabolism in which there was alternating depletion and reload of energy stores to a metabolism responding to overconsumption of calorically dense processed foods in combination with decreased energy expenditure due to physical inactivity. This mismatch has been linked to the growing epidemic of obesity and its associated comorbidities such as metabolic syndrome, type II diabetes mellitus, and nonalcoholic fatty liver disease. At the cellular level, the facile transition between fuel choices in response to nutritional circumstances at the level of the mitochondria is replaced by a rigid system characterized by continuous influx of surplus fuel in combination with decreased adenosine triphosphate (ATP) consumption. This imbalance creates reductive stress in the mitochondria and over the long term ultimately leads to irreversible damage to cellular macromolecules, organ dysfunction, and ultimately clinical disease.

FUEL SWITCHING IN THE ABSORPTIVE AND POST-ABSORPTIVE STATES

Metabolic flexibility describes the ease and rapidity by which metabolism transitions...
between the absorptive (fed) and post-absorptive (fasting) state. In the absorptive state, glucose and amino acids directly enter the blood across the enterocyte while lipids are first packaged into chylomicrons and are delivered into the blood after first traversing the lymphatics. Increased blood glucose levels signal release of insulin, which directs glucose to glycogen synthesis in the liver and skeletal muscle. Glucose entry into adipocytes is directed to glycerol 3-phosphate and triglyceride synthesis. Insulin promotes the entry of digested branch chain amino acids into skeletal muscle for protein synthesis.

In the absorptive state, glycolysis and pyruvate oxidation is the preferred pathway for energy needs (Figure 1). Glucose-induced rise in pyruvate levels exerts an inhibitory effect on pyruvate dehydrogenase complex and production of acetyl coenzyme A (CoA) that enters the citric acid cycle to undergo oxidative phosphorylation for generation of ATP. Increased production of citrate with subsequent export into the cytoplasm leads to increased levels of malonyl CoA. Malonyl CoA exerts an inhibitory effect on fatty acid β-oxidation and is used for fatty acid synthesis.

The post-absorptive state is characterized by a decrease in insulin levels and increased glucagon secretion in response to reductions in plasma glucose. Glucagon stimulates glycogenolysis causing release of glucose while reductions in insulin decrease transport of glucose into skeletal muscle and adipocytes; thereby ensuring adequate amounts of blood glucose availability for delivery to the brain and exclusively glycolytic tissues such as erythrocytes, the kidney medulla, bone marrow, and peripheral nerves. Liver glucose-6-phosphatase removes the phosphate group from glucose 6-phosphate generating free glucose, which is released directly into the bloodstream. Because skeletal muscle lacks glucose-6-phosphatase, muscle glycogen must first be metabolized to lactate, which is released into the circulation and resynthesized into glucose by the liver and kidney.

Decreased insulin levels also activate lipolysis making fatty acids available to serve as an alternative fuel for skeletal muscle. Oxidation of fatty acids generates acetyl CoA and nicotinamide adenine dinucleotide (NADH), which allosterically and through activation of pyruvate dehydrogenase kinase inhibits the catalytic activity of the pyruvate dehydrogenase complex, thereby ensuring that the small quantity of skeletal muscle glucose uptake is not completely oxidized via the citric acid cycle but is preferentially metabolized to pyruvate and lactate and converted back to glucose in the liver. Decreased entry of acetyl CoA into the citric acid cycle limits the supply of citrate available for export from the mitochondria to the cytoplasm, thereby lowering the levels of malonyl CoA. As a result, fatty acid oxidation is stimulated through increased carnitine

**ARTICLE HIGHLIGHTS**

- Normal human energy metabolism evolved from lifestyles in which energy supply was often preceded by prolonged periods of energy deficit. A survival advantage was given to the ability to rapidly transition between lipids as a mitochondrial fuel source during food deprivation to preferential use of glucose in the fed state.
- Physiology in the modern era is characterized by chronic overnutrition in which a continuous influx of fuel sources leads to impaired fuel switching in the mitochondria, ultimately causing cellular injury, organ dysfunction, and clinical disease.
- When energy supply exceeds demand across the mitochondria, as with a sedentary lifestyle, deposition of fat in non-adipose tissue occurs and is associated with insulin resistance and the metabolic complications of obesity.
- By contrast, skeletal muscle of trained endurance athletes is markedly insulin sensitive and has a high oxidative capacity similar to a fasting individual, despite having elevated lipid content.
- Remedies to restore fuel use and optimization of mitochondrial function can be achieved through exercise, caloric restriction, intermittent fasting, SGLT2 inhibitors, cold exposure, and hypobaric hypoxia.
palmitoyltransferase I activity, which controls the entry and oxidation of long chain fatty acids in the mitochondria. The shift to fatty acid mobilization and oxidation in the liver provides energy to fuel glucose production via the Cori cycle. The early reliance on Cori cycle activity in the post-absorptive state conserves protein by sparing the need for amino acid precursors for gluconeogenesis. As the duration of the post-absorptive state lengthens, muscle proteolysis is required to supply amino acids for liver gluconeogenesis.5

Thus, a generous supply of glucose in the fed state leads to increased glycolysis, glucose uptake and storage, and suppression of fatty acid oxidation, whereas fatty acid oxidation is the preferred source of fuel during fasting, sparing glucose for use by the brain. Metabolic intermediates arising from glucose oxidation are negative regulators of fat catabolism and vice versa. Preferential use of carbohydrate or lipid as fuel and switching between the two likely evolved from conditions where sustained periods of energy deficit preceded the absorptive state. Free communication and cooperativity between the competing fuel sources ensures that energy supply and demand are balanced at the level of the mitochondria. Currently, the partitioning of different energy sources is no longer separated due to chronic overnutrition without intervening periods of decreased food intake. Unremitting delivery of glucose simultaneously along with fatty acids creates an inflexible state in the mitochondria in which electrons are forced into the respiratory chain eventuating in mitochondrial and

![Diagram of metabolic pathways](image-url)
cellular injury. This deleterious effect is magnified when accompanied by less energy demand (inactivity). When energy supply exceeds energy demand across the mitochondria, a rigid and congested state is created predisposing to negative health outcomes. Insulin resistance may also play a primary role in creating this abnormality.

