INTRODUCTION

Overweight and obesity are defined by BMI, waist circumference, or waist-hip ratio. The World Health Organization (WHO) has classified BMI of 18.5 to 24.99 kg/m² as normal body weight, BMI ≥ 25 as overweight, and BMI ≥ 30 as obesity. In 2016, WHO reported that more than 1.9 billion adults had overweight (39% of the global population), of which 650 million had obesity (13% of the global population). Obesity affects all ages; more than 340 million children and adolescents worldwide have overweight or obesity (1). Overweight and obesity are usually caused by an imbalance between energy intake and energy expenditure, although genetics, gut microbiota, chronic stress, and side effects from medication (e.g., corticosteroids, older agents for treatment of neuropsychiatric diseases) are known contributors (2-4). Furthermore, overweight and obesity are strongly associated with insulin resistance and type 2 diabetes (T2DM) (5). Although whole-body energy metabolism is affected in individuals with overweight and obesity, this review will focus on adaptations in skeletal muscle energy metabolism, examining both in vivo and in vitro findings. In vivo results are mainly from studies on biopsies and muscle strips, whereas in vitro results are from cellular studies using skeletal muscle cells differentiated into myotubes. Satellite cells isolated from skeletal muscle biopsies proliferate to myoblasts that can be differentiated into multinucleated myotubes. Thus, the latter resembles mature skeletal muscle fibers. Several studies have shown that cultured human myotubes retain...
many metabolic characteristics of their muscle cell donors (6-13). Therefore, this is a unique model to distinguish between genetic and environmental factors in the etiology of obesity. This review describes four population groups based on BMI where possible: lean individuals = BMI < 25; individuals with overweight = 25 ≤ BMI < 30; individuals with obesity = 30 ≤ BMI < 40; and individuals with severe obesity = BMI ≥ 40.

**METABOLIC ALTERATIONS IN SKELETAL MUSCLE ASSOCIATED WITH OBESITY**

**Glucose and lipid metabolism**

The biochemical basis for insulin resistance may involve reduced glucose transport and/or defects in the intracellular handling of glucose in obesity (14-16). Basal uptake and oxidation of glucose were increased in myotubes from individuals with overweight compared with lean individuals (10). In contrast, an unchanged glucose oxidation in myotubes from donors with obesity compared with lean donors was reported (17). Myotubes from individuals with obesity, with or without T2DM, had decreased uptake and oxidation of glucose (Figure 1, unpublished results). In a recent study with cultured myotubes from a large number of donors, we examined how donor characteristics influenced energy metabolism in myotubes; no association between donor BMI and glucose uptake and oxidation was observed (18). Therefore, the described differences in basal glucose metabolism in individuals with obesity compared with lean individuals are inconsistent, possibly as a result of methodological differences.

With severe obesity, changes in skeletal muscle glucose metabolism, such as reduced insulin-mediated glucose uptake and oxidative metabolism as well as increased lactate production, have been observed (15,16). Impaired insulin-stimulated glucose uptake in skeletal muscle from individuals with severe obesity is accompanied by a deficiency in insulin receptor signaling, which may contribute to decreased insulin action (14,19). In skeletal muscle of individuals with severe obesity, several kinases are activated with subsequent serine phosphorylation of insulin receptor and insulin receptor substrate 1 (IRS1). These modifications depress insulin signal transduction and decrease stimulation of glucose transport, glycogen synthesis, glucose oxidation, and nonoxidized glycolysis (13,20). In myotubes from individuals with severe obesity, insulin stimulation of IRS1 tyrosine phosphorylation and protein kinase B (Akt) phosphorylation was shown to be blunted together with increased IRS1 serine phosphorylation (21). This impaired insulin signaling is greater in myotubes than in intact skeletal muscle (13,21). Another study with myotubes established from individuals with severe obesity who were nondiabetic showed a partitioning of glucose toward anaerobic glycolysis rather than oxidation in response to insulin stimulation due to a defective tricarboxylic acid cycle flux (22).

Changes in insulin-mediated metabolism are frequently seen when comparing primary human skeletal muscle cells from lean individuals versus individuals with obesity and T2DM (7,9). This has often been done to maximize the genetic difference, but findings from such studies may be due to the diabetes and/or obesity phenotype.

Several differences in lipid metabolism have been observed between skeletal muscle from lean individuals and individuals with obesity. Skeletal muscle of individuals with obesity typically displays a phenotype characterized by increased intramuscular/intramyocellular triacylglycerol (IMTG) (23-25) and reduced overall lipid oxidative capacity (26-30). Reduced fatty acid oxidation (FAO) was also observed in intact muscle strips and muscle homogenates from individuals with severe obesity compared with lean individuals and individuals with obesity (20). When comparing metabolism in muscle cells with these muscle strips and muscle homogenates, reduction in FAO was almost identical among the three groups (20). In addition, oxidation efficiency was equally reduced in myotubes and in vivo measurements in skeletal muscle (21). Lipid oxidation may be reduced in myotubes from individuals with severe obesity because of impairments at several levels of lipid metabolism, such as an increased mRNA expression of the lipogenic enzyme stearoyl-Coenzyme A (CoA) desaturase-1 and reduced mitochondrial content (13,20). Therefore, in obesity, metabolic capacity of skeletal muscle appears to be organized toward fatty acid (FA) esterification and storage rather than oxidation (25,31,32).

Some studies have suggested that reduced oxidative capacity in obesity and/or T2DM is attributable to decreased muscle mitochondrial content, probably due to impaired mitochondrial biogenesis and not intrinsic defects in mitochondrial FAO (33-36). There have also...
been findings indicating that existing mitochondria are unable to effectively oxidize substrates (26,37). In cultured myotubes from individuals with obesity, mitochondrial lipid oxidation was impaired (38). In skeletal muscle homogenates from individuals with obesity, mitochondrial lipid catabolic processes were decreased at several levels compared with lean individuals (30). FAO in obesity may be impaired at the level of carnitine palmitoyltransferase (CPT) 1, β-oxidation, and FA-derived respiration (32). Myotubes established from donors with obesity showed increased β-oxidation of palmitate as well as reduced oxidation efficiency (39). This was associated with an overall transcriptional upregulation of several β-oxidative enzymes and reduced mitochondrial respiratory chain complex II, III, and IV protein expression, indicating reduced mitochondrial function (39). In contrast, a study with myotubes and permeabilized myofibers showed no differences in mitochondrial respiratory function or content between lean individuals and individuals with obesity (40). It also was shown that mitochondrial network morphology as well as regulatory proteins in mitochondrial quality control was dysregulated in myotubes from individuals with severe obesity with or without T2DM, causing mitochondrial fragmentation (41).

When it comes to FA transport and storage, there are findings showing that skeletal muscle from individuals with obesity possessed a greater protein-facilitated apparatus for FA transport and uptake (42). This is concomitant with greater FA esterification to IMTG, resulting in higher IMTG levels (42). Myotubes from donors with obesity with or without T2DM demonstrated increased FA uptake and incorporation into complex lipids (39). Sparks et al. reported that FA incorporation into triacylglycerol (TAG) was perturbed in individuals with T2DM compared with BMI-matched control individuals, both in muscle tissue and cultured myotubes (43). Furthermore, total lipid accumulation, protein expression of FA translocase CD36, and FA esterification into complex lipids have been reported to be upregulated in myotubes from individuals with severe obesity (21). This indicates that reduced oxidation was not due to reduced FA uptake. Contrarily, myotubes from individuals with overweight showed reduced accumulation of FAs but no difference in uptake or oxidation compared with cells from lean individuals (10). We recently published that only FA uptake was increased by donor BMI when examining FA metabolism in myotubes from a large number of donors (18).

