

**OBESITY COMORBIDITY/ETIOLOGY AND PATHOPHYSIOLOGY**

# Lipotoxicity plays a key role in the development of both insulin resistance and muscle atrophy in patients with type 2 diabetes

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**Summary**

Insulin resistance and muscle mass loss often coincide in individuals with type 2 diabetes. Most patients with type 2 diabetes are overweight, and it is well established that obesity and derangements in lipid metabolism play an important role in the development of insulin resistance in these individuals. Specifically, increased adipose tissue mass and dysfunctional adipose tissue lead to systemic lipid overflow and to low-grade inflammation via altered secretion of adipokines and cytokines. Furthermore, an increased flux of fatty acids from the adipose tissue may contribute to increased fat storage in the liver and in skeletal muscle, resulting in an altered secretion of hepatokines, mitochondrial dysfunction, and impaired insulin signalling in skeletal muscle. Recent studies suggest that obesity and lipid derangements in adipose tissue can also lead to the development of muscle atrophy, which would make insulin resistance and muscle atrophy two sides of the same coin. Unfortunately, the exact relationship between lipid accumulation, type 2 diabetes, and muscle atrophy remains largely unexplored. The aim of this review is to discuss the relationship between type 2 diabetes and muscle loss and to discuss some of the joint pathways through which lipid accumulation in organs may affect peripheral insulin sensitivity and muscle mass.

**KEYWORDS**

insulin resistance, interorgan crosstalk, muscle atrophy, obesity

**Abbreviations:** 1RM, one repetition maximum; DAG, diacylglycerol; FGF-19, fibroblasts growth factor 19; FGF-21, fibroblasts growth factor 21; FXR, farnesoid X receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IMCL, intramyocellular lipid droplets; IκB, inhibitor of kappa B; mTOR, mammalian target of rapamycin; MuRF1, muscle RING finger 1; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor kappa B; PA, phosphatidic acid; PEDF, pigment epithelium-derived factor; PGC1α, peroxisome proliferator-activated receptor Gamma Coactivator 1-alpha; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptors gamma; PXR, pregnane X receptor; RBP4, retinol binding protein 4; ROS, reactive oxygen species; T2D, type 2 diabetes; TNFα, tumour necrosis factor α

## 1 | INTRODUCTION

The global prevalence of diabetes is rising rapidly. The number of adults with diabetes in the world increased from 108 million in 1980 to 422 million in 2014,<sup>1</sup> and by 2045, this number is expected to increase to 693 million.<sup>2</sup> Type 2 diabetes (T2D) is the most common form of this disease and accounts for 85% to 95% of the cases.