**IMPAIRED FUEL SWITCHING WITH CHRONIC OVERNUTRITION**

Changes in fuel preference as one transitions between feeding and fasting can be detected by changes in the respiratory quotient. This factor reflects cellular rates of CO₂ production relative to O₂ consumption decreasing toward 0.7 with greater amounts of fatty acid metabolism and increasing to greater than 1 when carbohydrate metabolism is dominant. Consumption of a meal enriched with carbohydrates elicits a surge in the respiratory quotient reflecting the predominance of glucose oxidation while the quotient falls in the post-absorptive period reflecting a shift to fatty acid oxidation. Conceptually, the magnitude of the oscillations are more pronounced in a metabolically healthy individual reflecting the ability to freely switch between the oxidative fuels. With overnutrition, the oscillations are blunted, as there is persistent oxidation of a mixture of carbon fuels.
Indirect calorimetry studies examining the balance of glucose and fatty acid uptake across the leg show disturbances in skeletal muscle fuel dynamics in obese subjects when compared with healthy controls. Using a hyperinsulinemic euglycemic clamp to mimic the fed state, glucose uptake in healthy subjects increases 10-fold with contributions from both storage and oxidation, whereas fatty acid uptake dramatically decreases, which is reflected by an increase in the respiratory quotient (RQ) from approximately 0.8 to 1.0. By contrast, glucose uptake is blunted in obese subjects during the clamp. The rate of fat oxidation in skeletal muscle of obese individuals is lower during fasting. The baseline RQ of 0.9 in obese subjects fails to change in response to the clamp suggesting an inflexible state where a mixture of fats and carbohydrates continue to be oxidized despite the changing nutritional context. A limitation of these studies is that they were conducted under clamped conditions rather than excess nutrient consumption. In addition, excessive nutrition modifies metabolic flexibility, which may be both a cause and consequence of disease.

Studies in both animal models and humans suggest feeding a high-fat diet causes an adaptive increase in β-oxidation without a parallel increase in enzymes of the tricarboxylic acid (TCA) cycle. This disconnect results in mitochondrial accumulation of incompletely oxidized lipid species reflected by measurable increases in acylcarnitines. Accumulation of these and other incompletely oxidized substrates cause mitochondrial stress and ultimately may lead to insulin resistance. However, if the high-fat diet is accompanied by increased energy demand, accumulation of incompletely metabolized substrates is minimized and insulin sensitivity is normalized (Figure 2).

Inflexibility in use of substrates causes disturbances in branch chain amino acid (BCAA) (leucine, isoleucine, and valine) homeostasis described in states of chronic overnutrition. Unlike other amino acids which are extensively metabolized in the liver following gastrointestinal absorption, low levels of hepatic mitochondrial branched chain aminotransferase allows these amino acids to be delivered into the systemic circulation where they are first metabolized in peripheral tissues. Increased levels of BCAA play a contributory role in development of insulin resistance in obese individuals. Reductions in adipocyte metabolism leads to increased circulating levels of BCAA. In the setting of overnutrition, glucose and fatty acid uptake by adipocytes may be the proximate cause of downregulated BCAA catabolic enzymes. Impaired brown adipose tissue activity contributes to elevated levels in an obese or diabetic state. The expanded pool of BCAA is shifted to the liver and, in particular, skeletal muscle as the primary site for metabolism. Metabolism in these tissues generates propionyl CoA and succinyl CoA, which can fill the citric acid cycle through the process of anaplerosis. This effect contributes to inefficient oxidation of fatty acids causing accumulation of incompletely oxidized metabolites of fatty acids and BCAA, particularly in the setting of high-fat diet. Glucose use in muscle is superfluous in the setting of nutrient excess leading to decreased glucose oxidation and ultimately glucose intolerance. In addition, accumulation of BCAA metabolites can constitutively activate mammalian target of rapamycin (mTOR) causing persistent insulin receptor substrate 1 phosphorylation by mammalian target of rapamycin complex 1 (mTORC1) resulting in an inhibitory effect on insulin signaling.

**CELLULAR ADVERSE METABOLIC CONSEQUENCE OF NUTRIENT EXCESS**

Under normal circumstances, flow of electrons to molecular oxygen, the final acceptor, leads to the pumping of protons across the inner mitochondrial membrane, generating a membrane potential and proton motive force that is subsequently used to drive the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate. The availability of ADP is the most important factor in determining the rate of oxidative phosphorylation. Availability of ADP is high under conditions of increased
ATP consumption whereas electron flow is reduced when ATP requirements are low. Each molecule of acetyl CoA generated from continuous oxidation of fatty acids, glucose, and amino acids is accompanied by production and delivery of reducing equivalents to the electron transport chain. When ATP consumption is low and ADP availability is reduced, this continuous influx of reducing equivalents increases the mitochondrial membrane potential and proton gradient eventually leading to leakage of electrons into the matrix (reducing equivalent supply > respiratory demand). 21,22 Rather than reducing oxygen to water, electrons react with oxygen to form superoxide anion followed by rapid conversion to hydrogen peroxide (H₂O₂) by superoxide dismutase.23 At low concentrations, superoxide production may be involved in cellular signal transduction, but at high concentrations, the radicals cause oxidative damage due to their high reactivity towards other cellular compounds.

In addition to production along the electron transport chain, H₂O₂ can also be produced by the pyruvate dehydrogenase complex.24,25 Under normal circumstances, the glutathione redox buffering network masks the majority of this production; however, under conditions of a high-fat diet, the scavenging system becomes oxidized increasing the emission rate of H₂O₂ from the enzyme complex. Additionally, an increase in the NADH/NAD⁺ ratio, as occurs with overnutrition and decreased activity, can increase H₂O₂ production by the alpha-ketoglutarate dehydrogenase complex.26 These additional sources of H₂O₂ under conditions of overnutrition create microenvironments conducive to injury.

Another consequence of the increasing NADH/NAD⁺ ratio is an inhibitory effect on several enzyme systems in the TCA cycle (Figure 2).27 This effect secondarily leads to accumulation of acetyl CoA due to slowing flux and decreasing substrate availability in the cycle. For example, use of acetyl CoA by citrate synthase decreases because this enzyme must first bind oxaloacetate, which can be limiting due to slowing of the cycle.28 The enzyme is also inhibited by succinyl CoA, which is generated in increasing amounts from metabolism of the branched chain amino acids valine and isoleucine, as occurs when mixtures of fuels are continuously being catabolized. As acetyl CoA accumulates, it can act as an acyl donor and through enzymatic and non-enzymatic mechanisms cause modification of proteins such as lysine acetylation, succinylation, and palmitoylation.29,30 The exact makeup of these modifications varies according to the constituents of the diet.31

As alluded to, there are buffering mechanisms identified which act to minimize deleterious effects of superoxide radical formation and protein modifications due to acetylation. These include glutathione and thioredoxin-reducing systems, the carnitine system, and the sirtuin family of NAD⁺-dependent deacylases.32-34 Despite protective systems, nutrient overload through oxidative stress and acetylation and protein modification compromise the integrity and plasticity of the fuel switch mechanisms leading to a sluggish metabolic response to nutritional cues.