Lower hormone-sensitive lipase (HSL) and higher adipose tri-glyceride lipase (ATGL) protein content was shown in skeletal muscle of individuals with obesity (44). This difference in lipase content was accompanied by lower diacylglycerol (DAG) hydrolyase and normal TAG hydrolyase activity, implying incomplete lipolysis (44,45). In line with this, myotubes from individuals with obesity showed markedly lower lipolysis and HSL protein expression compared with cells from lean individuals, indicating a primary defect acquired in obesity that persists in cultured myotubes (46). Moreover, it was shown that intrinsic defects in lipolysis and HSL expression coexisted with reduced insulin action in myotubes from individuals with obesity and T2DM (46). Therefore, it seems that TAG turnover may have been changed in obesity because of changes in lipase expression and regulation of lipolysis (47). Others have also described reduced lipid turnover and FAO rate in myotubes from donors with obesity with or without T2DM compared with lean individuals (8,13,38,48).

Another aspect to consider when discussing effects of obesity on skeletal muscle metabolism is fiber type. Skeletal muscles are composed of different fiber types that are all structurally, functionally, and metabolically different. The phenotypes are classified by contractile speed (slow- or fast-twitch) and histochemical staining for myofibrillar (myosin) adenosine triphosphatase (ATPase). The slow-twitch phenotype is type I, and the fast-twitch phenotype is type II (highest ATPase activity). Human skeletal muscle fibers mainly express three myosin heavy chain (MHC) isoforms: MHCß, MHC2A, and MHC2X (genes MYH7, MYH2, and MYH1, respectively) (49). MHCß-expressing fibers are slow, fatigue resistant, and oxidative type I muscle fibers; MHC2A-expressing fibers are fast oxidative type IIA muscle fibers; and MHC2X-expressing muscle fibers are fast glycolytic type IIX fibers (49). In individuals with obesity, a reduced amount of type I and an increased amount of type IIB muscle fibers have been observed compared with lean individuals (50). Interestingly, having more type I muscle fibers seemed to promote larger reductions in BMI in individuals with severe obesity undergoing a weight loss intervention (50). This indicates a relationship between obesity and skeletal muscle fiber type. Comparing cultured human myotubes from individuals with overweight and lean individuals, we did not observe any differences in fiber-type expression (10). Furthermore, an association between insulin sensitivity and the amount of oxidative type I skeletal muscle fibers has been suggested, as a lower expression of type I fibers has been observed in skeletal muscle biopsies from individuals with insulin resistance and T2DM compared with healthy individuals (51,52). However, there are discrepancies, as there has also been shown to be no differences in fiber-type distribution between skeletal muscle biopsies from lean, nondiabetic individuals and individuals with obesity and T2DM (53).

To summarize, metabolic disturbances in skeletal muscle associated with obesity include decreased insulin-mediated glucose uptake and insulin action, dysregulation of lipid metabolism, reduced mitochondrial substrate oxidation, and changes in mitochondrial network morphology. Specific metabolic adaptations with obesity are presented in Figure 2.

**Metabolic flexibility**

The ability to switch between substrates for fuel, depending on substrate availability, exercise intensity, and physiological conditions, represents an important feature of healthy skeletal muscle. This is referred to as metabolic flexibility (54-56), whereas loss of this ability is termed as metabolic inflexibility (54). Emerging evidence suggests that an inability to adapt to metabolic challenges, such as insulin stimulation or lipid exposure, may be an important aspect of the oxidative impairment in the obesity/diabetes phenotype (28,57-59). This is associated with reduced lipid oxidation and higher lipid accumulation in skeletal muscle (60), which may interfere with...
insulin signaling and function (Figure 2). Insulin resistance, obesity, and T2DM are linked to reduced FAO during fasting and impaired postprandial switch from lipid to glucose oxidation (28). This inflexibility has also been observed in individuals with impaired glucose tolerance (61), suggesting that inflexibility plays a role in early development of T2DM.

Inhibition of glucose oxidation by FAs (the Randle cycle) is mediated by inhibition of several glycolytic steps. Pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4), the dominant isoform in skeletal muscle, inhibits the pyruvate dehydrogenase (PDH) complex by phosphorylation, thereby switching fuel source from glucose to FAs (62). It was shown that PDH activity is regulated through acetylation-deacetylation catalyzed by sirtuins, and that impaired deacetylation reduces PDH function (63). Moreover, low carnitine availability may contribute to metabolic inflexibility and impaired glucose tolerance. Acetylcarnitine is synthesized by a conjugation of acetyl-CoA and free carnitine to prevent extreme fluctuations in the availability of acetyl-CoA and free CoA. Buffering of these CoA pools is especially important in order to reduce FA β-oxidation and prevent inflow of acetyl-CoA from FAO, which may reduce carbohydrate utilization (64).

Excess production of citrate from enhanced FAO escapes from mitochondria and inhibits the rate-limiting enzyme of glycolysis, 6-phosphofructo-1-kinase. This leads to an increase in glucose-6-phosphate, which eventually inhibits hexokinase and subsequently reduces glucose uptake and oxidation (65). The opposite, in which glucose suppresses FAO, is usually referred to as the reverse Randle cycle (66). Citrate that escapes from glucose oxidation is transported back to cytosol and converted to acetyl-CoA by ATP citrate lyase, which, in turn, is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC) 2. Malonyl-CoA inhibits CPT1 and thereby entry and oxidation of FAs in mitochondria (66,67). Thus, citrate signals both fed (high concentrations of glucose) and fasted (high concentrations of FAs) states.

In vitro suppressibility was inversely correlated with insulin sensitivity and metabolic flexibility in vivo, whereas adaptability was positively correlated with the same parameters (59). We have previously shown that myotubes from individuals with severe obesity and T2DM had lower adaptability for

**FIGURE 2** Metabolic alterations in skeletal muscle in obesity. Obesity is associated with numerous alterations in skeletal muscle metabolism, affecting both glucose and FA metabolism as well as insulin action. ATGL, adipose triglyceride lipase; CPT, carnitine palmitoyltransferase; DAG, diacylglycerol; FA, fatty acid; GLUT, glucose transporter; HSL, hormone-sensitive lipase; IMTG, intramuscular/intramyocellular triacylglycerol; IR, insulin receptor; IRS1, insulin receptor substrate 1; LDs, lipid droplets; MAG, monoacylglycerol; MCT, monocarboxylate transporter; PDC, pyruvate dehydrogenase complex; PDK4, pyruvate dehydrogenase lipoamide kinase 4; TAG triacylglycerol; TCA, tricarboxylic acid.
Ectopic lipid accumulation, lipotoxic lipids, and insulin resistance

A common consequence of obesity is the ectopic storage of FAs as TAG in lipid droplets (LDs) in skeletal muscle (IMTG, Figure 2). LD-coating proteins are essential for the formation and stability of these intracellular LDs. Perilipin (PLIN) 2 is an abundant LD-coating protein in skeletal muscle, as the majority of LDs are covered by PLIN2 (72). Interventions that increase muscle insulin sensitivity might be followed by increased PLIN2 protein expression (73), suggesting that PLIN2 might play a role in decreasing intramuscular lipid toxicity by promoting lipid storage. On the other hand, comparable muscular PLIN2 protein content has been observed in individuals with obesity with or without T2DM (74). We have studied the role of PLIN2 using myotubes from mice lacking PLIN2. PLIN2 was essential to protect the pool of skeletal muscle LDs to avoid uncontrolled hydrolysis of stored TAG and balance energy utilization of glucose and FAs (75).