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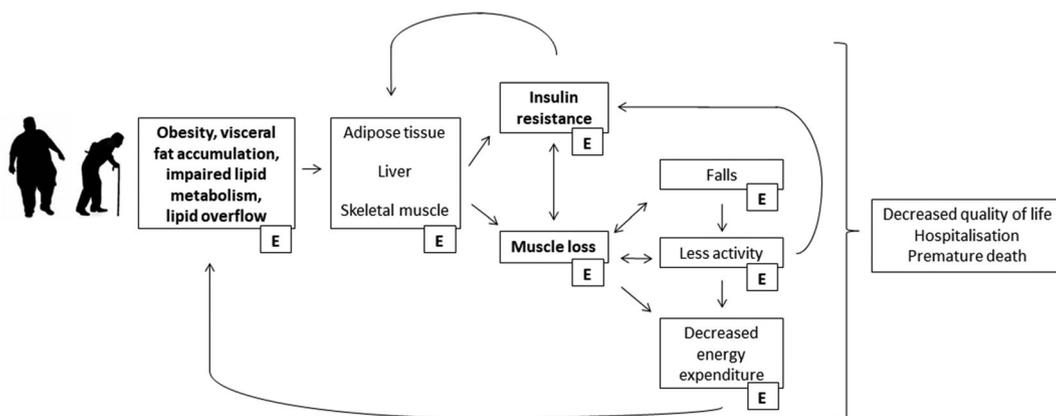
Muscle insulin resistance is one of the key features of T2D. In recent years, however, it has been increasingly recognized that there is also a deterioration in muscle mass and muscle strength in patients with T2D,<sup>3,4</sup> and this is independent of the length of disease, metabolic control, vitamin D status, and the presence of microvascular complications and pain.<sup>5</sup> In healthy individuals, muscle mass decreases at an annual rate of 1% to 2% after the age of 50.<sup>6</sup> This means that an average male person of 80 kg with 35 kg of muscle mass would lose 350 to 700 g a year, which is the equivalent of 7 to 14 kg over 20 years. Individuals with T2D have an accelerated ageing process, which places them at greater risk for developing frailty at an earlier age. We, as well as others, have shown that the problem of muscle loss is most striking in individuals with T2D who are older; it is estimated that 30% to 50% of patients with T2D older than 65 suffer from moderate to severe muscle loss, which is fourfold to fivefold higher than the general population older than 65.<sup>4,7,8</sup> Indeed, one of our previous studies showed that leg lean mass and appendicular skeletal muscle mass were 3% lower in patients with T2D compared with control subjects.<sup>4</sup> Muscle loss in patients with T2D is often not without consequences and can result in poor physical performance and decreased quality of life. A study performed in greater than 6000 participants showed that diabetes was associated with a two to three times increased odds of disability related to lower-extremity mobility, general physical activities, activities of daily living, instrumental activities of daily living, and leisure and social activities.<sup>9</sup> Patients enter a vicious cycle in which increased incidence of falls and hospitalization lead to more muscle loss, a further deterioration in quality of life, and premature death (Figure 1).<sup>10-12</sup> Given the steep rise in the number of patients with T2D, the number of people that are affected by muscle loss is expected to increase dramatically in the coming decades.

Studies estimated that approximately 80% of all individuals with T2D suffer from overweight or obesity.<sup>13</sup> This means there is increased fat storage in subcutaneous and visceral fat depots,<sup>14,15</sup> as well as lipid accumulation in ectopic fat depots, including skeletal

muscle and liver.<sup>15-18</sup> It is well established that obesity plays a crucial role in the development of insulin resistance.<sup>19</sup> In the past two decades, an increasing number of papers also linked obesity to a reduced muscle mass, and this phenomenon has been named "sarcopenic obesity (Figure 1)."<sup>20</sup> Sarcopenic obesity is now frequently observed and led to the suggestion that obesity not only causes insulin resistance but also plays a role in the development of muscle atrophy.<sup>21</sup> If this is true, this would mean that insulin resistance and muscle atrophy are "two sides of the same coin" and it would explain the simultaneous occurrence of insulin resistance and muscle atrophy in many patients with T2D. Notably, the combination of increased adipose tissue mass and muscle atrophy may aggravate cardiometabolic complications.<sup>22,23</sup> In this review, we will discuss the relationship between T2D and muscle mass loss, and we will discuss some of the pathways through which obesity may affect insulin sensitivity and muscle mass. To date, data on the link between T2D and muscle loss is still in its infancies, and a significant body of research comes from studies performed in older individuals, who are also characterized by increased adiposity or ectopic fat accumulation, insulin resistance, and muscle loss. Therefore, when information in patients with T2D is missing, observations made in older individuals will be reported. To conclude, this review will also briefly discuss strategies as a way to counteract insulin resistance and muscle mass loss.