**METABOLIC INFLEXIBILITY AND INSULIN RESISTANCE**

Metabolic inflexibility is associated with insulin resistance; however, which one causes the other is still unresolved. According to the Randle hypothesis, increasing the supply and oxidation of fatty acids generates more acetyl-CoA in the mitochondria and subsequently increases the amount of citrate available for transport into the cytosol.3,35 Citrate exerts a direct inhibitory effect on phosphofructokinase activity causing accumulation of glucose 6-phosphate, which in turn inhibits hexokinase activity, resulting in decreased net glucose uptake. In addition to inhibition of glycolysis and glucose oxidation, tissue accumulation of lipid and lipid-derived signaling molecules such as ceramides and diacylglycerol disrupt insulin signaling pathways causing decreased translocation of glucose transporter type 4 (GLUT4) into the cell membrane.36 Lipid accumulation is due to mitochondrial
dysfunction leading to deficiencies of oxidative metabolism and redirection of fatty acids away from oxidation and towards production of toxic lipid species. As previously mentioned, insulin resistance may be a cause of metabolic inflexibility and not a consequence.8,9

Insulin sensitivity is also influenced by energy expenditure.2 This relationship can be viewed as a continuum where the severity of insulin resistance increases as a function of carbon and electron supply relative to ATP demand across the mitochondria. Increasing supply without a change in demand impinges upon redox balance worsening insulin action. By contrast, when increased ATP demand is responsible for increased β-oxidation and supply of carbon and electrons, metabolic balance is maintained across the mitochondria and insulin sensitivity is preserved.

A central role for ATP demand in the genesis of insulin resistance comes from studies examining the effect of high-fat diet on skeletal muscle lipid metabolism. In the early stages of such a diet, key mitochondrial and peroxisomal enzymes are upregulated providing a mechanism for enhanced fat catabolism. Despite this increased capacity, ectopic lipid accumulates because supply exceeds demand. After several months of high fat intake, mitochondrial oxidative capacity is reduced.41 Prolonged exposure to such diets alters mitochondrial morphology to include decreased number, increased swelling, and distorted cristae. Similar morphologic changes are observed in obese humans with or without diabetes, suggesting that at some point in the etiology of diet-induced obesity and/or diabetes, mitochondrial architecture becomes distorted, leading to mitophagy and organelle dysfunction.42 The changes in mitochondrial morphology and function create a vicious cycle because skeletal muscle represents approximately 40% of body weight and approximately 10% to 25% of resting metabolic rate is due to energy expended to support mitochondrial proton leak.43 In some instances, mitochondrial dysfunction may precede the development of obesity. Without a decrease in energy intake, progressive reductions in number and function of muscle mitochondria worsen the imbalance between supply and demand secondary to decreased ATP requirements. Such changes resulting from chronic overnutrition and/or insulin resistance account for observations showing that skeletal muscle fat oxidation capacity is lower in obese compared with lean subjects.44,45 In this chronic phase, mitochondrial dysfunction and or intrinsic deficiencies in oxidative metabolism divert fatty acids away from oxidation toward production of toxic lipid species impinging on insulin signaling.

As previously mentioned, in the early stages of overnutrition there is evidence of increased but incomplete fatty acid oxidation. Accumulation of mitochondrial-derived acyl carnitine intermediates along with decreased citric acid cycle intermediates are consistent with incomplete fatty acid oxidation.46,47 Although defects in mitochondrial function have been implicated as a cause, an alternative explanation is lack of energy demand where the carbon load resulting from β-oxidation exceeds the drive for ATP synthesis.

Maneuvers designed to promote fatty acid oxidation and eliminate ectopic lipid accumulation should improve insulin resistance if defects in mitochondrial fatty acid oxidation is the root cause. Acetyl-CoA carboxylase is a critical enzyme which regulates fatty acid oxidation. This enzyme increases levels of malonyl CoA, which in turn down regulates activity of carnitine palmitoyltransferase (CPT-1) preventing entry and subsequent oxidation of fatty acids in the mitochondria. Studies in acetyl-CoA carboxylase knockout mice where fatty acid oxidation is increased have produced conflicting results regarding restoration of insulin sensitivity.48-50 In models where energy expenditure increases, there is improvement in insulin sensitivity whereas no benefit is seen when energy expenditure is unchanged. A similar link between insulin sensitivity and energy expenditure is found in experiments designed to activate the peroxisome
proliferator-activated family of receptors that stimulate expression of genes involved in transport and oxidation of fatty acids. In transgenic mouse models designed to overexpress peroxisome proliferator-activated receptor α (PPARα) or peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), diet-induced insulin resistance is actually worse despite a markedly enhanced capacity for fat oxidation in muscle. However, in cultured myotubes treated with dinitrophenol, which elevates energy demand by increasing proton conductance in the mitochondrial inner membrane, the deleterious effect of PPARα overexpression on glucose uptake is removed. These data suggest that PPARα overexpression creates a situation where flux through β-oxidation exceeds demand resulting in incomplete oxidation and ultimately contributing to decreased insulin action. Interestingly, rodents treated with PPARα or PPARβ/δ agonists have improved insulin sensitivity and lower rates of weight gain in response to high fat feeding.

Importantly, whole body energy expenditure is increased in these models providing a means to maintain balance across the mitochondria in a setting where fatty acid oxidation is increased.

The importance of matching flux rate through β-oxidation and energy demand as a determinant of insulin sensitivity is supported by additional observations. In lean but insulin-resistant PPARα transgenic mice, inhibiting CPT-1 activity blocks fatty acid oxidation and improves insulin sensitivity despite causing accumulation of lipid in muscle. Similarly, mice genetically engineered to have restricted flux through β-oxidation maintain normal insulin sensitivity despite increased accumulation of fat in muscle, liver, and adipose tissue. Taken together, conditions that increase flux through β-oxidation in the absence of a concomitant increase in energy demand lead to deleterious effects on mitochondrial redox balance and ultimately impinge on insulin activity. By contrast, if the increase in flux is in response to increases in energy demand, metabolic balance is maintained, abnormal reducing pressure on the respiratory system is avoided, and insulin activity is normal.

