Review Article

Targeting skeletal muscle mitochondrial health in obesity

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Metabolic demands of skeletal muscle are substantial and are characterized normally as highly flexible and with a large dynamic range. Skeletal muscle composition (e.g., fiber type and mitochondrial content) and metabolism (e.g., capacity to switch between fatty acid and glucose substrates) are altered in obesity, with some changes proceeding and some following the development of the disease. Nonetheless, there are marked interindividual differences in skeletal muscle composition and metabolism in obesity, some of which have been associated with obesity risk and weight loss capacity. In this review, we discuss related molecular mechanisms and how current and novel treatment strategies may enhance weight loss capacity, particularly in diet-resistant obesity.

Introduction

According to the World Health Organization, more than 1 billion adults are overweight and an additional 650 million adults are living with obesity. The global prevalence of obesity has tripled since the 1970s, with dramatic increases in the rates of childhood obesity [1–3]. Obesity is a major global public health concern as it is a risk factor for many cardiometabolic diseases including coronary heart disease, hypertension, Type 2 diabetes mellitus (T2D), and non-alcoholic fatty liver disease (NAFLD) [1,4–6]. Obesity is also associated with increased risk for many types of cancer, musculoskeletal disorders (e.g., osteoarthritis), and all-cause mortality [1,4,5,7–11]. The high healthcare costs associated with treating comorbidities associated with obesity demonstrate the critical need for weight loss strategies to manage this disease [12–14]. Achieving a modest weight loss of ≥5% has been shown to significantly modify risk factors for cardiometabolic disease [15]. Specifically, adjusted regression models in a retrospective analysis of electronic health records from patients with a history of obesity who had either maintained their current weight (i.e. not lost weight), lost >5% of body weight and subsequently regained weight, or lost and maintained >5% of their body weight, revealed that achieving a weight loss of 5–10% reduces T2D risk and lowers HbA1c, and that weight loss of 10–15% improves blood pressure and reduces plasma LDL and increases HDL [16]. Weight loss of >20% is generally observed with bariatric surgery and is further associated with decreased risk of cancer and increased life expectancy by up to 3 years [17].

Obesity is a complex, multifactorial disease involving the excess deposition of body fat, predominantly stored not only in adipose tissues but also ectopically in tissues such as liver and skeletal muscle. The expansion of adipose tissue drives increases in body mass and body mass index (BMI). Obesity is most commonly identified as a BMI exceeding 30 kg/m², while overweight is identified as a BMI greater than 25 kg/m² but less than 30 kg/m². The development of obesity is ultimately driven by an imbalance between dietary energy intake and energy expenditure. Thus, weight loss interventions require a negative energy balance, through reduced dietary energy intake, and/or increased energy expenditure (e.g., through exercise).
Figure 1. Potential factors contributing to adaptive thermogenesis during weight loss and resistance to diet-induced weight loss

Restricting energy intake can elicit metabolic adaptations in energy expenditure that oppose successful weight loss. The mechanisms that underlie adaptations in energy expenditure are associated with genetic factors, decreased activity of the sympathetic nervous system (SNS), impaired neuroendocrine signaling, changes in thyroid hormones, and variations in body composition (loss of fat free mass [FFM]). Moreover, resistance to diet-induced weight loss is associated with lower expression of OXPHOS and ribosomal genes, diminished glutathione redox, less type I muscle fibers, and decreased oxidative capacity. Figure created with BioRender.com.

Individuals with obesity often attempt multiple treatment approaches before seeking help from medical professionals; they frequently report using self-guided caloric restriction or popularized diet strategies, exercise, commercial weight-loss programs, and over-the-counter dietary supplements [18–20]. Once in the care of medical and allied health professionals, the focus is on behavioral strategies and pharmacotherapies. Behavioral programs frequently employ very low-calorie total meal replacements in combination with behavioral counseling. The statistics on sustained weight loss success with these approaches have been discouraging [21], and the hope is that more personalized approaches and novel pharmacotherapies will significantly improve weight loss outcomes. While greater degrees of weight loss and sustained weight loss are associated with bariatric surgeries, these approaches are invasive, not universally available, and typically are only for those with severe obesity, or obesity-associated comorbidities [22]. Long-term mortality significantly improves with surgical intervention [23]; however, there is still a significant risk of complications associated with bariatric surgery, including a 0.7% risk of mortality [24].

The objective of this review is to discuss the role of mitochondrial health and energy expenditure in the context of variability in weight loss success, and more specifically, factors associated with skeletal muscle metabolism that are associated with enhanced or perturbed weight loss success. While exercise is a major factor, and will be addressed here, other factors that augment energy expenditure of skeletal muscle will also be discussed.
Variability in weight loss success

Many studies have shown that there is substantial interindividual variability in weight loss response to traditional interventions [25,26], even after controlling for factors such as diet adherence, age, biological sex, exercise, and medications [25,27]. Max Wishnofsky first proposed the highly propagated idea that weight loss could be linearly quantified in caloric equivalents of a 3500-calorie deficit for one pound of body weight [28], suggesting that decreasing energy intake should lead to similar weight loss outcomes in patients following the same dietary intervention. The idea that low response to diet-induced weight loss is simply attributable to adherence was forwarded in a study by Lichtman et al., which described that individuals with a history of diet-resistant weight loss had large discrepancies in self-reported and actual energy intake and energy expenditure [29]. Remarkably complex mechanisms govern food intake control, and impairments in the perception of food intake can contribute to the development of obesity and make successful treatment more challenging [30,31]. However, a growing body of literature has since refuted the concept that poor response is simply due to poor adherence by demonstrating that weight loss after controlling for program adherence remains highly variable, in-part associated with complex metabolic adaptations in energy expenditure. In addition to the existence of adaptations in energy expenditure during weight loss, the recognition that obesity is a chronic disease has helped denounce the common bias suggesting that obese individuals resistant to weight loss simply lack discipline and/or have an inadequate perception of their food and/or activity habits [32]. While predictive models of weight loss are improving [33,34], there remains a great need for an improved understanding of the causes of variable weight loss success in order to identify more effective personalized treatment strategies.

Extensive research has examined genetic factors related to the propensity for obesity and weight loss. In the early 1990s, studies on monozygotic twins elucidated the heritability of body weight. Male twins overfed by 1000 kcal/day for 84 out of 100 days had similar intrapair variance in body weight and fat mass increases, but the variance between pairs of twins was significantly greater [35]. Similarly, monozygotic male twins who completed an exercise intervention in negative energy balance had similar anthropometric responses in body weight and regional fat distribution within a genotype but responses varied significantly between twin pairs [36]. The contribution of genetics to obesity susceptibility is also supported by adoptee studies, in which the BMI of adoptive children is more closely related to their biological parents than their adoptive parents [37]. In the 30 years following these classical studies, large-scale genome-wide association studies (GWAS) have identified ~100 loci associated with obesity [38]. The heritability of obesity is high and is estimated to be between 40 and 70% [38–40], with over 500 genetic loci associated with adiposity traits [41]. Recent genome-wide polygenic risk score computational models have been able to robustly predict BMI and obesity [42], providing new opportunities for obesity prevention and mechanistic insights into the pathophysiology of obesity. However, genetic variants associated with increased obesity susceptibility only explain >20% of BMI variation. There are a number of valid explanations for ‘missing heritability’. Despite the information provided by genome-wide association studies, many genetic variants with weak but real effects on a trait, such as BMI, do not reach GWAS significance ($P < 10^{-8}$). Array technologies also do not capture rare variants that are not in linkage disequilibrium with genotyped variants (the non-random association of alleles at different loci) [43]. Newer approaches that make use of whole genome sequencing (WGS) data and a methodology termed GREML (genomic-relatedness-based restricted maximum likelihood) have markedly increased the proportion of predicted heritability explained to date for traits such coronary heart disease, height, and BMI [44,45]. Additional and important layers exist including the epigenome and its interactions with the environment [44]. Finally, even in the context of high genetic risk, clearly lifestyle factors can markedly influence the phenotypic outcome, highlighting the influence of other biological, environmental, and lifestyle factors in the development of obesity [42].