It has been hypothesized that IMTG accumulates because of higher adiposity, whereas markers of intramyocellular lipotoxicity (i.e., DAG and ceramides) are determined within skeletal muscle (47). IMTG storage in untrained individuals is a strong predictor of insulin resistance and the development of T2DM (47,76-78). Higher levels of IMTG, when not matched by an increased oxidative capacity, have been associated with increased levels of lipotoxic intermediates that, in turn, could inhibit insulin signaling (47). IMTG per se does not cause insulin resistance, as endurance-trained athletes often have high IMTG levels but still show superior insulin sensitivity compared with sedentary individuals (24,79,80). The combination of a higher capacity for mitochondrial FAO as well as increased short-term storage of excess TAG may reduce intracellular lipid intermediates that negatively interfere with insulin signaling, thereby relieving insulin resistance (81,82). In fact, increased sequestering of incoming fat into IMTG has been shown to decrease lipid intermediates and improve insulin sensitivity in transgenic animals overexpressing diacylglycerol acyltransferase (DGAT) 1 in skeletal muscle (83) or lacking ATGL (47). Therefore, a mismatch between IMTG hydrolysis (lipolysis), FA re-esterification (lipid turnover), and mitochondrial FAO may increase the levels of lipotoxic intermediates with detrimental effects on insulin signaling and glucose metabolism (47,78,84).

Myokines in interorgan cross talk

Interorgan cross talk has a critical role in the maintenance of homeostasis and adaptation to external conditions. Peripheral organs produce and secrete numerous bioactive molecules, including hormones that enable autocrine, paracrine, or endocrine intercellular signaling. In the past years, the number of such signaling molecules produced by metabolic organs has increased dramatically, and the terms myokines, hepatokines, adipokines, and batokines have become common terms for the molecules produced by muscle, liver, and white and brown adipose tissue, respectively (85). Myokines regulate skeletal muscle mass and fiber switching and have profound effects on glucose and lipid metabolism. Myokines are proposed to play important roles in mediating beneficial health effects by increasing skeletal muscle mass upon exercise (86), as secretion of several myokines is dependent upon contraction (87). Obesity is associated with physical inactivity, which can lead to an altered myokine response. This could provide a molecular basis for understanding potential mechanisms for the association between sedentary behavior and many chronic diseases (88). Given that myokines contribute to favorable metabolic changes, identifying mediators linking physical activity to metabolic health could have potential as therapeutic targets for obesity and associated metabolic diseases (89).

Hypoxia, autophagy, and proinflammatory signaling pathways may modify skeletal muscle energy metabolism

Skeletal muscle oxidative metabolism in vivo may be lowered by hypoxia because of reduced perfusion (vascularization) in obesity, which may precede loss in muscle mitochondrial density and promote glycolytic energy utilization (90). Therefore, changes in control of skeletal muscle circulation and vascular dysfunction that occur with obesity may be implicated in impairment of skeletal muscle energy metabolism (91).
Moreover, autophagy regulates cellular energy metabolism as well as amino acid, glucose, and lipid turnover. Defects in autophagy homeostasis have been implicated in obesity (92). It has also been shown in obesity that proinflammatory and stress-activated signaling pathways are induced. These are important contributors in the pathogenesis of obesity and insulin resistance. On the molecular level, activating these pathways in skeletal muscle promotes apoptosis and atrophy of the muscle (93).

EFFECTS OF EXERCISE AND POSSIBLE PHARMACOLOGICAL TARGETS FOR TREATMENT THROUGH ACTION ON SKELETAL MUSCLE

Increasing daily energy expenditure to induce a negative energy balance is an efficient strategy in obesity management. Lifestyle interventions include a healthy diet with caloric restriction, increased daily physical activity, preferably regular exercise, and behavioral and motivational changes. To date, pharmacological treatments to reduce body weight are limited, much owing to adverse effects and limited efficacy. Despite choice of treatment, many individuals struggle to maintain and sustain weight loss and associated health benefits. The most intrusive treatment option for obtaining a significant weight loss is bariatric surgery. However, it may lead to complications, and its success is highly dependent on the patient’s motivation for maintaining the effect. In this review, neither bariatric surgery nor dietary interventions will be further discussed.

Exercise

Increased daily physical activity and regular exercise have been long recommended as part of weight loss therapy. A challenge when evaluating exercise intervention studies is the large variation in study setup. This includes exercise volume (duration and intensity); type of exercise included (aerobic, anaerobic, or a combination); whether it is in combination with another intervention (dietary, pharmacological, or motivational); number, age, gender, and degree of obesity of the participants; and whether a nonintervention control group is included.

Skeletal muscles are characterized by their ability to adapt and remodel in response to contraction, allowing the muscles to utilize substrates for ATP production more efficiently and thereby become more resistant toward fatigue. Energy metabolism, mitochondrial content and function, intracellular signaling, and gene transcription, as well as contractile proteins, are all affected by contraction (94).

During physical activity, skeletal muscle uses both circulating plasma glucose as well as stored glycogen as fuel sources. Contraction activates both oxidative and nonoxidative glucose disposal and glucose uptake. If exercise is maintained, skeletal muscle oxidative capacity and mitochondrial biogenesis will be further improved (95). It has been shown in both lean individuals and individuals with overweight that endurance exercise promotes a shuttling of dietary fat toward oxidation instead of storage. This is due to increased capacity for FA transport in skeletal muscle combined with increased mitochondrial FAO. Endurance exercise training has been shown to coordinately upregulate lipid oxidative capacity and lipolytic protein expression in the skeletal muscle of individuals with obesity. This physiological adaptation probably favors lipid oxidation and may alleviate lipotoxic lipid pressure in skeletal muscle (96). This is also aided by the finding that exercise training increases IMTG turnover and reduces content of DAG and cholesterol ester.

Acute exercise has immediate beneficial effects in individuals with obesity and T2DM, such as reduced IMTG resulting in stimulated lipolysis and lipid oxidation. However, muscle adaptations to long-term exercise programs are important for improving health outcomes and reversing insulin resistance as well as increasing expression of muscle PLIN and lipolytic enzymes. Furthermore, toxic lipid species such as ceramides are reduced upon physical exercise training, alleviating the inhibition of insulin signaling pathways and thereby contributing to improved insulin sensitivity (97). Fritzen et al. recently reviewed effects of dietary fat and different types of exercise on lipid metabolism, in particular FAO (32). They described aerobic exercise as an effective way to improve FAO and metabolic inflexibility in individuals with obesity, independent of insulin resistance, weight loss, and energy deficit (32).