MUSCLE LIPID CONTENT AND INSULIN RESISTANCE: THE ATHLETE’S PARADOX

Based on the foregoing discussion, deposition of fat in non-adipose tissue may or may not be associated with insulin resistance and metabolic complications of obesity (lipotoxicity). In the context of obesity and type 2 diabetes mellitus, intramyocellular triacylglycerol (IMTG) content correlates directly with muscle insulin resistance and serves as a strong predictor of diabetes risk. In contrast, the skeletal muscle of trained endurance athletes is markedly insulin sensitive and has a high oxidative capacity, despite having elevated lipid content. The greater insulin sensitivity despite an elevated IMTG deposition in the endurance-trained state is referred to as the athlete’s paradox.

Differences in muscle fiber type as well as size, subcellular compartmentalization, and functional and structural differences in lipid droplet characteristics may account for the observed differences between IMTG storage and skeletal muscle insulin sensitivity in the endurance-trained and insulin resistant state. In one study, mixed muscle lipid content was substantially greater in endurance athletes when compared with sedentary type 2 diabetes patients and their weight matched normoglycemic controls. Only approximately 40% of the greater mixed muscle lipid content was attributed to a higher proportion of type I muscle fibers in the endurance athletes compared with those with type 2 diabetes, whereas the remaining difference was explained by a significantly greater IMTG content in the type I muscle fibers of the trained athletes. Type 1 fibers are slow-twitch-oxidative fibers known to contain more total lipid and a greater number of mitochondria when compared with type 2 fast-twitch glycolytic fibers. Lipid accumulation in type 1 fibers serves as a readily available source of energy during exercise.
In patients with type 2 diabetes, lipid droplets are larger and are preferentially stored in the subsarcolemmal region of type II fibers, whereas endurance-trained subjects store lipid in a higher number of lipid droplets that are smaller in size and primarily located in the intermyofibrillar region of type I fibers. Increased amounts of the coating protein, perilipin 5, act to recruit and maintain close proximity of the intermyofibrillar droplets to mitochondria and protect against high-fat diet-induced lipotoxicity. Intramyocellular triacylglycerol content decreases during prolonged submaximal exercise, and analogously to glycogen, intramyocellular lipid content is increased in the trained state. In contrast to the dynamic nature of IMTG depletion and storage in the trained athlete, IMTG stores in the obese and/or type 2 diabetes patient are stagnant and a manifestation of a structural imbalance between plasma free fatty acid availability, fatty acid (FA) storage, and oxidation. Larger lipid droplet size limits accessibility to intramyocellular lipases and the subsarcolemmal location may cause physical hindrance to translocation of GLUT-4 into the cell membrane contributing to insulin resistance. The stagnant nature of IMTG leads to accumulation of triglyceride and FA metabolites such as ceramide that further contributing to insulin resistance.

**TREATMENT OF METABOLIC INFLEXIBILITY**

The fundamental goal in treating metabolic inflexibility is to relieve the reducing pressure and buildup of reactive oxidation species resulting from carbon overload across the mitochondria. A theoretical way to accomplish this goal is to prevent oxidative metabolism of one of three major fuel sources to lower carbon import to the mitochondria and alleviate substrate competition. Intermittent fasting as discussed below is the most clinically relevant way to limit mitochondrial carbon delivery. Alternatively, one can attempt to enhance the buffering capacity of the various systems that serve to mitigate mitochondrial reductive stress. These strategies center on augmenting antioxidant defense, regeneration of glutathione, carnitine-mediated acyl group buffering, and sirtuin-mediated protein deacylation. A

---

**FIGURE 3.** Therapeutic approaches to maintain balance of energy across the mitochondria. An increase in energy expenditure can relieve the increased membrane potential in the mitochondria created by positive energy balance. In contrast, increased flux through β-oxidation in the absence of an increase in energy demand will worsen the reducing pressure. Restoring or augmenting buffering mechanisms in the absence of a change in energy intake or expenditure is an alternative, albeit less desirable, strategy to improve mitochondrial function. SGLT2i = sodium glucose cotransporter 2 inhibitor.
third and arguably the most desirable strategy to address the energy imbalance brought on by nutrient surplus is to increase energy demand (Figure 3). This strategy ensures metabolic balance and insulin sensitivity are maintained in a setting where flux through β-oxidation is increased.

Exercise
Studies showing that a single bout of vigorous exercise can enhance insulin action for up to 24 hours suggest that increases in energy expenditure are evolutionary conserved mechanisms to ensure metabolic balance and substrate flexibility across the mitochondria. An acute bout of exercise stimulates translocation of GLUT4 into the cell membrane independent of insulin signaling.63,64 This effect is linked to activation of AMP-activated protein kinase in response to metabolic stress caused by muscle contraction and activation of the sarcoendoplasmic reticulum calcium transport ATPase pump due to changes in intracellular Ca2+. The potent effect of exercise to increase GLUT4 expression not only enhances insulin action but facilitates muscle glycogen storage following exercise training.65 The increase in energy demand brought about by exercise is also a potent stimulus to increase mitochondrial function and content.66 Studies in both humans and rodents have found strong associations between the level of physical activity and changes in mitochondrial content and function. Exercise training is more strongly associated with improvements in mitochondrial function while mitochondrial content as assessed by citrate synthase activity is more closely related to training volume.67,68 These beneficial mitochondrial adaptations are also evident following exercise training in older subjects.69

The changes in insulin signaling and mitochondrial dynamics noted above are consistent with the strong association described between exercise and metabolic flexibility. In a study of obese and lean young subjects given a high-fat diet over 3 days, skeletal muscle lipid oxidation increased in the lean but not obese individuals, indicative of an impairment in metabolic flexibility with obesity.70 This impairment was no longer seen following 10 consecutive days of aerobic exercise with obese individuals responding similarly to their lean counterparts to the increase in dietary lipid. Fatty acid oxidation was assessed by measuring 14CO2 production derived from the complete oxidation of palmitate in skeletal muscle biopsies. This technique may be an inadequate measure of FA oxidation. In a study of 24 older adults with prediabetes and obesity, 12 weeks of aerobic exercise training improved metabolic flexibility as measured by the difference between the fasting RQ and that measured while using a euglycemic hyperinsulinemic clamp.71 In a separate study, the insulin-induced shift in whole body substrate selection from fat to glucose oxidation as measured by the change in RQ was greater in trained subjects compared with sedentary counterparts.72 Exercise training also improves metabolic flexibility as measured by mathematical variances in insulin and nonprotein RQ.11,73 Using this approach, increased activity correlates with an increase in daily variance in RQ and is characterized by a large shift in fuel mix being oxidized in response to small changes in insulin. Taken together, the increase in energy demand with exercise is an effective way to alleviate substrate competition and restore carbon flow through the mitochondria and improve insulin sensitivity.