## 2 | MUSCLE LOSS IN INDIVIDUALS WITH T2D: CAUSE OR CONSEQUENCE?

T2D and muscle atrophy develop hand in hand, and it has previously been speculated that muscle loss might be a cause as well as a consequence of T2D. Studies suggesting a causal role for muscle loss in the development of metabolic disturbances are relatively scarce though,<sup>24,25</sup> and to our knowledge, there is only one study that evaluated the association between low muscle mass and incidence of T2D



**FIGURE 1** Individuals with obesity and older individuals experience an increase in lipid deposition in visceral and ectopic fat depots. This may affect metabolism in key organs including adipose tissue, liver, and skeletal muscle, which may result in the development of muscle insulin resistance and a decrease in muscle mass. Patients often enter a vicious cycle in which decreased activity levels and increased incidence of falls lead to more muscle loss, a deterioration in quality of life, and premature death. Exercise (E) is an effective strategy to reduce obesity and improve lipid metabolism. It will ameliorate muscle mass loss and the development of insulin resistance, and it will reduce the incidence of falls and improve quality of life

in a longitudinal manner.<sup>24</sup> Specifically, Son et al followed 6895 participants from Asian descent for a period of approximately 9 years and observed an inverse association between muscle mass index and the development of T2D. Main covariates included age, sex, urban or rural residence, family history of diabetes, hypertension, smoking status, education level, monthly income, physical activity, alcohol consumption, and diet. Also, additional adjustments for body fat mass, waist circumference, and body mass index (BMI) did not modify the relationship.<sup>24</sup> The assumption that muscle loss may play a causal role in the development of T2D is partly based on the fact that skeletal muscle is a major determinant of total energy expenditure.<sup>26</sup> Skeletal muscle accounts for approximately 80% of the variance in resting metabolic rate, whereas resting metabolic rate represents approximately 60% of total energy expenditure. Thus, if muscle mass decreases with age, total energy expenditure decreases accordingly, possibly leading to obesity, abdominal fat accumulation, and metabolic disturbances if energy intake is not properly reduced.<sup>27</sup> However, it is important to keep in mind that T2D is a complex and multifactorial disease and it would be an oversimplification to reduce such a complex system of closely interrelated mechanisms to simple cause-effect relationships. T2D is the resultant of insulin resistance, in combination with impaired beta-cell function and decreased glucose effectiveness.<sup>28,29</sup> Therefore, disturbances on the level of skeletal muscle only are unlikely sufficient for T2D to manifest, and derangements on the level of multiple organs are required. In agreement with this, a study found that in a model of severe muscle wasting, mice did not exhibit differences in metabolism compared to wild-type mouse.<sup>30</sup> Furthermore, it was shown that activation of nuclear factor kappa B (NF- $\kappa$ B) through muscle-specific transgenic expression of activated inhibitor of kappa B ( $\text{I}\kappa\text{B}$ ) kinase beta caused profound muscle wasting that resembles clinical cachexia, but insulin sensitivity, glucose tolerance, and glucose uptake in the extensor digitorum longus were not different between mice.<sup>30</sup> Also, a recent study in humans found that the prevalence of the metabolic syndrome was higher in individuals with low muscle mass, but this relationship was lost after controlling for fat mass.<sup>31</sup> Thus, overall, there seems to be limited evidence that low muscle mass alone leads to the development of T2D. Nonetheless, of considerable interest are two studies investigating the interplay between high fat and low muscle mass on cardiometabolic risk factors. The major finding of these studies was that participants with both high percent body fat and low skeletal muscle mass index had higher HbA1c compared with those with high percent body fat or low skeletal muscle mass index alone.<sup>22,25</sup> Furthermore, low muscle mass attenuated the exercise response on changes in visceral adipose tissue, insulin resistance, and triglyceride concentration in individuals that were affected by obesity, but not in individuals that were lean.<sup>22</sup> How exactly low muscle mass can act synergistically with increased adiposity to worsen metabolic control is an intriguing question and should be the topic of further investigation.