We and others have examined whether there is a skeletal muscle “memory” of in vivo exercise in cultured skeletal muscle cells. In 2017, we published results from a study examining effects of an in vivo exercise intervention on in vitro myotube metabolism (10). Lean individuals and individuals with overweight performed a 12-week exercise intervention consisting of extensive endurance and strength training. Both groups improved their maximal strength and insulin sensitivity from the intervention. Only lean individuals reduced their body fat percentage, whereas only individuals with overweight increased maximal oxygen uptake and reduced body weight and BMI. Visceral fat area also tended to be lower after the intervention in individuals with overweight. Furthermore, the combination of aerobic and anaerobic exercise-mediated changes in FA and glucose metabolism in myotubes. The exercise-induced changes were predominant in cells from individuals with overweight (10). The exercise-induced reduction in visceral fat in vivo correlated with increased mRNA expression of PDK4 in cultured myotubes, a correlation that was true for all participants combined and for participants with overweight alone. We did not observe any exercise-induced changes in insulin response in cultured myotubes; however, mRNA expression of IRS1 was decreased after exercise. Furthermore, increased DNA methylation within the first exon region of IRS1 was observed in myotubes after exercise. No exercise-induced changes in protein expression of IRS1 were observed.

Another study with a similar design has been performed. Individuals with obesity underwent an 8-week exercise intervention consisting of aerobic exercise, and skeletal muscle biopsies were taken before and after the study (98). Glucose metabolism was preserved in cultured myotubes from donors with obesity in response to aerobic exercise training. Oxidation of glucose, glycogen synthesis,
and inhibition of FAO by glucose were all enhanced in myotubes after the exercise intervention, and these changes were associated with differences in gene expression of glucose transporter (GLUT) 1, PDK4, and PDH E1 subunit α 1 (98). However, this study did not include control myotubes from lean individuals.

Over the years, electrical pulse stimulation (EPS) of skeletal muscle cells has been used as an in vitro exercise model to mimic features of in vivo muscle adaptation in response to physical activity (39,99,100). Although this approach has limitations, EPS provides a unique opportunity to study contraction-induced cellular responses under controlled conditions, which are difficult to study in vivo.

Myotubes from individuals with obesity respond differently compared with lean individuals when exposed to EPS (Figure 3). Improvements in FAO have only been observed in myotubes from lean individuals, whereas glucose metabolism was enhanced in myotubes from both lean individuals and individuals with obesity in response to EPS (99,101,102). Still, incorporation of FAs into complex lipids was higher in myotubes from donors with obesity compared with myotubes from lean individuals, and incorporation into DAG was increased by EPS. However, the defects in lipid handling seen in myotubes from individuals with obesity were not restored by EPS because of a lack of response on several metabolic features (impaired mitochondrial function, higher lipid accumulation, formation of acid-soluble metabolites, uptake, and oxidation of FAs). There is also evidence that EPS ameliorate insulin sensitivity in myotubes from individuals with severe obesity, in which insulin-stimulated phosphorylation of Akt and glycogen synthesis

**FIGURE 3** Effect of electrical pulse stimulation (EPS) of myotubes established from lean individuals versus individuals with obesity. An increase after treatment with EPS is indicated with ↑, whereas - indicates no effect of EPS. Akt, protein kinase B
positively correlated with BMI. Moreover, the expression of interleukin 6 (IL-6) from both myotubes from lean individuals and myotubes from donors with obesity has been observed to be upregulated with EPS (99,102).

Possible pharmacological targets and pharmaceutical agents

There is a limited repertoire of pharmaceutical agents on the market targeting obesity, and none specifically targets skeletal muscle. In the following sections, we highlight possible future pharmaceutical targets and drug candidates that could positively modify skeletal muscle energy metabolism (Figure 4).

Exercise mimetics

There is significant interest in the search for pharmacological compounds that can mimic effects of exercise, so-called exercise mimetics. Several exercise-mediated signaling cascades, leading to muscle adaptations to increase mitochondrial oxidative phosphorylation and cellular energy expenditure, can be targeted and are an attractive approach for this purpose.

Adenosine monophosphate-activated protein kinase (AMPK) is a key regulatory enzyme of cellular metabolism. In skeletal muscle, its activation promotes increased FAO by inactivating ACC2 and lowering malonyl-CoA, subsequently mitigating the inhibition of CPT1. Moreover, AMPK activation induces GLUT4 translocation to the plasma membrane, leading to increased glucose uptake. The increase in the AMP/ATP ratio during exercise is considered the primary signal for AMPK activation. However, studies have shown that IL-6 (myokine released during exercise) as well as leptin and adiponectin can induce AMPK activation (103). Given the favor.

tors by promoting AMP or calcium accumulation. Comprehensive reviews have been published (104,105).

Adenosine monophosphate-activated protein kinase (AMPK) is a key regulatory enzyme of cellular metabolism. In skeletal muscle, its activation promotes increased FAO by inactivating ACC2 and lowering malonyl-CoA, subsequently mitigating the inhibition of CPT1. Moreover, AMPK activation induces GLUT4 translocation to the plasma membrane, leading to increased glucose uptake. The increase in the AMP/ATP ratio during exercise is considered the primary signal for AMPK activation. However, studies have shown that IL-6 (myokine released during exercise) as well as leptin and adiponectin can induce AMPK activation (103). Given the favorable effects of AMPK activation in substrate homeostasis and mitochondrial biogenesis, AMPK-activating compounds are currently the most promising exercise mimetics. Numerous AMPK activators, acting by different mechanisms, have been developed. The compounds 5-aminoimidazole-4-carboxamide riboside (AICAR), salicylate, small compound thiopropiophenone A7769662, and benzimidazole derivatives (compound 911) directly bind to and activate AMPK without affecting ATP, AMP, or ADP levels. Biguanides (metformin), glitazones, and polyphenols (resveratrol) act as indirect AMPK activators by promoting AMP or calcium accumulation. Comprehensive reviews have been published (104,105).

Caffeine was reported to increase cytosolic calcium(2+) (Ca2+) levels in a similar way to what is seen in contracting muscle, inducing upregulation of cellular metabolism (106). The calcium/calmodulin-independent protein kinase type II (CaMKII) pathway is affected by exercise-mediated muscle contraction due to increased concentration in cytosolic Ca2+ released from the sarcoplasmic reticulum (SR) during excitation-contraction coupling. Results from our group (Figure 5, unpublished data) indicated that FAO was enhanced in human myotubes treated with caffeine. Although the concentrations of caffeine used here and in many similar studies are physiologically unattainable, it was demonstrated that comparatively lower concentrations of caffeine were sufficient to stimulate favorable changes in oxidative metabolism and mitochondrial biogenesis (107). Taken together, activating the CaMKII pathway could be a strategy to mimic exercise-mediated metabolic processes.

Possible pharmacological targets and pharmaceutical agents

There is a limited repertoire of pharmaceutical agents on the market targeting obesity, and none specifically targets skeletal muscle. In the following sections, we highlight possible future pharmaceutical targets and drug candidates that could positively modify skeletal muscle energy metabolism (Figure 4).

Exercise mimetics

There is significant interest in the search for pharmacological compounds that can mimic effects of exercise, so-called exercise mimetics. Several exercise-mediated signaling cascades, leading to muscle adaptations to increase mitochondrial oxidative phosphorylation and cellular energy expenditure, can be targeted and are an attractive approach for this purpose.