Intermittent Fasting
Calorie restriction is an additional way to limit carbon load and decongest the mitochondria. Energy-restricted diets ameliorate obesity and its complications including insulin resistance, dyslipidemia, and hypertension. Weight loss that accompanies continuous calorie restriction leads to an adaptive decrease in energy expenditure. Although metabolic slowing may have favorable effects on longevity by reducing oxidative stress, it also imposes a barrier to further weight loss.74,75 An increasingly popular alternative to continuous calorie...
restriction is intermittent fasting where no or few calories are consumed for periods ranging from 1 to several days followed by ad libitum intake on remaining days. The prolonged periods of energy restriction allow for a more complete cellular switch in fuel source from glucose to fat as liver glycogen is depleted and fatty acids are metabolized to ketone bodies. This approach is more reminiscent of the eating patterns of our human ancestors where the post-absorptive state triggered hunger and food-seeking behavior that took hours and sometimes days to satisfy. A survival advantage was given to individuals capable of a high level of physical performance under conditions of intermittent food deprivation. Intermittent fasting seems to provide additional benefits beyond those attributed to a reduction in caloric intake. In trials of overweight women assigned to either a 5:2 intermittent fasting regimen or a 25% reduction in daily caloric intake, intermittent fasting led to greater increases in insulin sensitivity and a larger reduction in waist circumference despite similar amounts of total weight loss when compared with reductions in caloric intake.

At the cellular level fasting elicits evolutionarily conserved adaptive responses to include increased expression of antioxidant defenses, mitochondrial biogenesis, autophagy, DNA repair, and downregulation of inflammation. Increases in the ratio of AMP and ADP to ATP cause activation of AMP-activated protein kinase, which in turn, triggers repair and inhibits anabolic processes. AMP-activated protein kinase activates autophagy by suppressing mTORC1 and stimulates de novo mitochondrial biogenesis through peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α)—dependent transcription. Starvation also increases the ratio of NAD⁺/NADH. NAD⁺ serves as a cofactor for sirtuins (NAD⁺-dependent...
deacetylase) which deacetylate forkhead box O (FOXO) transcription factor and PGC-1α factors, respectively, which are involved in stress resistance and mitochondrial biogenesis. In addition to operating as an alternative energy source, production of ketone bodies operate as endogenous histone deacetylase inhibitors allowing for increased FOXO-mediated antioxidant production. Metabolism of ketone bodies in the Krebs cycle leads to production of nicotinamide adenine dinucleotide phosphate and increases the ratio of reduced to oxidized glutathione providing additional defense against the toxicity of reactive oxygen species. Collectively, fasting stimulates autophagy and mitophagy and simultaneously inhibits mTOR-mediated protein synthesis pathways (Figure 4). These pathways allow for cells to remove oxidatively damaged proteins and mitochondria and recycle undamaged molecular constituents while temporarily reducing global protein synthesis to conserve energy and molecular resources.

**Sodium-Glucose Cotransport-2 Inhibitors**

The sodium-glucose cotransporter-2 inhibitors (SGLT2i) lower plasma glucose concentration by inhibiting Na⁺-glucose coupled transport in the proximal tubule. These drugs are now indicated for reducing heart failure hospitalization, cardiovascular death, and slowing the progression of chronic kidney disease in patients with and without type 2 diabetes mellitus. The forced excretion of glucose into the urine leads to metabolic and adaptive responses reminiscent of caloric restriction. Daily loss of 50 to 100 g of glucose into the urine leads to increased glucagon levels, a reduction in insulin secretion, increased fatty acid oxidation, and decreased intrahepatic lipid content. In patients both with and without diabetes these drugs shift energy metabolism from glucose to fat oxidation and increase endogenous glucose production and ketogenesis. The increase in glucagon/insulin ratio directs acetyl-CoA produced from β-oxidation to production of ketone bodies. This diversion of acetyl-CoA along with oxaloacetate directed to phosphoenolpyruvate for gluconeogenesis lowers TCA cycle intermediates, explaining the global reduction of mitochondrial ATP synthesis measured ex vivo in skeletal muscle biopsies of patients with type 2 diabetes who received with dapagliflozin. In a recent study of 26 patients with type 2 diabetes, the respiratory exchange ratio decreased to a greater extent from day to nighttime following dapagliflozin compared with placebo, suggesting improved metabolic flexibility. These metabolic findings suggest that the loss of calories in urine (negative energy balance) extending into the nighttime restores the fed to fasting cycle that is blunted under conditions of chronic overnutrition.

By mimicking a fasting-like state, SGLT2i’s have the potential to trigger molecular pathways involved in maintenance of optimal cellular function. Increases in plasma ketone concentration following SGLT2i may explain beneficial effects on cardiac function because they provide a readily available fuel source for oxidation by the myocardium. Increased levels of ketone bodies following SGLT2i exert a kidney protective effect by suppressing mTORC1 signaling in the proximal tubule. States of overnutrition lead to hyperactivation of mTORC1, which is strongly associated with structural injury in models of diabetic kidney disease. Ketone bodies also exert a favorable effect on cyst formation in rodent polycystic kidney disease accompanied by an inhibitory effect on mTORC1.

In addition to limiting the carbon load to the mitochondria by offloading calories in urine, SGLT2i increase energy expenditure by promoting beiging of adipocytes. These drugs have been shown to increase energy expenditure consistent with an increase in nonshivering thermogenesis. Increased sympathetic nerve activity, increases in fibroblast growth factor 21 levels, and increased brain-derived neurotrophic factor may play a role in the ability of these drugs to initiate the beiging process or activate existing brown fat depots. Cold exposure through activation of brown fat and the effects of hypobaric hypoxia to increase basal metabolic rate and suppress appetite are additional ways to improve metabolic flexibility.
CONCLUSION
Overconsumption of nutrients can be a precursor to metabolic inflexibility, mitochondrial dysfunction, and dysregulation of metabolic health. This review summarizes potential mechanisms by which excess fuel and insulin resistance may result in metabolic inflexibility, obesity, and dysregulation of cardiometabolic health. When energy supply exceeds energy demand across the mitochondria, a rigid and congested state is created predisposing to negative health outcomes. As the prevalence of obesity increases, understanding the origins of this disease becomes critical. Clinicians will be tasked to provide patients information regarding the health risks associated with obesity and insulin resistance. Educating patients about the deleterious effects overconsumption of nutrients combined with lack of exercise has on metabolism will be critical in our collective quest to correct this condition.