Our group has been investigating the factors that contribute to variability in weight loss in cohorts sampled from over 5000 patients who have completed the same intensively supervised 900 kcal/day meal replacement (OptiFast 900, Nestle) and behavioral program. We have shown that patients in the top quintile for weight loss (diet-sensitive, DS) have many physiological characteristics that distinguish them from patients in the lower quintile for weight loss (diet-resistant, DR). Many of these characteristics relate to skeletal muscle composition and metabolism including greater expression of genes involved in glucose and fatty acid metabolism, higher mitochondrial proton leak, and a greater antioxidant capacity in skeletal muscle from DS patients compared with DR patients [25,27,46,47]. Patients with diet-resistant obesity also exhibit lower weight loss capacity for up to 26-weeks following Roux-en-Y gastric bypass surgery [48], further supporting a biological basis for weight loss capacity.

The role of energy expenditure in weight loss success

Differences in energy expenditure and resting metabolic rate (RMR) can also contribute to the risk for obesity development [49]. Energy expenditure is classically defined as the sum of the basal metabolic rate, thermic effect of feeding,
Skeletal muscle from diet-resistant individuals has less mitochondrial proton leak and lower expression of genes involved in the electron transport chain and fatty acid metabolism compared to diet-sensitive individuals. Moreover, when challenged with a high-fat meal, skeletal muscle fatty acid oxidation and maximal oxidative phosphorylation was lower in DR individuals (see text for details). Figure created with BioRender.com.

Figure 2. Mitochondria-specific differences observed in skeletal muscle from diet-resistant versus diet-sensitive patients with obesity

Skeletal muscle from diet-resistant individuals has less mitochondrial proton leak and lower expression of genes involved in the electron transport chain and fatty acid metabolism compared to diet-sensitive individuals. Moreover, when challenged with a high-fat meal, skeletal muscle fatty acid oxidation and maximal oxidative phosphorylation was lower in DR individuals (see text for details). Figure created with BioRender.com.

and non-resting energy expenditure. Fat-free mass (FFM) is the most metabolically active component of body composition, and thus, the best predictor of resting metabolic rate [50,51]. Non-resting energy expenditure is the most variable component to total energy expenditure, and the metabolic variable most affected by weight change [52]. Non-resting energy expenditure can further be categorized into energy expenditure from thermoregulatory energy expenditure, exercise/voluntary physical activity and non-exercise activity thermogenesis (NEAT) that is related to non-volitional movements, such as fidgeting [53]. Thermoregulatory energy expenditure mechanisms include shivering and non-shivering thermogenesis. Energy expenditure fluctuates across the lifespan and, also, demonstrates high interindividual variation. Highlighted in a recent study with over 6000 participants in 29 different countries, analysis of doubly labelled water data revealed that total daily energy expenditure can vary ±20% even after accounting for
age, fat-free mass, and sex [54]. The large interindividual variation in energy expenditure has a strong genetic component, with highly similar RMRs observed between monozygotic versus dizygotic twins [53], and siblings within the same family [49]. Starting in late childhood, low RMR is associated with a greater weight gain in adolescence [56]; these observations persist in adulthood, with a continued association between low 24-h energy expenditure and weight gain over 2 years [49]. When obesity develops, there can be increases in both total energy expenditure and RMR [57–60], which are largely due to increases in FFM that accompany the increase in body weight [58].

Reducing energy intake can elicit an evolutionarily conserved adaptive decline in RMR to preserve energy during times of dietary energy deficits and starvation [61,62]. This adaptive thermogenesis was elucidated as part of the Minnesota semistarvation study in which 24 weeks of semistarvation was found to reduce resting energy expenditure by 15.5%, independent of changes in body mass and FFM [63]. While interventions that have used caloric restriction as a tool to induce weight loss in individuals with obesity generally use moderate reductions in caloric intake, more recent studies have also demonstrated that on a low-calorie diet compared to individuals who lose weight quickly [73–75]. Diet-induced weight loss can prompt the undesirable loss of FFM [76], exacerbating weight-loss-induced adaptive thermogenesis. Interindividual differences in energy metabolism likely underlie the considerable variability in adaptive thermogenesis that may enhance or perturb weight loss capacity.

The molecular mechanisms for adaptive thermogenesis and resistance to diet-induced weight loss are vast and involve metabolic and neuroendocrine factors. Upon achieving a greater than 10% weight loss, there are substantial changes to sympathetically nervous system (SNS) activity, notable decreases in thyroid hormones and leptin, and increases in skeletal muscle work efficiency (energy cost per muscle contraction) [61,62]. Total mass, contractile activity and metabolism of skeletal muscle greatly influence weight loss capacity. Skeletal muscle accounts for 40–45% of total body mass, and when the body is at rest, skeletal muscle is responsible for approximately 20% of resting energy expenditure (this proportion increases with exercise) [77,78]. Beyond its overall energy demands, skeletal muscle is the primary site of postprandial glucose uptake [79] and plays a major role in maintaining whole-body glucose homeostasis [80]. Skeletal muscle also serves as a reservoir for amino acids permitting synthesis of new proteins during fasting periods or supporting energy transduction via proteolysis when other macronutrient sources are depleted [81].

Skeletal muscle and mitochondrial bioenergetics

Skeletal muscle metabolism can rapidly adapt to meet energy demands and relies heavily on mitochondrial oxidative metabolism to transduce macronutrient energy into adenosine triphosphate (ATP) [82]. Oxidation of energy substrates provides reducing equivalents (i.e., electrons) that then drive the activity of the mitochondrial electron transport chain (ETC). The electrons can enter at complexes I–III (CI–CIII) of the ETC, and the flow of electrons coincides with the pumping of protons by CI, CII and CIV from the matrix to the intermembrane space, thereby contributing to the generation of proton motive force (PMF) across the inner membrane. PMF is comprised of two components: a charge gradient (mitochondrial membrane potential, Δψm) and a chemical gradient (ΔpH). Positively charged ions generate the Δψm charge gradient, which has a much larger contribution to PMF than the chemical separation that determines the ΔpH [83,84]. The return of protons to the matrix, down the electrochemical gradient via the F1F0-ATP synthase produces ATP and decreases PMF [85].

In most cells of the body, OXPHOS yields 90% of the ATP needed to support cellular work; however, the efficiency of mitochondrial ATP production can be highly variable due to the uncoupling of energy substrate oxidation from ATP synthesis through a process termed proton leak. When ATP demand is low, protons can migrate across the inner membrane into the matrix, independently from the ATP synthase, leading to increased activity of the ETC, consuming oxygen in the process. Proton leak is thought to be a protective redox regulation mechanism, preventing
excess formation of reactive oxygen species (ROS) by allowing electron flow through the ETC, rather than a build-up and escape of electrons to form superoxide anion.

The mechanisms of proton leak are complex and include both basal and inducible forms of proton conductance. In skeletal muscle proton leak, uncoupling is thought to be responsible for up to 50% of resting muscle energy expenditure, and this proportion is thought to decrease as ATP demands increase [86]. At the protein level, the only UCP expressed in skeletal muscle is UCP3, but it is expressed at approximately two orders of magnitude lower than the levels of UCP1 in brown adipose tissue mitochondria. UCP1 is well-recognized for its role in mediating proton leak uncoupling in brown and beige adipose tissues in many mammals [87–89], and its activity in adult humans is associated with reduced risk for many chronic metabolic diseases [90,91]. Adenine nucleotide translocase (ANT), an abundant mitochondrial inner membrane protein in skeletal muscle that supplies ADP from the cytosol to the mitochondrial matrix in exchange for ATP (i.e. ADP/ATP exchange). ANT also mediates proton leak (basal and fatty acid-activated) independently of ADP/ATP exchange and may facilitate the permeability transition pore opening through mitochondrial depolarization [86,92–95]. Fatty acids act as co-factors for H+ transport by ANT and may bind to the reputed ADP/ATP binding site as H+ transport by ANT is negatively regulated by ANT-mediated ADP/ATP exchange [93,96,97]. Taken together, these data are consistent with the conclusion that ANT has two distinct and partially competing functions: fatty acid-activated proton leak and ADP/ATP exchange. Post-translational modifications of ANT through acetylation, and glutathionylation have been reported and require further investigation in light of the recently identified reciprocal activities of ADP/ATP transport versus H+ leak [98,99].

If proton leak is responsible in humans (as in rats) for upwards of 50% of energy expenditure in resting muscle, and as muscle energy expenditure is responsible for roughly 20% of RMR, then proton leak in muscle alone could be responsible for approximately 10% of RMR in humans [100–102]. A role for low proton leak in muscle in the context of diet-resistant obesity is supported by results from our group, and is discussed below.

Declines in skeletal muscle mitochondrial content have repeatedly been associated with obesity as evidenced by 20–60% reductions in mitochondrial surface area (using electron microscopy), and significantly lower expression of various mitochondrial genes and proteins [103–105]. It is well established that impaired skeletal muscle mitochondrial function is characteristic of obesity, and is reflected by impaired fatty acid metabolism, lower activity of mitochondrial enzymes, and increased H2O2 emission [106–113].