Adenosine monophosphate-activated protein kinase (AMPK) is a key regulatory enzyme of cellular metabolism. In skeletal muscle, its activation promotes increased FAO by inactivating ACC2 and lowering malonyl-CoA, subsequently mitigating the inhibition of CPT1. Moreover, AMPK activation induces GLUT4 translocation to the plasma membrane, leading to increased glucose uptake. The increase in the AMP/ATP ratio during exercise is considered the primary signal for AMPK activation. However, studies have shown that IL-6 (myokine released during exercise) as well as leptin and adiponectin can induce AMPK activation (103). Given the favorable effects of AMPK activation in substrate homeostasis and mitochondrial biogenesis, AMPK-activating compounds are currently the most promising exercise mimetics. Numerous AMPK activators, acting by different mechanisms, have been developed. The compounds 5-aminoimidazole-4-carboxamide riboside (AICAR), salicylate, small compound thiopropiophenone A7769662, and benzimidazole derivatives (compound 911) directly bind to and activate AMPK without affecting ATP, AMP, or ADP levels. Biguanides (metformin), glitazones, and polyphenols (resveratrol) act as indirect AMPK activators by promoting AMP or calcium accumulation. Comprehensive reviews have been published (104,105).

Caffeine was reported to increase cytosolic calcium(2+) (Ca2+) levels in a similar way to what is seen in contracting muscle, inducing upregulation of cellular metabolism (106). The calcium/calmodulin-independent protein kinase type II (CaMKII) pathway is affected by exercise-mediated muscle contraction due to increased concentration in cytosolic Ca2+ released from the sarcoplasmic reticulum (SR) during excitation-contraction coupling. Results from our group (Figure 5, unpublished data) indicated that FAO was enhanced in human myotubes treated with caffeine. Although the concentrations of caffeine used here and in many similar studies are physiologically unattainable, it was demonstrated that comparatively lower concentrations of caffeine were sufficient to stimulate favorable changes in oxidative metabolism and mitochondrial biogenesis (107). Taken together, activating the CaMKII pathway could be a strategy to mimic exercise-mediated metabolic processes.

Peroxisome proliferator-activated receptors (PPARs) are known to govern energy demand during physical activity. Three subtypes of PPARs have been identified in mammals, wherein PPARδ is the main subtype in skeletal muscle. Upregulation of PPARδ is one of several exercise-induced pathways contributing to oxidative remodeling in skeletal muscle (108). The role of PPARδ in skeletal muscle has been linked to regulation of lipid metabolism. Moreover, evidence suggests that overexpression or activation of PPARδ induces increased oxidative type I muscle fibers and improved insulin sensitivity, similar to exercise-induced adaptations (108-110). The PPARδ-selective agonist GW501516 increased expression of genes involved in FA metabolism and oxidative capacity but failed to increase endurance performance in supplemented mice (108). Interestingly, combining GW501516 treatment with exercise elicited a robust improvement in gene expression involved in the regulation of FA uptake and storage, as well as endurance capacity, reflecting cross talk between exercise and PPARδ signaling. Moreover, we have shown that PPARδ-activation in human myotubes enhances mitochondrial FA oxidative capacity and reduces glucose utilization through a switch in substrate preference from glucose to FAs (111). Despite these promising effects of GW501516 on energy metabolism, available data on safety are inadequate.

Tetradecylthioacetic acid (TTA), a modified FA and pan-PPAR agonist, has shown positive effects on promoting skeletal muscle energy metabolism. Treatment of myotubes with TTA limited FA accumulation and increased both complete FA and glucose oxidation by improving mitochondrial function (112).

Combined, new and suitable PPARδ agonists may be considered for clinical evaluation and future drug development, but potential detrimental side effects must be carefully considered and examined.

Adaptive thermogenic mechanisms

Skeletal muscles are a major contributor to basal metabolic rate. Increasing energy expenditure in muscle through shivering and nonshivering thermogenic mechanisms can affect whole-body energy metabolism and counteract weight gain (113). Only muscle-specific nonshivering thermogenic mechanisms are addressed in this review.

It is believed that futile Ca2+-cycling by the sarcoplasmic/ endoplasmic reticulum calcium ATPase (SERCA) pump is the primary source for thermogenesis in skeletal muscle. The SERCA pump is responsible for the removal of Ca2+ from the cytoplasm and back to the SR to promote muscle relaxation. In vivo and in vitro studies have reported that binding of sarcolipin to SERCA
can induce futile cycling of SERCA from Ca^{2+} transport, mediating heat production from an increase in ATP hydrolysis (113,114). Furthermore, increased mitochondrial Ca^{2+} influx due to elevated cytosolic Ca^{2+} levels stimulates Ca^{2+}-dependent signaling pathways and recruitment of PGC1α to promote mitochondrial biogenesis and oxidative metabolism in muscle (115). Therefore, interventions based on a modulation of SERCA could provide a promising therapeutic strategy to increase energy expenditure and management of whole-body energy metabolism in the absence of exercise training.
Acute exposure to caffeine results in enhanced fatty acid oxidation in myotubes. Cultured human myotubes from *musculus vastus lateralis* were incubated with different concentrations of caffeine for 4 hours in the presence of [1-14C] oleic acid (18.5 kBq/mL, 100 μM) using a substrate oxidation assay. Oxidation (CO2) was measured by scintillation counting and normalized to cell protein content. Values are presented as mean ± SEM from myotubes obtained from three donors (n = 3). *Statistically significant compared with untreated (control) myotubes (p < 0.05, paired t test)

**Sirtuins and sirtuin activators**

The class III histone deacetylases sirtuins (SIRTs), with seven identified isoforms, display astonishing enzymatic activities. The nuclear sirtuins (SIRT1, SIRT6, and SIRT7) regulate activity of central transcription factors and cofactors, which are almost universally expressed across tissues and respond cellulary, dependent upon energy demand. The mitochondrial sirtuins (SIRT3, SIRT4, and SIRT5) regulate, as the name indicates, activity of mitochondrial enzymes and respond to fasting and caloric restriction. SIRT2 is, on the other hand, mainly located in the cytoplasm (121). SIRT1 has been shown to regulate adiponectin expression, insulin secretion, oxygen consumption, and mitochondrial capacity; repress PPAR γ-activity; lower plasma glucose; and improve insulin sensitivity. In myotubes, the SIRT1-activator SRT1720 increased FAO (6). As all of these metabolic parameters are often dysregulated in overweight and obesity, SIRT1 has become a target of interest to treat these conditions. However, whether such SIRT-activators hold a place therapy still remains unanswered, as efficacy and safety are a challenge; studies have indicated potential critical negative effects with regards to tumorigenesis (122).

**Fibroblast growth factors**

The family of fibroblast growth factors (FGFs), such as FGF19, FGF21, and FGF23, act as endocrine regulators with many physiological effects on whole-body energy homeostasis, glucose, and lipid metabolism (123). These polypeptides are expressed in many metabolically active organs, interact with various tissues (124), and are investigated for the treatment of obesity and insulin resistance. FGF21 is secreted by skeletal muscle and may act as an autocrine factor. An enhanced expression in human myotubes was observed and was associated with cell differentiation and mitochondrial dysfunction (125). Moreover, FGF21 exposure of human myotubes and isolated muscle *in vitro* increased basal and insulin-stimulated glucose uptake (126). The pharmaceutical industry has developed a large number of FGF21 analogs and mimetics that have biological actions similar to endogenous FGF21 but with improved pharmacokinetic properties (123). Although the function of FGF21 is complicated and still debated because of its different sites of production and biological actions, this molecule may be used as a drug or drug target to combat obesity in the future (124). Moreover, it is quite likely that other myokines with therapeutic potential for treating obesity and associated metabolic abnormalities will be explored in the future (89).