In contrast to the few studies that suggest a direct role for muscle loss in relation to the development of T2D, there are more studies that argue that muscle loss is a consequence of T2D.<sup>32-35</sup> A longitudinal study showed that the decline in muscle mass in subjects with T2D happens twice as fast compared with those without diabetes, with

the greatest declines in lean mass in persons with undiagnosed diabetes.<sup>8</sup> Also, in patients undergoing chronic dialysis who were followed over the course of a year, the presence of diabetes was the most significant independent predictor of muscle loss.<sup>35,36</sup> The reason behind the increased prevalence of muscle loss in individuals with T2D is still under debate. Some studies found that the decrease in muscle strength was related to the severity of neuropathy but not to degree of nephropathy or retinopathy, suggesting that the decrease in muscle strength may be, at least partly, due to neuropathy.<sup>32,37,38</sup> It has also been suggested that insulin resistance may contribute to the development of muscle loss. Insulin plays an important role in the regulation of muscle protein metabolism by direct activation of the translation machinery and via vasodilatory actions; thus, it has been hypothesized that insulin resistance may lead to a decreased anabolic response, resulting in muscle loss. Several studies would support this hypothesis. For example, it was shown that chronic intake of sucrose induced insulin resistance and accelerated muscle loss in old rats.<sup>39</sup> In a longitudinal study in 3000 older men without diabetes, it was shown that lean mass loss over the course of approximately 5 years was more pronounced, while fat mass gain was less pronounced in men that were insulin resistant as compared with men that were insulin sensitive.<sup>34</sup> Treatment with metformin resulted in a significant improvement in insulin sensitivity in adults with newly diagnosed T2D, and this went along with an improvement in lean-to-fat ratio.<sup>40</sup> Women with obesity showed a blunted protein anabolic response to hyperinsulinaemia.<sup>41</sup> And it was also found that lipid-induced insulin resistance was associated with an impaired skeletal muscle protein synthetic response to amino acid ingestion in healthy young men.<sup>42</sup> However, whether the changes in muscle mass are due to changes in insulin sensitivity per se is difficult to say. Animal research indicated that mice can develop insulin resistance without subsequently developing muscle atrophy.<sup>43</sup> Furthermore, in the studies discussed above,<sup>34,39-42</sup> there was a simultaneous change in adiposity or ectopic lipid accumulation. It is therefore also possible that the insulin resistance is merely a reflection of adiposity and that muscle mass changes due to increased lipid availability.