Skeletal muscle mitochondrial bioenergetics and weight loss

Skeletal muscle fiber composition and the efficiency of skeletal muscle mitochondrial energy transduction have been linked to weight loss characteristics in individuals with obesity (Figure 1) [102]. Adults with obesity have lower proportions of mitochondria-dense type I fibers compared with proportions in muscle of lean controls [114–117], and the proportion of type I fibers contributes to obesity susceptibility [118,119]. Moreover, a recent systemic review revealed a negative relationship between the proportion of type I fibers and BMI, and a positive relationship between the proportion of type II fibers and BMI, with no clear relationship between type Ia fiber proportion and obesity [120]. The proportion of type I fibers also contributes to weight loss capacity, with a high degree of weight loss following bariatric surgery strongly associated with type I fiber content in rectus abdominis muscle [114]. Similarly, vastus lateralis muscle from DS patients with obesity has a greater proportion of type I fibers and fiber hypertrophy compared with both DR patients and lean controls [27]. Altogether, findings support the conclusion that variability in skeletal muscle fiber composition and size are associated with obesity risk and weight loss.

While it is clear that skeletal muscle mitochondrial content is decreased with obesity [103,104], a direct link between skeletal muscle mitochondrial content and propensity for weight loss has yet to be observed. However, substantial evidence supports the conclusion that mitochondrial oxidative capacity may drive weight loss success. Gene set enrichment analysis of repeated skeletal muscle biopsies obtained from patients undergoing a hypocaloric diet revealed that DS patients who lost an average of 49% more weight than DR patients had significantly higher enrichment of gene transcripts encoding mitochondrial ETC proteins [27]. Higher enrichment of mitochondrial ETC transcripts was even observed in circulation prior to weight loss, suggesting that oxidative capacity could predict weight loss success [121]. The differences in ETC transcripts between DR and DS individuals translate into functional differences in skeletal muscle, where maximal mitochondrial respiration and complex I+II OXPHOS are lower in muscle biopsies of DR versus DS participants (Figure 2) [46]. However, lower enrichment of mitochondrial ETC transcripts in DR muscle did not translate into decreased expression of key ETC subunits in primary myotubes isolated from DR individuals [47].

Skeletal muscle energy transduction efficiency is also important. Differences in mitochondrial proton leak uncoupling have been associated with weight loss capacity. Proton leak was found to be 50% higher in mitochondria...
isolated from rectus femoris muscle of DS individuals who achieved high rates of diet-induced weight loss compared to DR individuals with obesity [25]. Enhanced proton leak in DS muscle appears to be cell autonomous as proton leak is also higher in primary myotubes isolated from DS individuals [47]. The difference in skeletal muscle mitochondrial proton leak between DS and DR has been attributed to altered expression of UCP3, as UCP3 mRNA expression is greater in isolated mitochondria from DS rectus femoris muscle [25]. When the human form of UCP3 is over-expressed in mouse skeletal muscle by ∼2.5-fold above normal, mice are protected from diet-induced obesity and display metabolic characteristics of enhanced fatty acid oxidation [122–125]. Genetic polymorphisms may play a key role in determining UCP3 function, as a recent meta-analysis concluded that -55C/T polymorphism in UCP3 protects from obesity and T2D [126,127]. Moreover, evidence from murine models indicates that ADP sensitivity is impaired in skeletal muscle from mice with diet-induced obesity [128], suggesting that ANT-mediated ADP/ATP exchange is decreased in obesity. However, ANT-mediated proton leak may remain intact in obesity due to the accumulation of intramuscular fatty acids which may promote H⁺ transport [107,129,130]. These data support the conclusion that differences in mitochondrial uncoupling in muscle impact propensity for obesity and weight loss.

There is evidence to support the possibility that blunted capacity for fat oxidation contributes to obesity and the capacity for weight loss. In response to a defined high-fat meal (∼35% of daily kcal requirements based on indirect calorimetry; >60% calories from fat), diet-resistant women exhibit lower fatty acid clearance, suggesting that lower fatty acid oxidation may contribute to the diet-resistant phenotype [46]. Low resting respiratory exchange ratio (RER, VCO2/VO2 i.e., carbohydrate versus fat oxidation) has previously been identified in women with a high rate of weight loss [131]; whereas high resting RER has been linked to weight gain [132]. However, meta-analyses of studies associating 24-h RQ and body weight have not confirmed this hypothesis [133]. Ex vivo studies at the level of skeletal muscle have shown that fatty acid oxidation is lower in DR muscle [46], which may be attributed to decreased fatty acid availability and reduced fatty acid mobilization from adipose tissue [134,135].

With the increasing availability of omics platforms, researchers have a greater capacity to investigate differences in protein and metabolite patterns that could explain weight loss variability. In a large cohort of adults with obesity, predictive models of baseline parameters identified plasma metabolites that could explain up to 57% of variation in weight loss success prior to diet-induced weight loss. Circulating concentrations of branched-chain amino acids (BCAAs), tyrosine, specific lipid species, and citrate have consistently been linked to weight loss success in principal component analyses of plasma and serum metabolites [73,131,136]. There is a clear pattern for a metabolic basis of variations in body weight; however, much research is still required to translate this to novel and personalized approaches for the treatment of obesity.

**Interventions to boost skeletal muscle mitochondrial bioenergetics for diet-resistant obesity**

While comprehensive weight loss programs that focus on decreasing caloric intake can result in a 5–8% weight reduction in many individuals [21], it is clear that diet-centric approaches do not work for all patients, and that maintenance of the reduced body weight is the exception rather than the norm. Bariatric surgery is the most effective treatment strategy and typically results in 20–45% weight loss 12 months following Roux-en-Y gastric bypass (RYGB). Moreover, the weight loss is maintained in 70% of patients for over 7 years [137]. However, as discussed, the invasive and irreversible nature of this treatment, as well as the lifelong changes to food intake and diet supplements, mean that this approach is not available or desirable for many with obesity. Table 1 summarizes possible interventions to boost skeletal muscle bioenergetics in diet-resistant obesity.

**Exercise**

The use of exercise in the absence of dietary approaches as a means to induce weight loss has a controversial history in obesity treatment. Despite abundant research demonstrating the beneficial effects of exercise in mitigating the risk of cardiometabolic disease and all-cause mortality [138–141], exercise is often overlooked as a primary treatment for obesity. Exercise is well recognized for its beneficial effects on skeletal muscle glucose homeostasis and has repeatedly been shown to enhance insulin sensitivity in individuals with obesity [142–144]. However, caloric restriction is ultimately more effective at inducing weight loss than exercise is alone [145–152]. Given the above-described deficits in mitochondrial energetics in DR obesity, exercise interventions may be particularly beneficial in DR obesity; on the other hand, if the deficits are ‘hard-wired’ (e.g., genetically), then exercise interventions may not be of particular benefit to those with DR obesity.
Table 1 Interventions to boost skeletal muscle mitochondrial bioenergetics