**DGAT1 and DGAT2 inhibitors**

Accumulation of TAG from excess FA in skeletal muscle is associated with obesity-induced insulin resistance. Reducing TAG synthesis and modifying lipid turnover could be considered as potential therapeutic targets to overcome dysregulation in lipid metabolism. DGAT1 and DGAT2 are enzymes that catalyze the final step of TAG synthesis. Our recent study has shown that acute inhibition of DGAT1 and DGAT2 using selective enzymatic inhibitors had distinct effects on lipid metabolism in myotubes (127). Inhibition of DGAT1, the most abundant candidates

**Liver X nuclear receptors**

The liver X receptors (LXRs) α and β are ligand-dependent nuclear receptors important for the regulation of cholesterol and lipid metabolism, glucose metabolism, and insulin sensitivity. Chronic exposure of human myotubes to an LXR agonist was shown to increase uptake and oxidation of both FAs and glucose (118). The expression of uncoupling protein 2 and 3 was elevated in muscle cells exposed to LXR agonists, suggesting that a greater proportion of energy may be dissipated as heat. Therefore, modulating the LXR signaling pathway in skeletal muscle may have therapeutic potential against obesity and T2DM (119,120).
isoform in skeletal muscle, resulted in an almost totally abolished incorporation of exogenous FAs into TAG and LDs. De novo FA synthesis was also reduced. In contrast, an increased proportion of TAG was observed by DGAT2 inhibition. Simultaneously, DGAT2 inhibition promoted a robust reduction of TAG in absence of exogenous FAs, indicating that DGAT2 plays a role in de novo synthesis of TAG from glycerol-3-phosphate. Interestingly, DGAT activities played a role in determining the rate of FAO from exogenous FAs. Furthermore, inhibition of DGATs also modified glucose metabolism in myotubes; surprisingly, glucose uptake increased after DGAT1 inhibition.

CONCLUSION

Given that skeletal muscles are the body’s largest organs, modifying skeletal muscle energy metabolism will affect whole-body energy homeostasis, which is of great importance to combat obesity. Current effective strategies apart from bariatric surgery include dietary approaches and exercise interventions. There are few pharmacological treatment options available, with none acting directly on skeletal muscle to enhance energy metabolism. This is being continuously examined and, with further research and development, new and perhaps even more potent drugs against obesity might enter the market.

KEY POINTS FOR FUTURE SKELETAL MUSCLE RESEARCH IN OBESITY

- Elucidate mechanisms regulating lipid storage and utilization as well as mitochondrial function.
- Elucidate mechanisms regulating metabolic flexibility.
- Elucidate the role of adaptive thermogenic mechanisms (futile cycles).
- Identify drug candidates that could modify lipid storage and turnover, increase mitochondrial function, and activate futile cycles.

ACKNOWLEDGMENTS

Figures were made using GraphPad Prism version 9 (experimental results; GraphPad Software, San Diego, California) or BioRender.com (summary figures; BioRender, Toronto, Ontario, Canada).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ORCID

Abel M. Mengeste https://orcid.org/0000-0001-8900-7660
Arild C. Rustan https://orcid.org/0000-0003-0963-5363
Jenny Lund https://orcid.org/0000-0002-0744-8268

REFERENCES

1. World Health Organization. Obesity and overweight. Updated June 9, 2021. Accessed July 1, 2020. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight
2. Tamashiro KL, Sakai RR, Shively CA, Karatsoreos IN, Reagan LP. Chronic stress, metabolism, and metabolic syndrome. Stress. 2011;14(5):468-474.
3. Moran CP, Shanahan F. Gut microbiota and obesity: role in aetiology and potential therapeutic target. Best Pract Res Clin Gastroenterol. 2014;28(4):585-597.
4. Verhaegen AA, Van Gaal LF. Drugs affecting body weight, body fat distribution, and metabolic function—mechanisms and possible therapeutic or preventive measures: an update. Curr Obes Rep. 2021;1:1-13.
5. Haslam D. Obesity and diabetes: the links and common approaches. Prim Care Diabetes. 2010;4(2):105-112.
6. Aas V, Bakke SS, Feng YZ, et al. Are cultured human myotubes far from home? Cell Tissue Res. 2013;354(3):671-682.
7. Gaster M, Petersen I, Højlund K, Poulsen P, Beck-Nielsen H. The diabetic phenotype is conserved in myotubes established from diabetic subjects: evidence for primary defects in glucose transport and glycogen synthase activity. Diabetes. 2002;51(4):921-927.
8. Gaster M, Rustan AC, Aas V, Beck-Nielsen H. Reduced lipid oxidation in skeletal muscle from type 2 diabetic subjects may be of genetic origin: evidence from cultured myotubes. Diabetes. 2004;53(3):542-548.
9. Henry RR, Abrams L, Nikouлина S, Ciaraldi TP. Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects: comparison using human skeletal muscle cell cultures. Diabetes. 1995;44(8):936-946.
10. Lund J, Rustan AC, Løvsletten NG, et al. Exercise in vivo marks human myotubes in vitro: training-induced increase in lipid metabolism. PLoS One. 2017;12(4):e0175441. doi:10.1371/journal.pone.0175441
11. Lund J, Helle SA, Li Y, et al. Higher lipid turnover and oxidation in cultured human myotubes from athletic versus sedentary young male subjects. Sci Rep. 2018;8(1):17549. doi:10.1038/s41598-018-35715-7
12. Lund J, S. Tangen D, Wiig H, et al. Glucose metabolism and metabolic flexibility in cultured skeletal muscle cells is related to exercise status in young male subjects. Arch Physiol Biochem. 2018;124(2):119-130.
13. Houmard JA, Pories WJ, Dohm GL. Is there a metabolic program in the skeletal muscle of obese individuals? J Obes. 2011;2011:250496. doi:10.1155/2011/250496
14. Dohm GL, Tapscott EB, Pories WJ, et al. An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. J Clin Invest. 1988;82(2):486-494.
15. Elton C, Tapscott E, Pories W, Dohm G. Effect of moderate obesity on glucose transport in human muscle. Horm Metab Res. 1994;26(04):181-183.
16. Friedman JE, Caro JoséF, Pories WJ, Azevedo JL, Dohm G. Glucose metabolism in incubated human muscle: effect of obesity and non-insulin-dependent diabetes mellitus. Metabolism. 1994;43(8):1047-1054.
17. Gaster M. Metabolic flexibility is conserved in diabetic myotubes. J Lipid Res. 2007;48(1):207-217.
18. Aas V, Thoresen GH, Rustan AC, Lund J. Substrate oxidation in primary human skeletal muscle cells is influenced by donor age. Cell Tissue Res. 2020;382(3):599-608.
19. Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ, Dohm GL. Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. J Clin Invest. 1995;95(5):2195-2204.
20. Houmard JA, Pories WJ, Dohm GL. Severe obesity: evidence for a deranged metabolic program in skeletal muscle? Exerc Sport Sci Rev. 2012;40(4):204-210.
21. Bell JA, Reed MA, Consitt LA, et al. Lipid partitioning, incomplete fatty acid oxidation, and insulin signal transduction in primary human muscle cells: effects of severe obesity, fatty acid incubation, and fatty acid translocase/CD36 overexpression. J Clin Endocrinol Metab. 2010;95(7):3400-3410.
22. Zou K, Hinkle JM, Park S, et al. Altered tricarboxylic acid cycle flux in primary myotubes from severely obese humans. *Int J Obes (Lond).* 2019;43(4):895-905.

23. Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism.* 2000;49(4):467-472.

24. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab.* 2001;86(12):5755-5761.

25. Simoneau J-A, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J.* 1999;13(14):2051-2060.

26. Mogensen M, Sahlin K, Fernstrom M, et al. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes.* 2007;56(6):1592-1599.

27. Anderson EJ, Lustig ME, Boyle KE, et al. Mitochondrial H2O2 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.

28. Kelley DE, Goodpaster B, Wing RR, Simoneau J-A. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol Endocrinol Metab.* 1999;277(6):E1130-E1141.

29. Huvel MW, Berggren JR, Cortright RN, et al. Skeletal muscle lipid metabolism with obesity. *Am J Physiol Endocrinol Metab.* 2003;284(4):E741-E747.

30. Kim J-Y, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2000;279(5):E1039-E1044.

31. Bandypadhyay GK, Yu JG, Ofrecio J, Olefsky JM. Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. *Diabetes.* 2006;55(8):2277-2285.

32. Fritzen AM, Lundsgaard A-M, Kiens B. Tuning fatty acid oxidation in skeletal muscle with dietary fat and exercise. *Nat Rev Endocrinol.* 2020;16(12):683-696.

33. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Krausnæ R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia.* 2007;50(4):790-796.

34. Lefort N, Glancy B, Bowen B, et al. Increased reactive oxygen species production and lower abundance of complex I subunits and carnitine palmitoyltransferase 1B protein despite normal mitochondrial respiration in insulin-resistant human skeletal muscle. *Diabetes.* 2010;59(10):2444-2452.

35. Holloway GP, Thrush AB, Heigenhauser GJF, et al. Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. *Am J Physiol Endocrinol Metab.* 2007;292(6):E1782-E1789.

36. Holloway GP, Bonen A, Spriet LL. Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals. *Am J Clin Nutr.* 2009;89(1):4555-4625.

37. Phelix E, Schrauwen-Hinderling VB, Mensink M, et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. *Diabetes.* 2008;57(11):2943-2949.

38. Boyle KE, Zheng D, Anderson EJ, Neuffer PD, Houmard JA. Mitochondrial lipid oxidation is impaired in cultured myotubes from obese humans. *Int J Obes (Lond).* 2012;36(8):1025-1031.

39. Lævsletten NG, Rustan AC, Laurens C, Thoresen GH, Moro C, Nikolić N. Primary defects in lipid handling and resistance to exercise in myotubes from obese donors with and without type 2 diabetes. *Appl Physiol Nutr Metab.* 2020;45(2):169-179.

40. Fisher-Wellman KH, Weber TM, Cathey BL, et al. Mitochondrial respiratory capacity and content are normal in young insulin-resistant obese humans. *Diabetes.* 2014;63(1):132-141.

41. Gundersen AE, Kugler BA, McDonald PM, Veraska A, Houmard JA, Zou K. Altered mitochondrial network morphology and regulatory proteins in mitochondrial quality control in myotubes from severely obese humans with or without type 2 diabetes. *Appl Physiol Nutr Metab.* 2020;45(3):283-293.

42. Bonen A, Parolin ML, Steinberg GR, et al. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. *FASEB J.* 2004;18(10):1144-1146.

43. Sparks LM, Bosma M, Brouwers B, et al. Reduced incorporation of fatty acids into triacylglycerol in myotubes from obese individuals with type 2 diabetes. *Diabetes.* 2014;63(5):1583-1593.

44. Jocken JWE, Moro C, Goossens GH, et al. Skeletal muscle lipid content and activity in obesity and type 2 diabetes. *J Clin Endocrinol Metab.* 2010;95(12):5449-5453.

45. Badin P-M, Louche K, Mailar A, et al. Altered skeletal muscle lipase expression and activity contribute to insulin resistance in humans. *Diabetes.* 2011;60(6):1734-1742.

46. Kase ET, Feng YZ, Badin P-M, et al. Primary defects in lipolysis and insulin action in skeletal muscle cells from type 2 diabetic individuals. *Biochem Biophys Acta.* 2015;1851(9):1194-1201.

47. Laurens C, Moro C. Intramyocellular fat storage in metabolic diseases. *Harm Mol Biol Clin Investig.* 2016;26(1):43-52.

48. Gaster M. Reduced lipid oxidation in myotubes established from obese and type 2 diabetic subjects. *Biochem Biophys Res Comm.* 2009;382(4):766-770.

49. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev.* 2011;91(4):1447-1531.

50. Tanner CJ, Barakat HA, Dohm GL, et al. Muscle fiber type is associated with obesity and weight loss. *Am J Physiol Endocrinol Metab.* 2002;282(6):E1191-E1196.

51. Oberbach A, Bossenz Y, Lehmann S, et al. Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. *Diabetes Care.* 2006;29(4):895-900.

52. Marín P, Andersson B, Krotkiewski M, Björntorp P. Muscle fiber composition and capillary density in women and men with NIDDM. *Diabetes Care.* 1994;17(5):382-386.

53. Chomentowski P, Coen PM, Radiková Z, Goodpaster BH, Toledo FGS. Skeletal muscle mitochondria in insulin resistance: differences in intermyofibrillar versus subsarcolemmal subpopulations and relationship to metabolic flexibility. *J Clin Endocrinol Metab.* 2011;96(2):494-503.

54. Kelley DE, Reilly JP, Veneman T, Mandarino LJ. Effects of insulin on skeletal muscle glucose storage, oxidation, and glycolysis in humans. *Am J Physiol Endocrinol Metab.* 1990;258(6):E923-E929.

55. Thoresen GH, Hessevik NP, Bakke SS, Aas V, Rustan AC. Metabolic switching of human skeletal muscle cells in vitro. *Prostaglandins Leukot Essent Fatty Acids.* 2011;85(5):227-234.

56. Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. *J Clin Invest.* 2005;115(7):1699-1702.

57. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes.* 2000;49(5):677-683.

58. Boyle KE, Canham JP, Consitt LA, et al. A high-fat diet elicits differential responses in genes coordinating oxidative metabolism in skeletal muscle of lean and obese individuals. *J Clin Endocrinol Metab.* 2011;96(3):775-781.
62. Jeong JY, Jeoung NH, Park K-G, Lee I-K. Transcriptional regulation of pyruvate dehydrogenase kinase. Diabetes Metab J. 2012;36(5):328-335.

63. Bruce CR, Thrush AB, Mertz VA, et al. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. Am J Physiol Endocrinol Metab. 2006;291(1):E99-E107.