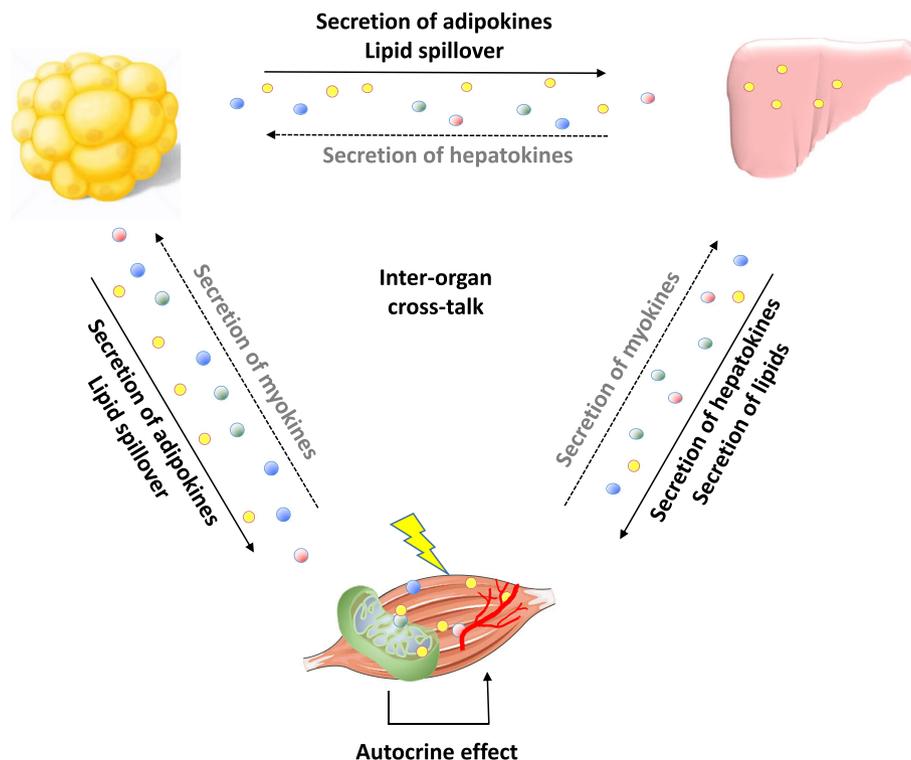
### 3 | DETERMINANTS OF MUSCLE MASS

Muscle mass is regulated by the delicate balance between muscle protein synthesis and muscle protein breakdown. Food—and protein in particular—is an important stimulator of muscle protein synthesis. The postprandial muscle protein synthetic response to feeding is regulated on a number of levels, including dietary protein digestion and amino acid absorption, splanchnic amino acid retention, postprandial insulin release, skeletal muscle tissue perfusion, amino acid uptake by muscle, and intramyocellular signalling.<sup>44</sup> Muscle contraction is a very potent anabolic stimulus that can further increase basal as well as postprandial muscle protein synthesis rates. Interestingly, the anabolic response to food and exercise is blunted in older individuals.<sup>45</sup> This may be due to a delay in amino acid digestion or absorption, or to a decrease in physical activity levels. Alternatively, it could be

caused by excess adiposity or circulating lipid levels. It is estimated that approximately 80% of all individuals with T2D are affected by overweight or obesity,<sup>13</sup> which leads to increased lipid deposition in visceral<sup>14,15</sup> and ectopic fat depots, including skeletal muscle and liver.<sup>15-18</sup> In addition, many patients with T2D have decreased physical activity levels,<sup>46</sup> which contributes to the accumulation of fat. While ectopic fat accumulation in individuals with T2D is usually the result of obesity, fat infiltration in organs in older individuals can happen independent of weight changes.<sup>47</sup> Nevertheless, there are indications in both groups that expansion of adipose tissue, as well as lipid accumulation in other organs, plays an important role in the development of muscle atrophy. To test the hypothesis that the development of anabolic resistance can be caused by excess lipids, a study was performed in which young healthy volunteers received a 7-hour saline or intralipid infusion on two randomized occasions.<sup>42</sup> The authors observed that excess lipid availability per se induced insulin resistance of skeletal muscle glucose metabolism as well as anabolic resistance of amino acid metabolism, and given the randomized crossover design, it could be concluded that this was independently of any changes in amino acid handling or physical activity levels.<sup>42</sup> It is therefore likely that an increase in lipid levels in older people and people with T2D contribute to the development of muscle atrophy. In the following paragraphs, several possible pathways will be discussed.

### 3.1 | Expansion of the adipose tissue

Besides being a lipid buffering organ, adipose tissue is a major endocrine organ and is known to secrete hundreds of adipokines.<sup>48</sup> Adipokines are important regulators of metabolism, and expansion of adipose tissue leads to a change in the secretion of adipokines, which have been linked to the development of insulin resistance in skeletal muscle through interorgan crosstalk (Figure 2).<sup>49,50</sup> Adipokines that are well-known to modulate metabolism and insulin sensitivity include leptin,<sup>51</sup> retinol binding protein 4 (RBP4),<sup>52</sup> adiponectin,<sup>53</sup> resistin,<sup>54</sup> and pigment epithelium-derived factor (PEDF).<sup>55</sup> Macrophages are also believed to be an important contributor of insulin resistance.<sup>56,57</sup> Macrophages account for up to 40% of adipose cell content in individuals with obesity compared with only 10% in lean humans<sup>58</sup> and secrete pro-inflammatory cytokines such as interleukin (IL) 6, IL1 $\beta$ , granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ).<sup>59</sup> In addition, macrophage infiltration in adipose tissue leads to adipose tissue dysfunction, thereby contributing to the altered secretion pattern of adipokines. It is interesting to note that the secretion pattern of adipokines and cytokines not only changes with increased adiposity but also varies across fat depots. Specifically, the secretion products of the mesenteric and omental adipose tissue depots are more strongly associated with metabolic complications of obesity compared with the secretion products of the