| Intervention                  | Mode of action and adaptations to skeletal muscle metabolism | Clinically relevant weight loss | Status and remarks | Safety and adverse effects | References |
|------------------------------|-------------------------------------------------------------|--------------------------------|-------------------|---------------------------|------------|
| **Lifestyle**                |                                                             |                                |                   |                           |            |
| Aerobic exercise training    | ↑ Energy expenditure                                         | Aerobic exercise alone, low weight loss | Recommended in most weight loss programs | Non-serious adverse events related to muscle pain and fatigue | [146,147,151,369,370] |
|                             | ↑ Fat mass                                                   |                                 |                   |                           |            |
|                             | ↑ Mitochondrial content and function                         |                                 |                   |                           |            |
| Resistance exercise training | ↑ Protein translation                                        | Resistance Exercise alone, no weight loss; Resistance exercise + diet high | Recommended in most weight loss programs | Non-serious adverse events related to muscle pain and fatigue | [212,217,370,371] |
|                             | ↑ FFM                                                        |                                 |                   |                           |            |
| Cold exposure                | ↑ Energy expenditure                                         | Insufficient evidence           |                   |                           | [372]      |
|                             | ↑ Heat production                                            |                                 |                   |                           |            |
|                             | ↑ Proton leak                                                |                                 |                   |                           |            |
|                             | ↑ AMPK activation                                            |                                 |                   |                           |            |
|                             | ↑ Energy expenditure                                         |                                 |                   |                           |            |
|                             | ↑ Heat production                                            |                                 |                   |                           |            |
|                             | ↑ Proton leak                                                |                                 |                   |                           |            |
|                             | ↑ SERCA Ca²⁺ cycling                                        |                                 |                   |                           |            |
|                             | ↑ UCP content and proton leak                                |                                 |                   |                           |            |
| (2,4)-Dinitrophenol (DNP)    | ↑ Energy expenditure                                         | High                           | Not approved for weight loss treatment | Major safety concerns; hyperthermia, mortality | [266]      |
| (3–5 mg/kg/day; 75–300 mg/day)| ↑ Heat production                                            |                                 |                   |                           |            |
|                             | ↑ Proton leak                                                |                                 |                   |                           |            |
| Salsalate (3–4 g/day)        | Animal studies:                                             | No                             | Not approved for weight loss. Human trials show increased or no change in body weight | Side effects include tinnitus, headache, rash, gastrointestinal disturbances | [273,275,373] |
|                             | ↑ AMPK activation                                            |                                 |                   |                           |            |
|                             | ↑ Energy expenditure                                         |                                 |                   |                           |            |
|                             | ↑ Heat production                                            |                                 |                   |                           |            |
|                             | ↑ Proton leak                                                |                                 |                   |                           |            |
| Niclosamide, niclosamide    | Animal studies:                                             | Insufficient evidence in humans | Not approved for weight loss treatment; Approved as anthelmintic | Generally well tolerated; nausea, gastrointestinal disturbances, dizziness, pruritus | [277,278,374] |
| ethanolate and niclosamide   | ↑ Proton leak                                                |                                 |                   |                           |            |
| piperazine                   | ↑ Fatty acid oxidation                                       |                                 |                   |                           |            |
|                             |                                                             |                                 |                   |                           |            |
| **Mitochondrial uncouplers** |                                                             |                                |                   |                           |            |
| Amphetamine                  | ↓ Energy intake                                              | High                           | Approved for short-term use | Side effects include paresthesia, cardiovascular abnormalities, nausea | [263,377,378] |
| Methamphetamine              | ↑ Energy expenditure                                         |                                 |                   |                           |            |
|                             | ↑ SNS activity                                               |                                 |                   |                           |            |
|                             | ↑ Thyroid hormones                                           |                                 |                   |                           |            |
|                             | ↑ Catecholamine and monoamines                              |                                 |                   |                           |            |
|                             | ↑ (α and β)-Adrenergic receptor stimulation                 |                                 |                   |                           |            |
|                             | ↑ Skeletal muscle contractions                               |                                 |                   |                           |            |
|                             | ↑ Motor stimulation                                          |                                 |                   |                           |            |
|                             | ↑ SERCA activity                                             |                                 |                   |                           |            |
|                             | ↑ UCP activity                                               |                                 |                   |                           |            |
| Sympathomimetic agents       | ↓ Energy intake                                              | High                           | Approved for short-term use | Side effects include paresthesia, cardiovascular abnormalities, nausea | [263,377,378] |
| Amphetamine Congeners        | ↑ SNS activity                                               |                                 |                   |                           |            |
| e.g. Amfepramone, Diethylpropion, Mazindol Phentermine (15–30 mg), Phentermine/topiramate, Phenmetrazine (3 x 25 mg/day), Phendimetrazine (6 x 35 mg) | ↑ Release of monoamines | (α and β)-Adrenergic receptor stimulation |                      |                      |            |
|                             | ↑ Motor stimulation                                          |                                 |                   |                           |            |
| Sibutramine (10–20 mg)       | ↓ Energy intake                                              | High                           | Withdrawn          | Major safety concerns; Hypertension, increased risk of heart attack and stroke, mortality | [377,379,380] |
|                             | ↑ SNS activity                                               |                                 |                   |                           |            |
|                             | ↑ Energy expenditure                                         |                                 |                   |                           |            |
|                             | ↑ (α and β)-Adrenergic receptor stimulation                 |                                 |                   |                           |            |

Continued over
| Intervention | Mode of action and adaptations to skeletal muscle metabolism | Clinically relevant weight loss | Status and remarks | Safety and adverse effects | References |
|--------------|-------------------------------------------------------------|-------------------------------|-------------------|---------------------------|------------|
| Serotonergic agents | ↓ Energy intake ↑ Serotonin release ↑ Serotonin reuptake ↑ (α and β)-Adrenergic receptor stimulation ↑ Glucose uptake, lactate production (Fenfluramine) | High | Withdrawn | Major safety concerns; increased risk of cardiovascular events, valvulopathy, pulmonary hypertension | [377,381] |
| Thyroid Hormones T3 (18–117 μg/70 kg/day) | ↑ Energy expenditure Muscle: ↑ ATP turnover ↑ Proton leak ↑ UCP3 | Insufficient evidence in euthyroid patients | Not approved for weight loss treatment; Approved for hypothyroidism | Safety concerns for euthyroid patients. Adverse effects include thyrotoxicosis, cardiovascular abnormalities, decreased bone density, muscle catabolism | [382,383] |
| Metformin (500–3000 mg/day) | ↓ Energy intake ↑ AMPK activation ↑ Fatty acid oxidation | Medium | Not approved for weight loss treatment | | [335,384] |
| β-Adrenoceptor agonists e.g Isoproterenol, Isoprenaline (non-selective β-adrenoceptor agonists) Mirabegron (β3-adrenoceptor agonist) Formoterol (β2-adrenoceptor agonist) GLP-1 agonists Liraglutide (3 mg/day) Semaglutide (2.4 mg subcutaneous injection/week) Caffeine (60–600 mg/day) (L)-Carnitine (1.8–4 g/day) Ephedrine/Ephedra (20–150 mg/day) Conjugated Linoleic acid 2.4–6 g/day Green tea Green tea catechins (141–1207 mg/day) | ↑ β-Adrenergic receptor stimulation ↑ Energy expenditure ↑ Lipolysis ↑ Fatty acid oxidation ↑ Lean mass ↑ Energy intake ↑ Energy expenditure ↑ Fatty acid oxidation ↓ Muscle loss ↑ Energy expenditure ↑ SNS activity ↑ Catecholamines ↑ Fatty acid oxidation ↑ Lipolysis ↑ Energy expenditure ↑ Fatty acid oxidation | High | Approved for weight loss treatment | Nausea, diarrhea, constipation | [386,387] |
| Supplements | | | | | |
| Caffeine (60–600 mg/day) | ↑ Energy expenditure ↑ SNS activity ↑ Catecholamines ↑ Fatty acid oxidation | Low | Approved for sale as a dietary supplement | Generally well tolerated in moderate doses. Mild side effects include sleep disturbances, increased blood pressure, diuresis, nausea, and gastrointestinal discomfort. Toxic at doses of 15 mg/kg | [360–362,388] |
| (L)-Carnitine (1.8–4 g/day) | ↑ Fatty acid oxidation | Low | Approved for sale as a dietary supplement | Generally well tolerated. Mild side effects include nausea and gastrointestinal disturbances | [367,368] |
| Ephedrine/Ephedra (20–150 mg/day) | ↑ Energy expenditure ↑ SNS activity ↑ Fatty acid oxidation | Low | Not approved for sale as a dietary supplement | Major safety concerns. Adverse effects include psychiatric symptoms, gastrointestinal disturbances, cardiovascular abnormalities and events, mortality | [352,353] |
| Conjugated Linoleic acid 2.4–6 g/day | ↑ Lipolysis | Low | Approved for sale as a dietary supplement | Generally well tolerated. Mild side effects include gastrointestinal discomfort | [354] |
| Green tea Green tea catechins (141–1207 mg/day) | ↑ Energy expenditure ↑ Fatty acid oxidation | Low | Approved for sale as a dietary supplement | Generally well tolerated. Mild side effects include nausea, gastrointestinal discomfort, increased blood pressure | [353,356] |

Interventions that target energy expenditure and skeletal muscle metabolism and their associated weight loss efficacy in humans. Clinically relevant weight loss scale: low = 0–2 kg weight loss, medium = 2–5 kg weight loss, high ≥ 5 kg weight loss. SERCA, sarcoplasmic/endoplasmic reticulum Ca2+ ATPase; SNS, sympathetic nervous system; UCP3, uncoupling protein 3.
Exercise evokes ATP demand to support the energy needs during muscle contractile activity. The three most ATP demanding cellular processes include the sarcolemmal Na+/K+ ATPase, Ca²⁺ reuptake into the sarcoplasmic reticulum by the Ca²⁺ ATPase, and actin-myosin cross-bridge cycling by myosin ATPase. The substrate source for ATP supply during exercise is largely determined by exercise intensity and duration. Intense, short-term exercise is fuelled by anaerobic (non-mitochondrial) pathways that facilitate the breakdown of phosphocreatine and glycogen to produce ATP. As the intensity decreases and the duration of exercise increases, oxidative phosphorylation becomes the primary source of ATP for contracting muscle. The metabolic demand for ATP during exercise is accompanied by the activation of intracellular stress signaling pathways in skeletal muscle, including production of ROS [153], release of proinflammatory myokines [154], calcium (Ca²⁺) [155], and the unfolded protein response [156,157]. These molecular stressors elicit physiological adaptations that subsequently enhance mitochondrial oxidative capacity [158–161]. Resistance and aerobic exercise training promote different, complementary but potentially interfering adaptations in skeletal muscle that improve skeletal muscle metabolism [162].