ACKNOWLEDGMENT
Drs Palmer and Clegg have equally contributed to the writing of this manuscript.

Abbreviations and Acronyms: BCAA, branch chain amino acid; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; SGLT2, sodium-glucose cotransporter-2 inhibitor; H2O2, hydrogen peroxide

Potential Competing Interests: The authors report no potential competing interests.

Correspondence: Address to Biff F. Palmer, MD, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390 USA (biff.palmer@utsouthwestern.edu).

REFERENCES
1. Freese J, Klement R, Ruiz-Núñez B, Schwarz S, Lötzerich H. The sedentary (r)evolution: have we lost our metabolic flexibility? F1000Res. 2017;6:1787. https://doi.org/10.12688/ f1000research.12724.2
2. Muoio Deborah M. Metabolic inflexibility: when mitochondrial indecision leads to metabolic gridlock. Cell. 2014;159(6):1253-1262. https://doi.org/10.1016/j.cell.2014.11.034
3. Randle Pj. Metabolic fuel selection: general integration at the whole-body level. Proc Nutr Soc. 1995;54(4):317-327. https://doi.org/10.1079/pnu950057
4. McGarry JD, Mannuerts GP, Foster DW. A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J Clin Invest. 1977;60(1):265-270. https://doi.org/10.1172/JCI108764
5. Palmer BF, Clegg DJ. Starvation ketosis and the kidney. Am J Nephrol. 2012;35(2):467-478. https://doi.org/10.1159/000347055
6. van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochim Biophys Acta. 2002;1582(2):313-322. https://doi.org/10.1016/S0304-419X(02)00260-1
7. Muoio DM, Neuerer PD. Lipid-induced mitochondrial stress and insulin action in muscle. Cell Metab. 2012;15(5):595-605. https://doi.org/10.1016/j.cmet.2012.04.010
8. van de Weijer T, Sparks L, Phielix E, et al. Relationships between mitochondrial function and metabolic flexibility in type 2 diabetes mellitus. PLoS One. 2013;8(2):e51648. https://doi.org/10.1371/journal.pone.0051648
9. Song J, Alves T, Befroy D, et al. Dissociation of muscle insulin resistance from alterations in mitochondrial substrate preference. Cell Metab. 2020;32(5):726-735.e5. https://doi.org/10.1016/j.cmet.2020.09.008
10. Starlien L, Oakes ND, Kelley DE. Metabolic flexibility. Proc Nutr Soc. 2004;63(2):363-368. https://doi.org/10.1079/PNS2004349
11. Bergouignan A, Antoun E, Marniken I, et al. Effect of contrasted levels of habitual physical activity on metabolic flexibility. J Appl Physiol (1985). 2013;114(3):371-379. https://doi.org/10.1152/japplphysiol.00458.2012
12. Kelley DE, Goodpaster B, Wing RR, Simonneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol. 1999;277(6):E130-E141. https://doi.org/10.1152/ajpendo.1999.277.6.E130
13. Koves TR, Ulsher JR, Noland RC, et al. Mitochondrial over-load and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab. 2008;7(1):45-56. https://doi.org/10.1016/j.cmet.2007.10.013
14. Sparks LM, Xie H, Koza RA, et al. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes. 2005;54(7):1926-1933. https://doi.org/10.2337/diabetes.54.7.1926
15. Bilal MC, Neinast MD, Arany Z. Whole-body metabolic fate of branched-chain amino acids. Biochem J. 2017;474(4):765-776. https://doi.org/10.1042/BCJ20200666
16. Herman MA, She P, Peroni OD, Lynch CJ, Kahn BB. Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. J Biol Chem. 2010;285(15):11348-11356. https://doi.org/10.1074/jbc.M109.075184
17. Yoneshiro T, Wang Q, Tajima K, et al. BCAA catabolism in brown fat controls energy homeostasis through SLC25A44. Nature. 2015;527(7611):614-619. https://doi.org/10.1038/nature15866
18. Neinast MD, Jang C, Hui S, et al. Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. Cell Metab. 2019;29(2):417-429.e41. https://doi.org/10.1016/j.cmet.2018.10.013
19. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab. 2012;15(5):606-614. https://doi.org/10.1016/j.cmet.2012.01.024
20. Yoon M-S. The emerging role of branched-chain amino acids in insulin resistance and metabolism. Nutrients. 2016;8(7):405. https://www.mdpi.com/2072-6643/8/7/405
21. Heer CD, Brenner C. Letting off electrons to cope with metabolic stress. Nat Metab. 2020;2(6):485-486. https://doi.org/10.1038/s42255-020-0207-8
22. Qiu H, Schlegel V. Impact of nutrient overload on metabolic homeostasis. Nutr Rev. 2018;76(9):693-707. https://doi.org/10.1111/nure.12643
23. Anderson EJ, Lustig ME, Boyle KE, et al. Mitochondrial H2CO2 emission and cellular redox state link excess fat intake to...
insulin resistance in both rodents and humans. J Clin Invest. 2009;119(3):573-581. https://doi.org/10.1172/JCI37048

24. Zhang S, Hulver MW, McMillan RP, Clene MA, Gilbert ER. The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. Nutr Metab (Lond). 2014;11(1):10. https://doi.org/10.1186/s13006-014-0021-1

25. Fisher-Wellman KH, Gilliam LAA, Lin CT, Cathey BL, Lark DS, Darrell Neuffer P. Mitochondrial glutathione depletion reveals a novel role for the pyruvate dehydrogenase complex as a key H2O2-emitting source under conditions of nutrient overload. Free Radic Biol Med. 2013;65:1-12. https://doi.org/10.1016/j.freeradbiomed.2013.09.008

26. Trettet L, Adam-Vizi V. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. J Neurosci. 2004;24(36):7771-7778. https://doi.org/10.1523/JNEUROSCI.1842-04.2004

27. Muose DM. Intramuscular triacylglycerol and insulin resistance: guilty as charged or wrongly accused. Biochim Biophys Acta. 2010;1801(3):281-288. https://doi.org/10.1016/j.bbalip.2009.11.007

28. Kuz LC, Naika T, Stein R, et al. Effects of changes in three catalytic residues on the relative stability of some of the intermediates and transition states in the citrate synthase reaction. Biochemistry. 1998;37(7):9724-9737. https://doi.org/10.1021/bi980253z