**FIGURE 2** Schematic overview of interorgan crosstalk between adipose tissue, liver, and skeletal muscle, leading to muscle insulin resistance and decrease of muscle mass. Increased lipid deposition in visceral and ectopic fat depots include skeletal muscle and liver. Increased lipid levels may lead to a change in the secretion pattern of cytokines, which may lead to muscle insulin resistance and a decrease in muscle mass via interorgan crosstalk. Lipids and cytokines may also affect mitochondrial function and vascularization in skeletal muscle, which contributes to the problem. Full arrows indicate pathways discussed in this review. Broken arrows indicate pathways outside the scope of this review

subcutaneous fat depots.<sup>60-62</sup> This is consistent with the fact that individuals with T2D have increased visceral fat depots<sup>14,15</sup> and are characterized by increased inflammatory profile and increased insulin resistance. Several studies show that inflammatory cytokines and adipokines are also related to decreased muscle mass and strength (Figure 2).<sup>63</sup> In humans, high levels of IL-6 (greater than 5 pg/mL) was associated with a twofold to threefold greater risk of losing more than 40% of muscle strength.<sup>64</sup> Furthermore, individuals that performed low on a physical activity test were characterized by smaller muscle volume and lower muscle strength and had higher levels of interleukin 1 $\beta$ , 6, 10, 12, 13, TNF $\alpha$ , IL-6, and GM-CSF compared with individuals that performed well on the physical activity test.<sup>65-67</sup> Concentrations of the anti-inflammatory adipokine adiponectin were found to be decreased in individuals with sarcopenia,<sup>66</sup> while levels of the pro-inflammatory adipokine leptin<sup>68</sup> were increased. Although there seems to be a lot of evidence to suggest that adipokines and cytokines contribute to lower muscle mass and strength, the studies mentioned here are cross-sectional studies, and therefore, it is difficult to differentiate between cause and consequence. Nevertheless, it has been shown in diet-induced obese mice that administration of quercetin reduced levels of inflammatory cytokines and macrophage accumulation in the skeletal muscle, along with reduced transcript and protein levels of the specific atrophic factors, Atrogin-1 and muscle RING finger 1 (MuRF1), and protected as such against the reduction of muscle mass and muscle fibre size.<sup>69</sup> Furthermore, in 2015, Pellegrinelli et al demonstrated for the first time that the secretome of human obese adipocytes directly decreased the expression of contractile proteins in myotubes, consequently inducing atrophy,<sup>70</sup> and this latter study provides evidence for a direct link between the adipose tissue secretome and the development of muscle loss. In support of this, also, O'Leary et al recently found that the secretome of human obese subcutaneous adipose tissue impaired the myogenesis of old myoblasts, an effect that was mediated via resistin-induced activation of NF $\kappa$ B.<sup>71</sup> Interestingly, the secretome of obese subcutaneous adipose tissue did not impair myogenesis of young myoblasts,<sup>71</sup> suggesting that the effect of obesity on muscle mass may be particularly harmful in older individuals.

### 3.2 | Lipid accumulation in the liver

Liver steatosis is clinically defined as a hepatic triglyceride content that exceeds 5% of the total liver weight<sup>72</sup> and is present in many individuals with obesity and in patients with T2D. Studies showed that approximately 60% of liver triglyceride content originates from lipolysis in adipose tissue. It is likely that in individuals with obesity, enlarged adipose tissue depots as well as insulin resistance of the adipose tissue contribute to fat disposition in the liver, thereby providing a link between obesity, insulin resistance, and liver steatosis. The liver on its turn also secretes a wide range of lipids, including high levels of triacylglycerols via the secretion of very-low-density lipoproteins.<sup>73</sup> These lipids can accumulate in skeletal muscle and contribute to the development of insulin resistance (see following paragraph). In addition, lipids secreted by the liver can also play an important role in

the development of atherosclerosis and increase the risk for cardiovascular problems and other complications such as nephropathy, retinopathy, and neuropathy. Neuropathy can result in decreased muscle strength, decreased physical activity, and muscle atrophy. Importantly, impaired vascular function also hampers glucose and insulin delivery to the muscle, which leads to a worsening of the hyperglycaemic state and a further deteriorating health.