**Aerobic exercise**

Aerobic exercise training is traditionally associated with improved skeletal muscle oxidative capacity and bioenergetic metabolism. A single aerobic exercise bout initiates mitochondrial biogenesis through transient increases in mRNA and protein expression of peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC1α), along with PGC1α translocation to the nucleus [163,164]. PGC1α orchestrates mitochondrial biogenesis by interacting with and activating additional transcription factors that promote expression of nuclear- and mitochondrial-encoded genes involved in β-oxidation, OXPHOS, and antioxidant enzymes [161,165–168]. With prolonged endurance training, the induction of mitochondrial biogenesis and synthesis of new mitochondrial proteins result in increases in skeletal muscle mitochondrial content [163,169–171] that are stoichiometrically associated with exercise-induced improvements in mitochondrial respiratory capacity [172]. There is some evidence from muscle-specific loss of PGC1α rodent models suggesting that PGC1α can be dispensable for exercise-induced increases in mitochondrial content and function [173], suggesting that redundant pathways confer exercise-induced improvements in mitochondrial health. However, human studies generally agree that PGC1α plays a central role in facilitating the exercise-induced improvements in oxidative capacity. In addition to initiating transcription for mitochondrial proteins, aerobic exercise training has profound affects on mitochondrial ribosomal protein translation [172], mitochondrial supercomplex formation [174], and intrinsic mitochondrial function. Together, enhanced mitochondrial density and function contribute to increases in skeletal muscle oxidative capacity that correlate with improvements in cardiorespiratory fitness [163,169–171].

AMP-activated protein kinase (AMPK) and p38 MAP kinase are also activated in skeletal muscle with aerobic exercise and are intimately linked to PGC1α to support increases in mitochondrial content and oxidative capacity [175–180]. In response to aerobic exercise, AMPK and p38 MAPK phosphorylate and directly activate PGC1α, and also indirectly activate other transcription factors to support mitochondrial biogenesis, including myocyte enhancer factor 2 (MEF2), ATF-2, and p53 [179,180]. In addition to contributing to exercise-induced mitochondrial biogenesis, AMPK also improves skeletal muscle glucose and fatty acid metabolism by enhancing substrate uptake and oxidation. Moreover, in rodent models and untrained humans, aerobic exercise has been shown to increase UCP3 mRNA expression in skeletal muscle [181,182], which seems counterintuitive, but may be associated with an enhanced capacity for fatty acid oxidation and the associated need to minimize fatty acid oxidation associated ROS [122,183,184].

Beyond induction of mitochondrial biogenesis, aerobic exercise is associated with enhanced mitochondrial networking which also confers improvements to oxidative capacity. Mitochondria form as an interconnected network and can adapt and reorganize to meet the bioenergetic demands of a cell. Mitochondria appear smaller in skeletal muscle from individuals with obesity, and the mitochondrial reticulum length is shorter [185,186]. A single aerobic exercise bout increases the number of electron-dense contact sites between adjacent mitochondrial membranes [187] and augments mRNA expression of mitofusins 1 and 2 (MFN1 and MFN2) [188]. Aerobic exercise training promotes elongation of the mitochondrial reticulum by enhancing the ratio of fusion to fission proteins [189]. Moreover, endurance training enhances mitophagy, a protective mechanism that clears damaged mitochondria to maintain the health of the mitochondrial network and prevent cellular apoptosis [190,191]. The energetic imbalance imposed by exercise activates AMPK while suppressing mTOR leading to the induction of mitophagy through the PTEN-induced putative kinase protein 1 (PINK1)/Parkin pathway [192–195]. Overall, the exercise-induced adaptations in mitochondrial networking improve mitochondrial turnover, quality, and function.
Resistence exercise

Resistance training robustly increases anabolic signaling and promotes skeletal muscle hypertrophy as evidenced by increases in cross-sectional area of myofibers [196–198]. While long-term aerobic exercise training can also promote hypertrophy particularly in untrained individuals [199], a recent meta-analysis validated the superiority of resis-tance training at promoting skeletal muscle hypertrophy [200]. Resistance exercise-induced skeletal muscle hypertrophy is primarily driven by enhanced myofibrillar protein synthesis [201] and increases in translational capacity via ribosomal biogenesis [202].

There is some evidence suggesting that resistance exercise can enhance mitochondrial function, but not content [203]. However, while increases in skeletal muscle mitochondrial respiration have been observed following 12 weeks of resistance training in the absence of changes in markers of mitochondrial content [203], other studies have failed to report this relationship [204].

Aerobic and/or resistance exercise and weight loss

Weight-loss interventions that combine exercise training with caloric restriction demonstrate that exercise can enhance weight loss capacity compared to caloric restriction alone [147,150–152,205]. Even after RYGB bariatric surgery, higher levels of exercise/physical activity are associated with greater reductions in body weight and fat mass [206]. When comparing the effects on mitochondrial oxidative capacity in patients with obesity, diet-induced weight loss fails to elicit improvements in mitochondrial capacity, whereas diet combined with exercise enhances muscle mitochondrial content and ETC activity [207–209].

While most weight loss studies have focused on aerobic exercise training [210], the combination of aerobic and resistance exercise training may prove to be particularly useful during caloric restriction in DR patients with obesity. Aerobic exercise plus caloric restriction promotes greater decreases in adipose tissue than dieting alone [211] and is likely more effective at promoting decreases in body weight and fat mass than resistance exercise [212,213]. At the molecular level, aerobic exercise training may improve the previously observed deficits in skeletal muscle oxidative capacity in DR individuals by promoting mitochondrial biogenesis. Aerobic exercise can also increase the proportion and cross sectional area of type I fibers [214,215], which may be particularly useful in individuals with diet-resistant obesity who have been shown to have lower proportions and cross sectional area of type I fibers in vastus lateralis muscle [27]. In contrast, resistance exercise would maintain lean body mass and preserve myocellular quality during weight loss [211,216]. Within skeletal muscle, resistance training may enhance protein synthesis and ribosomal biogenesis in DR individuals, who have been shown to have lower ribosomal protein transcripts in whole blood and in skeletal muscle [27]. In line with the idea that a combined aerobic and resistance exercise program may preferentially benefit DR individuals with obesity, when examining the effects of diet plus aerobic and/or resistance exercise on weight loss outcomes, interventions using diet plus combined aerobic/resistance exercise training generally are the most effective at inducing weight loss, improving physical function, and preserving lean body mass [216,217].

Cold exposure

The discovery of functional brown adipose tissue (BAT) in adult humans has renewed interest in exploiting cold-induced thermogenesis to treat obesity. The use of positron-emission tomographic (PET-CT) imaging using radiolabeled tracers demonstrated that BAT is generally expressed in fairly low amounts of cold-induced thermogenesis to treat obesity. The use of positron-emission tomographic and computed tomographic (PET-CT) imaging using radiolabeled tracers demonstrated that BAT is generally expressed in fairly low amounts of cold-induced thermogenesis to treat obesity. The use of positron-emission tomographic and computed tomographic (PET-CT) imaging using radiolabeled tracers demonstrated that BAT is generally expressed in fairly low amounts of cold-induced thermogenesis to treat obesity. The use of positron-emission tomographic and computed tomographic (PET-CT) imaging using radiolabeled tracers demonstrated that BAT is generally expressed in fairly low amounts of cold-induced thermogenesis to treat obesity. The use of positron-emission tomographic and computed tomographic (PET-CT) imaging using radiolabeled tracers demonstrated that BAT is generally expressed in fairly low amounts of cold-induced thermogenesis to treat obesity.
receptors inhibits thermogenesis through the coupled actions of G\textsubscript{i} proteins that inhibit β-adrenergic receptor stimulation of adenylyl cyclase [221,228].