29. Choudhary C, Wienert BT, Nishida Y, Verdin E, Mann M. The protease Mpa regulates the proteolytic degradation of proteins in the chemical conditions of the mitochondrial matrix. J Biol Chem. 2013;288(40):29036-29045. https://doi.org/10.1074/jbc.M113.486753

30. Wagner GR, Payne RM. Widespread and enzyme-independent, nepsilon-acetylation and nepsilon-succinylation of proteins in the chemical conditions of the mitochondrial matrix. J Biol Chem. 2013;288(40):29036-29045. https://doi.org/10.1074/jbc.M113.486753

31. Meyer JG, Softie S, Baitsy N, et al. Temporal dynamics of liver mitochondrial protein acetylation and succinylation and metabolites due to high fat diet and/or excess glucose or fructose. PLoS One. 2018;13(12):e208973. https://doi.org/10.1371/journal.pone.0208973

32. Malloux RJ, Jin X, Willmore WG. Redox regulation of mitochondrial function with emphasis on cysteine oxidation reactions. Redox Biol. 2014;2:123-139. https://doi.org/10.1016/j.redox.2013.12.011

33. Noland RC, Kaves TR, Seiler SE, et al. Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. J Biol Chem. 2009;284(34):22840-22852. https://doi.org/10.1074/jbc.M108.032888

34. Jing E, O'Neill BT, Rardin MJ, et al. Sirt3 regulates metabolic flexibility of skeletal muscle through reversible enzymatic deacetylation. Diabetes. 2013;62(4):1304-1317. https://doi.org/10.2337/db12-1650

35. Jenkins CM, Yang J, Sims HF, Gross RW. Reversible high affinity inhibition of phosphofructokinase-1 by acetyl-CoA: a mechanism integrating glycolytic flux with lipid metabolism. J Biol Chem. 2011;286(14):11937-11950. https://doi.org/10.1074/jbc.M110.203661

36. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med. 2016;371(12):1311-1322. https://doi.org/10.1056/NEJMra1501035

37. Hancock CR, Han DH, Chen M, et al. High-fat diets cause insulin resistance despite an increase in muscle mitochondrial function. Proc Natl Acad Sci U S A. 2008;105(22):7815-7820. https://doi.org/10.1073/pnas.0802057105

38. Turner N, Bruce CR, Beale SM, et al. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. Diabetes. 2007;56(8):2085-2092. https://doi.org/10.2337/db07-0093

39. Kaves TR, Li P, An J, et al. Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. J Biol Chem. 2005;280(39):33588-33598. https://doi.org/10.1074/jbc.M507062200

40. Noland RC, Woodfield TL, Whitfield BR, et al. Peroxisomal-mitochondrial oxidation in a rodent model of obesity-associated insulin resistance. Am J Physiol Endocrinol Metab. 2007;293(4):E986-E1001. https://doi.org/10.1152/ajpendo.00399.2006

41. Bernard C, Durand A, Peyrol S, et al. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J Clin Invest. 2008;118(2):789-800. https://doi.org/10.1172/JCI32601

42. Chomontowski P, Coen PM, Radikova Z, Goodpaster BH, Toledo FG. Skeletal muscle mitochondria in insulin resistance: differences in intermyo-filarin versus subsarcomasternal subpopulations and relationship to metabolic flexibility. J Clin Endocrinol Metab. 2011;96(2):494-503. https://doi.org/10.1210/jc.2010-0822

43. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev. 1997;77(3):731-758. https://doi.org/10.1544/0384.1

44. Adams SH, Hoppel CL, Lakhi KH, et al. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetes. Diabetes. 2005;54(10):2944-2950. https://doi.org/10.2337/db04-1135

45. Hulver MW, Berggren JT, Cortright RN, et al. Skeletal muscle lipid metabolism with obesity. Am J Physiol Endocrinol Metab. 2003;284(4):E741-E747. https://doi.org/10.1152/ajpendo.00389.2002

46. Mihalik SJ, Goodpaster BH, Kelley DE, et al. Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity (Silver Spring). 2010;18(9):1695-1700. https://doi.org/10.1038/oby.2009.510

47. Adams SH, Hoppel CL, Lakhi KH, et al. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. 2009;139(6):1073-1081. https://doi.org/10.3945/jn.108.103754

48. Choi CS, Savage DB, Abu-Elheiga L, et al. Continuous fat oxidation in acetyl-CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity. Proc Natl Acad Sci U S A. 2007;104(42):16480-16485. https://doi.org/10.1073/pnas.0705930104

49. Olson DP, Pulinukumti T, Cline GW, Shulman GI, Lowell BB. Gene knockout of Acc2 has little effect on body weight, fat mass, or food intake. Proc Natl Acad Sci U S A. 2010;107(16):7598-7603. https://doi.org/10.1073/pnas.0913492107

50. Hoehn KL, Turner N, Swarbrick MM, et al. Acute or chronic upregulation of mitochondrial fatty acid oxidation has no net effect on whole-body energy expenditure or adiposity. Cell Metab. 2010;11(1):70-76. https://doi.org/10.1016/j.cmet.2009.11.008

51. Choi CS, Befroy DE, Codelia R, et al. Paradoxical effects of increased expression of PGC-1 alpha on muscle mitochondrial function and insulin-stimulated muscle glucose metabolism. Proc Natl Acad Sci U S A. 2008;105(50):19926-19931. https://doi.org/10.1073/pnas.0810339105

52. Finck BN, Berman-Mitchaci C, Han DH, et al. A potential link between muscle peroxisome proliferator-activated receptor-alpha signaling and obesity-related diabetes. Cell Metab. 2005;1(2):133-144. https://doi.org/10.1016/j.cmet.2005.01.006

53. Tanaka T, Yamamoto I, Iwazaki S, et al. Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic...
syndrome. Proc Natl Acad Sci U S A. 2003;100(26):15924-15929. https://doi.org/10.1073/pnas.0330698100

54. Guerre-Millo M, Gervois P, Raspe E, et al. Peroxisome proliferator-activated receptor alpha improves insulin sensitivity and reduces adiposity. J Biol Chem. 2000;275(22): 16638-16642. https://doi.org/10.1074/jbc.275.22.16638

55. Guerre-Millo M, Rouault C, Poulain P, et al. PPAR-alpha null mice are protected from high-fat diet-induced insulin resistance. Diabetes. 2001;50(12):2809-2814. https://doi.org/10.2337/diabetes.50.12.2809