81. Bruce CR, Thrush AB, Mertz VA, et al. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. Am J Physiol Endocrinol Metab. 2006;291(1):E99-E107.

82. Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest. 2007;117(6):1690-1698.

83. Liu L, Zhang Y, Chen N, Shi X, Tsang B, Yu Y-H. Upregulation of myocellular DGAT1 augments triglyceride synthesis in skeletal muscle and protects against fat-induced insulin resistance. J Clin Invest. 2007;117(6):1679-1689.

84. Watt MJ, Hoy AJ. Lipid metabolism in skeletal muscle: generation of adaptive and maladaptive intracellular signals for cellular function. Am J Physiol Endocrinol Metab. 2012;302(11):E1315-E1328.

86. Argi ës JM, Campos N, Lopez-Pedrosa JM, Rueda R, Rodriguez-Manãs L. Skeletal muscle regulates metabolism via interorgan crosstalk: roles in health and disease. J Am Med Dir Assoc. 2016;17(9):789-796.

87. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012;8(8):457-465.

88. Ahima RS, Park HK. Connecting myokines and metabolism. Endocrinol Metab (Seoul). 2015;30(3):235-245.

89. Guo A, Li K, Xiao Q. Sarcopenic obesity: myokines as potential diagnostic biomarkers and therapeutic targets? Exp Gerontol. 2020;139:111022. doi:10.1016/j.exger.2020.111022.

90. Stapleton PA, James ME, Goodwill AG, Fribbee JC. Obesity and vascular dysfunction. Pathophysiology. 2008;15(2):79-89.

91. Limberg JK, Morgan BJ, Schrage WG. Peripheral blood flow regulation in human obesity and metabolic syndrome. Exerc Sport Sci Rev. 2016;44(3):116-122.

92. Zhang Y, Sowers JR, Ren J. Targeting autophagy in obesity: from pathophysiology to management. Nat Rev Endocrinol. 2018;14(6):356-376.

93. Sishi B, Loos B, Ellis B, Smith W, du Toit EF, Engelbrecht A-M. Diet-induced obesity alters signalling pathways and induces atrophy and apoptosis in skeletal muscle in a prediabetic rat model. Exp Physiol. 2011;96(2):179-193.

94. Coffey VG, Hawley JA. The molecular bases of training adaptation. Sports Med. 2007;37(9):737-763.

95. Thyfault JP, Bergouignan A. Exercise and metabolic health: beyond skeletal muscle. Diabetologia. 2020;63(8):1464-1474.

96. Louche K, Badin P-M, Montastier E, et al. Endurance exercise up-regulates lipolytic proteins and reduces triglyceride content in skeletal muscle of obese subjects. J Clin Endocrinol Metab. 2013;98(12):4863-4871.

97. Zacharewicz E, Hesselink M, Schrauwen P. Exercise counteracts lipotoxicity by improving lipid turnover and lipid droplet quality. J Intern Med. 2018;284(5):505-518.

98. Bourlier V, Saint-Laurent C, Louche K, et al. Enhanced glucose metabolism is preserved in cultured primary myotubes from obese donors in response to exercise training. J Clin Endocrinol Metab. 2013;98(9):3739-3747.

99. Feng YZ, Nikoliã n B, Bakke SS, et al. Myotubes from lean and severely obese subjects with and without type 2 diabetes respond differently to an in vitro model of exercise. Am J Physiol Cell Physiol. 2015;308(7):C548-C556.

100. Nikoliã n N, Gã rgens SW, Thoresen GH, Aas V, Eckel J, Eckardt K. Electrical pulse stimulation of cultured skeletal muscle cells as a model for in vitro exercise–possibilities and limitations. Acta Physiol. 2017;220(3):310-331.

101. Ë lsvsetten NG, Rustan AC, Laurens C, Thoresen GH, Moro C, Nikoliã n N. Primary defects in lipid handling and resistance to exercise in myotubes from obese donors with and without type 2 diabetes. Appl Physiol Nutr Metab. 2020;45(2):169-179.

102. Lamberd S, Taube A, Schober A, et al. Contractile activity of human skeletal muscle cells prevents insulin resistance by
inhibiting pro-inflammatory signalling pathways. *Diabetologia*. 2012;55(4):1128-1139.

103. Hardie DG, Sakamoto K. AMPK: a key sensor of fuel and energy status in skeletal muscle. *Physiology (Bethesda)*. 2006;21:48-60.

104. Hardie DG. Regulation of AMP-activated protein kinase by natural and synthetic activators. *Acta Pharm Sin B*. 2016;6(1):1-19.

105. Kim J, Yang G, Kim Y, Kim J, Ha J. AMPK activators: mechanisms of action and physiological activities. *Exp Mol Med*. 2016;48(4):e224. doi:10.1038/emm.2016.16

106. Ojuka EO, Jones TE, Han DH, Chen MA, Holloszy JO. Raising Ca2+ in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle. *FASEB J*. 2003;17(6):675-681.

107. Schnuck JK, Gould LM, Parry HA, et al. Metabolic effects of fatty acids and synthetic activators. *Acta Pharm Sin B*. 2016;6(1):1-19.

108. Narkar VA, Downes M, Yu RT, et al. AMPK and PPARs: mechanisms of action and regulation by mitochondria-driven signalling. *Front Physiol*. 2019;10:419. doi:10.3389/fphys.2019.00419

109. Wang Y-X, Zhang C-L, Yu RT, et al. Regulation of muscle fiber type and running endurance by PPARβ. *PLoS Biol*. 2004;2(10):e294. doi:10.1371/journal.pbio.0020294

110. Feng YZ, Nikolić N, Bakke SS, et al. PPARβ activation in human myotubes increases mitochondrial fatty acid oxidative capacity and reduces glucose utilization by a switch in substrate preference. *Arch Physiol Biochem*. 2014;120(1):12-21.

111. Periasamy M, Herrera JL, Reis FCG. Skeletal muscle thermogenesis and its role in whole body energy metabolism. *Diabetes Metab J*. 2017;41(5):327-336.

112. Archer A, Laurencikiene J, Ahmed O, et al. Skeletal muscle as a target of LXR agonist after long-term treatment: focus on lipid homeostasis. *Am J Physiol Endocrinol Metabolism*. 2018;306(5):E494-E502.

113. Song J, Yang B, Jia X, et al. Distinctive roles of sirtuins on diabetes, protective or detrimental? *Front Endocrinol (Lausanne)*. 2018;9:724. doi:10.3389/fendo.2018.00724

114. Narkar VA, Downes M, Yu RT, et al. AMPK and PPARα agonists are exercise mimetics. *Cell*. 2008;134(3):405-415.

115. Tanaka T, Yamamoto J, Iwasaki S, et al. Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci*. 2003;100(26):15924-15929.

116. Degirolamo C, Sabbà C, Moschetta A. Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. *Nat Rev Drug Discov*. 2016;15(1):51-69.

117. Ribas F, Villarroya J, Hondares E, Giralt M, Villarroya F. FGF21 expression and release in muscle cells: involvement of MyoD and regulation by mitochondria-driven signalling. *Biochem J*. 2014;463(2):191-199.

118. Løvsletten NG, Vukov J, Rustan AC, Lund J. Skeletal muscle energy metabolism in obesity. *Obesity (Silver Spring)*. 2021;29:1582-1595. https://doi.org/10.1002/oby.23227