In addition to UCP1-mediated thermogenesis in brown adipocytes, substrate cycling also contributes to enhanced energy expenditure in BAT and is thought to function independent from UCP1. For example, creatine kinase B liberates excess ADP derived from ATP in a 1 ATP:1 creatine stoichiometric relationship, creating a futile creatine-dependent ADP/ATP substrate cycle that stimulates thermogenic respiration in brown adipocytes [229,230]. Similarly, lipid cycling from the resynthesis of TAGs and calcium cycling are also thought to contribute to thermogenesis in BAT independently from UCP1 [231,232].

Low levels of UCP1 were originally hypothesized to be important in obesity susceptibility [233], and the benefits of BAT were recently highlighted by Becher et al. who demonstrated that individuals with BAT have a lower prevalence of cardiometabolic diseases and improved blood glucose and lipid profiles [90]. However, individuals with obesity demonstrate blunted responses to cold exposure, including reduced sympathetic responsiveness, and lower BAT glucose uptake during cold exposure [234]. Moreover, adipocytes from individuals with obesity display lipolytic catecholamine resistance [235]. Overfeeding and cold exposure evoke similar interindividual variation patterns in energy expenditure and metabolism, through diet-induced, and cold-induced thermogenesis, respectively [236,237].

Studies have attempted to target β-adrenergic receptors with sympathomimetics to directly stimulate BAT without the need for cold exposure but have yielded mixed results. Both the systemic infusion of isoprenaline and the intramuscular injection with ephedrine fail to elicit glucose uptake by BAT in humans [238,239]. Ephedrine administered orally has been shown to enhance BAT glucose uptake in lean adults, but not individuals with obesity [240]. Direct activation of β\textsubscript{3}-adrenergic receptors through oral administration of mirabegron (50 and 200 mg), a medication approved for treating overactive bladders, increases energy expenditure in humans [222,241]. Despite increases in energy expenditure, mirabegron failed to stimulate BAT oxidative metabolism in humans and induced undesirable cardiovascular responses owing to contaminant non-selective β-adrenergic receptor activation, including increased heart rate and blood pressure [222]. To avoid the unwanted effects on the cardiovascular system, most studies have employed cold exposure to investigate BAT thermogenesis in humans. However, as a method to study the contributions of BAT, cold exposure is somewhat limited by the inevitable shivering activity that evokes contractions of skeletal muscles, even when measures are taken to minimize shivering activity [242,243]. Cold-induced increases in metabolic rate are closely associated with shivering intensity [243]. During mild cold exposure, shivering preferentially recruits type I muscle fibers in proximal muscle groups for contraction [243] and evokes the mild uncoupling of skeletal muscle mitochondria [244]. These contractions are accompanied by large increases in glucose uptake with mild cold exposure, with skeletal muscle accounting for 50% more glucose turnover than BAT during acute cold exposure [243]. Thus, while the cold-induced rate of glucose uptake in BAT is high relative to its small volume, skeletal muscle is the predominant site of glucose disposal during cold exposure [243]. Results from animal studies have supported the idea that skeletal muscle plays a central role in cold-induced thermogenesis even in the absence of shivering (i.e., non-shivering thermogenesis). Skeletal muscle mitochondrial uncoupling has been linked to increases in metabolic rate in several species such as dogs [245], pigeons [246], fur seals [247], rodents [248], and penguin chicks [249]. Furthermore, animal studies have demonstrated that alterations in mitochondrial coupling are accompanied by the stimulation of skeletal muscle angiogenesis [250], and high rates of calcium (Ca\textsuperscript{2+}) cycling, an ATP-dependent process in the sarcoplasmic reticulum [251,252].

Beyond proton leak and mitochondrial oxidative capacity, there is emerging evidence to support a role for the sarcoplasmic/endoplasmic reticulum Ca\textsuperscript{2+} ATPases (SERCAs) in cold-induced thermogenesis. SERCAs are a family of membrane-bound P-type ATPases that utilize energy derived from ATP hydrolysis to translocate Ca\textsuperscript{2+} against the chemical gradient from cytosol into the sarcoplasmic reticulum [253,254]. Calcium is released from the sarcoplasmic reticulum into the cytoplasm via the ryanodine receptors to initiate a muscle contraction. Following contraction, SERCA ion pumps rapidly facilitate reuptake of calcium into the sarcoplasmic reticulum [255]. However, SERCA-mediated ATP hydrolysis can be uncoupled from Ca\textsuperscript{2+} transport, resulting in a futile cycle controlled by two membrane phosphoproteins: phospholamban and sarcolipin [256]. Data from rodent studies have confirmed a role for sarcolipin-mediated thermogenesis, as mice null for sarcolipin are unable to maintain their body temperature during an acute cold challenge [257]. Furthermore, overexpression of sarcolipin confers resistance against diet-induced obesity and induces mitochondrial biogenesis [258,259], suggesting that SERCA uncoupling could be exploited to enhance skeletal muscle energy expenditure in obesity.

Importantly, many of the molecular adaptations to cold exposure in skeletal muscle appear to diverge in response to either acute or prolonged cold exposure. UCP3 mRNA and protein expression are rapidly up-regulated in rat skeletal muscle within one day of cold exposure; however, prolonged cold exposure is associated with UCP3 downregulation [260]. In humans, UCP3 mRNA expression decreases after 60 h of mild cold exposure, without affecting UCP3 protein
content [261]; however, authors found that interindividual differences in UCP3 protein were closely associated with individual differences in 24-h energy expenditure [261]. When comparing the effects of acute cold exposure versus four weeks of cold acclimation on mitochondrial bioenergetics, proton leak dependent respiration was 4.4-fold higher than baseline following acute cold exposure, but this effect was eliminated with prolonged cold acclimation despite similar levels of whole-body substrate utilization, consistent with the idea of inter-tissue adaptations (e.g., muscle and BAT) [262]. Cold exposure, or mimetics of cold exposure may prove to be useful in boosting both skeletal muscle and BAT thermogenesis.

**Pharmacotherapy**

Research over the past two decades has markedly improved our understanding of the neuroendocrine basis of obesity, and this has led to the development of various anti-obesity medications. Before the development of newer age anti-obesity medications, there were repeated cycles of launching and withdrawing medications due to unforeseen adverse effects. Anti-obesity medications can generally be classified into five groups: (1) mitochondrial uncouplers, (2) appetite suppressants, (3) medications that promote nutrient malabsorption, (4) hormone replacement therapies, and (5) diabetes medications that also promote weight loss. Not all of these classes exert known effects on skeletal muscle metabolism and therefore only some will be addressed here [see detailed anti-obesity medication reviewed by Muller et al. [263]].

**Mitochondrial uncouplers**

As this review focuses to a large extent on variable efficiency of mitochondrial energetics, it is relevant to discuss past and current pharmacologic approaches to induce mitochondrial uncoupling. In the late 1880s, a weak lipophilic acid commonly used to manufacture explosives and dyes, [2,4]-dinitrophenol (DNP), was shown to markedly enhance energy expenditure in dogs. Tainter and Cutting then demonstrated that 300–400 mg/day of DNP could induce rapid weight loss by enhancing resting metabolism in humans [264,265]. DNP is a weak uncoupler that induces diffusion of protons across the inner membrane (i.e., bypassing ATP synthase) and thus increasing oxidative metabolism. Recently, Bertholet et al. challenged the idea that protonophores induce proton leak through protein-independent mechanisms by demonstrating that protonophores, including DNP, activate ANT and UCP1 to facilitate protonophoric activity [96]. DNP (300 mg) was then shown to induce weight loss averaging 6.3 kg in 113 patients with obesity with minimal adverse effects. Anti-obesity medications can generally be classified into five groups: (1) mitochondrial uncouplers, (2) appetite suppressants, (3) medications that promote nutrient malabsorption, (4) hormone replacement therapies, and (5) diabetes medications that also promote weight loss. Not all of these classes exert known effects on skeletal muscle metabolism and therefore only some will be addressed here [see detailed anti-obesity medication reviewed by Muller et al. [263]].