56. Li X, Li Z, Zhao M, et al. Skeletal muscle lipid droplets and the metabolic syndrome. Am J Physiol Endocrinol Metab. 2018;355(10):E1292-E1298. https://doi.org/10.1152/ajpendo.2018.03.003

57. van Loon LJ, Koopman R, Manders R, van der Weegen W, van Daemen S, Gemmink A, Brouwers B, et al. Distinct lipid droplet programming in mice and the athlete's paradox. Cells. 2019;8(3):249. https://doi.org/10.3390/cells8030249

58. van Loon LJ, Koopman R, Manders R, van der Weegen W, van Kratersen GP, Keizer HA. Intramyocellular lipid content in males and highly trained endurance athletes. J Sport Med Phys Fit. 2012;62(3):258-265. https://doi.org/10.24014/jspm.2012.02.019

59. Redman LM, Smith SR, Burton JH, Martin CK, Ilyassova D, Ravussin E. Metabolic slowing and reduced oxidative damage with sustained caloric restriction support the rate of living and oxidative damage theories of aging. Cell Metab. 2016;27(4): 805-815.e4. https://doi.org/10.1016/j.cmet.2016.09.011

60. de Cabo R, Mattson MP. Effects of intermittent fasting on health, aging, and disease. N Engl J Med. 2019;381(2):2541-2551. https://doi.org/10.1056/NEJMra1905136

61. Di Francesca A, Di Germanio C, Bernier M, de Cabo R. A time to fast. Science. 2013;342(6416):770-775. https://doi.org/10.1126/science.1239789

62. Harvie MN, Pegington M, Mattson MP, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. Int J Obes (Lond). 2011;35(5): 714-727. https://doi.org/10.1038/ijob.2011.17

63. Harvie M, Wright C, Pegington M, et al. The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women. Br J Nutr. 2013;110(8):1534-1547. https://doi.org/10.1017/S0007114513000792

64. Veech RL, Bradshaw PC, Clarke K, Curtis W, Pavlosky R, King MT. Ketone bodies mimic the life span extending properties of caloric restriction. JUBI Life. 2017;6(5):305-314. https://doi.org/10.1002/jubi.1627

65. Puchakala P, Crawford PA. Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. Cell Metab. 2017;25(2):262-284. https://doi.org/10.1016/j.cmet.2016.12.022

66. Newman JC, Verdin E. Ketone bodies as signaling metabolites. Trends Endocrinol Metab. 2021;32(5):42-52. https://doi.org/10.1016/j.tem.2021.03.002

67. Rojas-Morales P, Pedraza-Chaverri J, Tapia E. Ketone bodies, stress response, and redox homeostasis. Redox Biol. 2020;31:101395. https://doi.org/10.1016/j.redox.2020.101395

68. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377(7):643-657. https://doi.org/10.1056/NEJMoa1611925

69. Heepink HJJ, Stefansson BV, Correa-Rotter R, et al. Dapagliflozin in patients with chronic kidney disease. N Engl J Med. 2020;383(15):1436-1446. https://doi.org/10.1056/NEJMoa2024816

70. Palmer BF, Clegg DJ. Euglycemic ketoacidosis as a complication of SGLT2 inhibitor therapy. Curr Am Soc Nephrol. 2021;16(8):1284-1291. https://doi.org/10.2215/CIN.17621120
type 2 diabetes. Diabetes Care. 2016;39(11):2036-2041. https://doi.org/10.2337/dc15-2688

88. Ferrannini E, Baldi S, Frascerra S, et al. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. Diabetes. 2016;65(5):1190-1195. https://doi.org/10.2337/db15-1356

89. Op den Kamp YJM, de Ligt M, Dautzenberg B, et al. Effects of the SGLT2 inhibitor dapagliflozin on energy metabolism in patients with type 2 diabetes: a randomized, double-blind crossover trial. Diabetes Care. 2021;44(6):1334. https://doi.org/10.2337/dc20-2887

90. Esterline RL, Vaag A, Oscansson J, Vora J. Mechanisms in endocrinology: SGLT2 inhibitors: clinical benefits by restoration of normal diurnal metabolism? Eur J Endocrinol. 2018;178(4):R113-R125. https://doi.org/10.1530/EJE-17-0832

91. Packer M. Mitigation of the adverse consequences of nutrient excess on the kidney: a unified hypothesis to explain the renoprotective effects of sodium-glucose cotransporter 2 inhibitors. Am J Nephrol. 2020;51(4):289-293. https://doi.org/10.1159/000506534

92. Inoki K, Mori H, Wang J, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. J Clin Invest. 2011;121(6):2181-2196. https://doi.org/10.1172/JCI44771

93. Tamita I, Kume S, Sugahara S, et al. SGLT2 inhibition mediates protection from diabetic kidney disease by promoting ketone body-induced mTORC1 inhibition. Cell Metab. 2020;21(3):404-419.e6. https://doi.org/10.1016/j.cmet.2020.06.020

94. Torres JA, Kruger SI, Broderick C, et al. Ketosis ameliorates renal cyst growth in polycystic kidney disease. Cell Metab. 2019;30(6):1007-1023.e5. https://doi.org/10.1016/j.cmet.2019.09.012

95. Xu L, Nagata N, Chen G, et al. Empagliflozin reverses obesity and insulin resistance through fat browning and alternative macrophage activation in mice fed a high-fat diet. BMJ Open Diabetes Res Care. 2019;7(1):e000783. https://doi.org/10.1136/bmjdrc-2019-000783

96. Matthews JR, Herat LY, Magno AL, Gorman S, Schlach MP, Matthews VB. SGLT2 inhibitor-induced sympathoexcitation in white adipose tissue: a novel mechanism for beiging. Biomedicines. 2020;8(11):514. https://doi.org/10.3390/biomedicines8110514

97. Lin B, Kebuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. Cardiovasc Diabetol. 2014;13(1):148. https://doi.org/10.1186/s12933-014-0148-1

98. Palmer BF, Clegg DJ. Non-shivering thermogenesis as a mechanism to facilitate sustainable weight loss. Obes Rev. 2017;18(8):819-831. https://doi.org/10.1111/obr.12563

99. Palmer BF, Clegg DJ. Ascent to altitude as a weight loss method: the good and bad of hypoxia inducible factor activation. Obesity (Silver Spring). 2014;22(2):311-317. https://doi.org/10.1002/oby.20499

100. Hanssen MJ, van der Lans AA, Brans B, Hoes J, et al. Short-term cold acclimation recruits brown adipose tissue in obese humans. Diabetes. 2016;65(5):1179-1189. https://doi.org/10.2337/db15-1372