Since then, there has been interest in the development of novel mitochondrial uncouplers with improved tolerability and a wider dynamic range. Several promising compounds have been investigated, but poor absorption and adverse side effects have presented challenges [268]. Recent studies, for example, have focused on BAM15 ((2-fluorophenyl)6-[(2-fluorophenyl)amino](1,2,5-oxadiazolo[3,4-e]pyrazin-5-yl)amine), a mitochondrial-targeted uncoupler that prevents and reverses diet-induced obesity in rodents [269,270]. High-fat diet-fed mice that were treated with 0.1% or 0.15% BAM15 (w/w) exhibited higher whole-body oxygen consumption, had lower RERs, and gained less fat mass compared with untreated mice [269,270], and effects were partly attributable to elevated hepatic lipid oxidation [270]. Moreover, BAM15 enhanced skeletal muscle mitophagy and mitochondrial content, in old mice fed a HF diet that also exhibited attenuated age-related declines in muscle mass and strength [271]. Recently, SHC517 was identified as a more potent derivative of BAM15 that was able to prevent diet-induced obesity and improve glucose tolerance in mice [272]. While these compounds have demonstrated promise in rodent models for the prevention of diet-induced obesity, caution is warranted for concerns related to potential toxicities (i.e., narrow safety windows) and abuse.

A handful of approved medications can also act as protonophores and induce mild mitochondrial uncoupling. The glucose-lowering prodrug salsalate can directly activate the β1 subunit of AMPK, but also enhances mitochondrial uncoupling at clinical concentrations [273]. Rodent studies have demonstrated that salsalate can enhance skeletal muscle thermogenesis and attenuate diet-induced weight gain [274]; however, clinical trials examining the glucose lowering effects of salsalate have reported no change in body weight [275,276]. The anthelmintic medication niclosamide and its salt derivatives, niclosamide ethanalamine and nicloasmide piperazine, have shown promising effects at preventing diet-induced obesity by promoting mitochondrial uncoupling and enhancing lipid oxidation in rodents [277,278]. While niclosamide and its derivatives are tolerable for short-term use [279,280], future studies examining the long-term safety are needed. Altogether, mitochondrial-targeted uncouplers that have less safety concerns may be beneficial at enhancing skeletal muscle metabolism by increasing proton leak in individuals with obesity.
**Appetite suppressants**

Many weight loss medications act to increase satiety and lower caloric intake through the modulation of monoamine neurotransmitters [281]. Centrally acting agents increase satiety by modulating serotonin, noradrenaline, or dopamine in the hypothalamus to block catecholamine reuptake. There are two main classes of centrally acting appetite suppressants that are approved for short-term use: sympathomimetics and serotoninergic agents.

Soon after DNP was withdrawn, amphetamine (benzedrine sulphate) was widely used to induce weight loss mainly by suppressing hunger [282–284]. Amphetamine was successful at inducing weight loss, but the highly addictive drug led to adverse psychiatric effects, physical dependence and abuse. Sympathomimetic agents were developed as phenylethylamine derivatives, which are structurally similar to amphetamine, but without the α-methylated side chain. These phenylethylamine derivatives are synthesized from tyrosine and act on hypothalamic and limbic regions of the brain to stimulate the release of norepinephrine and dopamine and increase satiety. In addition to increased satiety, certain sympathomimetic agents may also increase energy expenditure by stimulating β-adrenergic receptors and delaying gastric emptying, such as phentermine and mazindol [285]. The peripheral effects of sympathomimetic medications on skeletal muscle are relatively unexplored; however, there is evidence to suggest that amphetamines may enhance skeletal muscle thermogenesis. For example, rodents administered methamphetamine or 3,4-methylenedioxyamphetamine (MDMA) exhibit increases in skeletal muscle temperature [286], and increases body temperature correlate with UCP3 protein expression [287]. Moreover, UCP3 knockout mice display a blunted hyperthermic response to MDMA, and pharmacological blockade of SERCA-mediated Ca²⁺ release attenuates methamphetamine-induced hyperthermia [288]. Since diet-resistant obesity exists after controlling for compliance to a 900 kcal/day meal replacement diet [25], it is likely that appetite suppressants would not benefit DR obesity to a greater degree than DS obesity. However, the stimulation of β-adrenergic receptors by certain sympathomimetic agents could enhance skeletal muscle metabolism. In both rodent models and humans, individuals with increased susceptibility to weight gain often display decreased adrenergic-dependent thermogenic capacity [289].

Skeletal muscle also contains β₁-, β₂-, and β₃-adrenergic receptors, with β₂-adrenergic receptors as the predominant muscle isoform [290]. Density of β-adrenergic receptors is higher in type I fibers, and correlates with succinate dehydrogenase activity, a marker of oxidative capacity [291]. Similar to the mechanism in BAT, stimulation of β-adrenergic receptors in skeletal muscle activates PKA via cAMP, which in turn phosphorylates the RyR1, leading to SERCA-mediated Ca²⁺ release and generation of contractile force [292,293]. Pharmacological blockade of β-adrenergic receptors in humans is associated with decreases in isokinetic endurance and lower thermogenesis in vastus lateralis biopsies [294]. β-adrenergic receptor agonism in humans is associated with increases in glucose uptake, lipolysis, and skeletal muscle hypertrophy, resulting from both increases in protein synthesis and suppression of catabolic pathways [295–297]. Evidence from in vitro studies suggests that β₂-adrenergic receptor activation enhances mitochondrial function in myotubes [298]. However, chronic administration of β₂-agonists is associated with increases in the proportions type II fibers in rodents, and decreases in oxidative enzyme activity [299,300], although there is some evidence that deleterious shifts in fiber type can be mitigated with low-intensity exercise [301]. In contrast, mirabegron, the β₃-adrenergic receptor agonist, has recently been shown to promote fiber-type switching to type I fibers and reduces skeletal muscle content [302]. Altogether, activation of β-adrenergic receptors by sympathomimetic agents could theoretically enhance thermogenesis in individuals with diet-resistant obesity, particularly when combined with exercise to mitigate shifts in fiber type.

**Thyroid hormones**

Thyroid hormones have repeatedly been associated with body weight, BMI, and mitochondrial uncoupling thermogenesis [303–307]. Thyroid-stimulating hormone (TSH) is produced by the pituitary and acts as the primary signal for thyroxine (T₄) and triiodothyronine (T₃) production and release by follicular cells in the thyroid gland. T₄ is an iodine-containing tyrosine-based precursor hormone that is converted into T₃ [308]. T₃ binds to nuclear thyroid hormone receptors (TR) to up-regulate the transcription of T₃-responsive genes. T₃ and T₄ increase basal energy expenditure by modulating major metabolic pathways, augmenting ATP turnover, and uncoupling mitochondrial oxidative phosphorylation. Hypothyroidism has long been associated with weight gain; whereas patients with hyperthyroidism frequently present with weight loss, and thus, desiccated thyroid, thyroxine, and triiodothyronine have historically been used to treat obesity [309,310]. Similarly, weight loss is associated with small decreases in T₃ [311–317]. Supplementation with triiodothyronine during a hypocaloric liquid diet has been shown to enhance diet-induced weight loss [318]. Similarly, patients with high baseline levels of free T₃ achieve significantly greater weight loss following bariatric surgery [319]. The actions of thyroid hormones appear to be partially mediated by leptin [320].
In skeletal muscle, T3 also augments the expression and activity of Na+/K+ ATPase in the plasma membrane and the Ca²⁺ ATPase of the sarcoplasmic reticulum via SERCA1 [321,322]. In skeletal muscle, T3 also increases TCA cycle flux and promotes mitochondrial uncoupling [323]. Supplementation with 75 μg of T3 upregulates mRNA expression of UCP3 and adenine nucleotide translocases 1 and 2 in vastus lateralis muscle of healthy volunteers [324]. Moreover, thyroid hormones enhance lipolysis and mobilization of triglycerides from adipose tissue, and lipid utilization [325]. In rodents, hypothyroidism and hyperthyroidism cause decreased and increased levels, respectively, of ‘energy wasting’ mitochondrial proton leak in hepatocytes [326]. Induction of mitochondrial proton leak uncoupling in skeletal muscle by T3 occurs in vivo [323] and in ex vivo preparations [327–330]. Because DR obesity is associated with lower OXPHOS, decreased fatty acid utilization, and lower mitochondrial uncoupling, a greater understanding of the effects of thyroid hormones on muscle physiology in obesity, particularly DR obesity, is needed.

**Diabetes medications: metformin, DPP4 inhibitors, and GLP-1**

Metformin is well recognized as the first line of pharmacotherapy for patients with Type 2 diabetes and pre-diabetes. Despite its introduction in 1950, the exact mechanism of metformin is not fully understood, but many of its actions are associated with the activation of AMPK and inhibition of complex I [331]. Metformin does not directly activate AMPK [332] but rather is thought to modulate the ADP:ATP ratio leading to AMPK activation [331]. The AMPK-dependent actions of metformin have been linked to increases in skeletal muscle mitochondrial fatty acid oxidation and decreased expression of lipogenic genes [333,334]. Recent large-scale meta-analyses have revealed that metformin can reduce body weight and adiposity, which has been attributed to decreased energy intake [335,336].

Newer aged diabetes medications, Liraglutide and Semaglutide, are agonists of gut-derived peptide hormones (incretins) that have recently been approved in many countries for the treatment of obesity. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretins secreted by intestinal L and K cells into the hepatic portal system to facilitate glucose-stimulated insulin secretion [337,338]. Both GLP-1 and GIP are rapidly inactivated by the enzymatic removal of the N-terminal dipeptide by dipeptidyl peptidase 4 (DPP4) [339]. The discovery that GIP activity was impaired in patients with T2D [340] lead to the pharmacological development of incretin analogues, DPP4 inhibitors, and receptor agonists for the treatment of T2D. DPP4 inhibitors are generally considered to be weight neutral, as they are associated with variable results on body weight [341,342]. However, GLP-1 receptor agonists can elicit weight loss. GLP-1 receptors are involved in appetite regulation and are highly expressed in the brain [343]. As such, GLP-1 receptor agonism induces weight loss by enhancing satiety signals. Indeed, early investigative studies demonstrated that intravenous infusion with GLP-1 suppressed appetite, decreased energy intake and delayed gastric emptying in individuals with obesity and in healthy adults [344–347]. Clinical studies then demonstrated that GLP-1 receptor agonists, such as Liraglutide, can aid in weight loss when combined with a hypocaloric diet [348]. Specifically, when combined with behavioral therapy, GLP-1 agonists are associated with a 5–7% weight loss after 12 months, with greater success at higher doses. GLP-1 agonists exert anabolic effects within muscle and can ameliorate muscle atrophy to preserve lean body mass in mice [349], and have recently been shown to overcome anabolic resistance to feeding in older humans [350]. The appetite suppressing effects of GLP-1 agonists would likely confer equal benefits to DS and DR patients, while the anabolic effects of GLP-1 on skeletal may prove to be helpful in those with DR obesity.

**Supplements**

Dietary supplements hold appeal because they can be easier to implement than large lifestyle changes such as diet and exercise. Supplements are also often readily available without a prescription and many are associated with prodigious claims regarding weight loss. Approximately 15% of adults have reported using non-prescription dietary supplements to promote weight loss [351]. In contrast with the regulation of prescription medications, weight loss supplements often contain multiple ingredients and many clinical trials involving dietary supplements are often of poor methodological quality. Of the widely available supplements, there is some evidence to support weight-loss claims for caffeine, (L-)carnitine, conjugated linoleic acid, green tea, and ephedrine. However, meta-analyses have concluded that ephedrine [352,353] and conjugated linoleic acid [354] can elicit weight loss of less than 1 kg, for which the clinical relevance is low.

The effects of green tea on weight loss are attributed to caffeine and catechins in green tea. When examining the effects of green tea catechins on weight loss many studies are confounded by the presence of caffeine in green tea. While green tea extract has been shown to increase thermogenesis and fat oxidation in humans [355], meta-analyses have determined that green tea does not elicit significant weight loss [353,356]. However, caffeine has been associated with modest weight loss in both short-term clinical interventions and large-scale epidemiological studies. Caffeine...
is a methylxanthine that increases activity of the sympathetic nervous system by acting as a competitive inhibitor of phosphodiesterase and adenosine, leading to increases in intracellular cyclic adenosine monophosphate (cAMP) and suppressed catecholamine release. The resulting caffeine-induced increase in SNS activity has been shown to promote lipolysis, enhance fatty acid oxidation, and cause short-term increases in energy expenditure in a dose-dependent manner [357]. Caffeine is also an agonist of ryanodine receptors and can stimulate calcium ion flux in the sarcoplasmic reticulum [358], which may enhance skeletal muscle thermogenesis through increased SERCA activity [359]. Most clinical studies that have examined the efficacy of caffeine on weight loss have been of short duration and many have used caffeine in combination with other ingredients. However, a recent meta-analysis of RCTs determined that caffeine consumption is associated with an average weight loss of <2 kg after 4 weeks [360]. Moreover, data from cross sectional and observational studies have suggested that increased caffeine intake is associated with less weight gain in the long-term [361], and may also be beneficial for the maintenance of weight loss [362].

(L-)-Carnitine (L-b-hydroxy-c-N-trimethylaminobutyric acid) is an endogenous compound that plays an essential role in lipid metabolism by acting as a cofactor to facilitate long-chain fatty acid transport across the mitochondrial membrane via formation of acylcarnitines [363]. L-Carnitine also participates in glucose metabolism by maintaining the acetyl-CoA/CoA ratio. Intramitochondrial free CoA availability increases as fatty acids are transported into mitochondrion via carnitine, and the rise in free CoA stimulates the pyruvate dehydrogenase complex [364]. More than 95% of total carnitine is stored in skeletal muscle, and chronic oral L-carnitine with simultaneous carbohydrate ingestion can increase intramuscular carnitine concentrations and alter fuel utilization during exercise [365,366]. Meta-analyses of randomized controlled trials have concluded that L-carnitine is associated with modest weight loss between 1.2 and 1.3 kg and an average of 2.08 kg of fat mass [367,368]. We have previously demonstrated that patients with DR obesity have a lower capacity for fatty acid oxidation, reflected by less fatty acid-supported respiration in permeabilized skeletal muscle and greater circulating long- and medium-chained acylcarnitines following a high fat meal [46]. Thus, (L-)carnitine supplementation may enhance the capacity for fat oxidation.

**Conclusion**
The use of caloric restriction and very low-calorie diet programs often yield considerable variability in weight loss success. Understanding the metabolic processes that contribute to overall energy expenditure and adaptive thermogenesis will aid in the development of novel treatment strategies for individuals who fail to respond adequately to diets despite documented adherence. With new technologies and integrative -omics platforms, future research avenues may lead to more personalized lifestyle and nutrition treatment strategies for more effective weight loss outcomes. Interventions that enhance energy expenditure, mitochondrial biogenesis, and mitochondrial uncoupling may benefit individuals with diet-resistant obesity. Exercise is often given low priority in weight loss programs but is beneficial in offsetting adaptive decreases in metabolic rate. Cold exposure and sympathomimetic drugs (appetite suppressants) and supplements (caffeine and ephedrine) may stimulate β-adrenergic receptors to enhance energy expenditure during weight loss, but effects are generally modest. Mitochondrial uncoupers can be effective at enhancing energy expenditure through futile processes but most have deleterious side effects that currently limit their use. The development of new anti-obesity drugs and weight loss agents could exploit processes such as uncoupling in skeletal muscle to enhance energy expenditure.

**Competing Interests**
The authors declare that there are no competing interests associated with the manuscript.

**Funding**
CIHR FDN-143278 (M.E.H.).

**CRediT Author Contribution**
Chantal A. Pileggi: Visualization, Writing—original draft, Writing—review & editing. Breana G. Hooks: Visualization, Writing—original draft, Writing—review & editing. Ruth McPherson: Writing—original draft, Writing—review & editing. Robert Dent: Writing—original draft, Writing—review & editing. Mary-Ellen Harper: Writing—original draft, Writing—review & editing.

**Abbreviations**
ADP, adenosine diphosphate; AMPK, AMP-activated protein kinase; ANT, adenine nucleotide translocase; ATP, adenosine triphosphate; BAT, brown adipose tissue; BMI, body mass index; cAMP, cyclic adenosine monophosphate; DNP, (2,4)-dinitrophenol; DPP4, dipeptidyl peptidase 4; DR, diet resistant; DS, diet sensitive; ETC, electron transport chain; FFM,
fat-free mass; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; MFN1/2, mitofusin 1 and 2; NEAT, non-exercise activity thermogenesis; OXPHOS, oxidative phosphorylation; PGC1α, peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α; PMF, proton motive force; RER, respiratory exchange ratio; RMR, resting metabolic rate; ROS, reactive oxygen species; RQ, respiratory quotient; RYGB, Roux-en-Y gastric bypass; SERCA, sarcoplasmic/endoplasmic reticulum Ca2+ ATPase; SNS, sympathetic nervous system; T2D, type 2 diabetes; TSH, thyroid-stimulating hormone; UCP, uncoupling protein.

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