In recent years, there has also been a growing interest regarding the role of liver-secreted proteins (ie, hepatokines) in relation to the development of insulin resistance.<sup>74,75</sup> We showed that the protein secretory profile of hepatocytes is altered with steatosis and that this altered profile leads to inflammation and insulin resistance in muscle cells (Figure 2).<sup>76</sup> We also identified fetuin B as a liver-secreted protein that is increased in individuals with obesity and in patients with liver steatosis and that impairs glucose uptake in mice.<sup>76</sup> Examples of other liver-derived endocrine factors that have been linked to insulin resistance and impaired glucose metabolism include fetuin A,<sup>77</sup> adropin,<sup>78</sup> angiopoietin-like protein 6,<sup>79</sup> and selenoprotein P.<sup>80</sup> In the last few years, liver-secreted proteins have also been linked to muscle wasting (Figure 2). For example, individuals with high levels of  $\alpha$ 1-antichymotrypsin were 40% less likely to experience loss of muscle strength and tended to have a smaller decline in muscle mass compared with those with low levels of  $\alpha$ 1-antichymotrypsin.<sup>64</sup> In addition, Cystatin C and Beta-2-microglobulin, two liver-secreted proteins, were found to be positively associated with the development of severe muscle loss<sup>81-83</sup>; fetuin A was identified as a predictor of sarcopenic left ventricular dysfunction<sup>84</sup>; and tissue iron levels were elevated in muscle loss, which went along with an increase in transferrin, another liver-secreted protein. Interestingly, secretion of Cystatin C and Beta-2-microglobulin, fetuin A, and transferrin is increased from a fatty liver,<sup>76</sup> suggesting a role for liver steatosis in the development of muscle loss. Unfortunately, research regarding the role of hepatokines is only in its infancies, and future studies are needed to identify the mechanisms through which certain hepatokines regulate insulin resistance and muscle loss.

### 3.3 | Lipid accumulation in skeletal muscle

Intramyocellular lipid droplets (IMCLs) are lipid droplets present in skeletal muscle tissue. IMCLs are dynamic functional organelles, involved in lipid metabolism, vesicle trafficking, and cell signalling, and they are covered and surrounded by lipases and lipid droplet coating proteins that play an important role in controlling the balance between IMCL storage, mobilization, and oxidation (for extended reviews on this topic, please refer to previous works<sup>85-89</sup>). While IMCL is important for energy metabolism, lipid overflow from the expanded adipose tissue and the liver leads to increased fat deposition in skeletal muscle. In addition, also, resistin has been shown to promote IMCL storage in primary human myotubes.<sup>71</sup> Importantly, increased IMCL storage results in the accumulation and dysregulation of detrimental lipid intermediates such as diacylglycerols (DAGs) and ceramides, and those intermediates lead to insulin resistance through activation of

protein kinase C (PKC).<sup>90-100</sup> High levels of DAG and ceramides have also been suggested to play a role in the development of muscle loss.<sup>16,101-103</sup> In C2C12 and L6 myotubes, treatment with the fatty acid palmitate induced ceramide accumulation, and this was associated with increased expression of pro-atrophic genes such as atrogen-1/MAFbx, increased levels of FoxO3, upregulated eIF2 $\alpha$  phosphorylation, and decreased protein synthesis.<sup>16,101,104</sup> Conversely, blocking ceramide synthesis prevented muscle atrophy, and this went along with improved mammalian target of rapamycin (mTOR) signalling, suppressed levels of Foxo3, and decreased atrogen-1/MAFbx expression.<sup>103</sup> Rivas and colleagues found that obese animals had significantly higher storage of ceramide and DAG compared with lean, and there was an attenuated insulin response in components of the mTOR anabolic signalling pathway.<sup>102</sup> Interestingly, conversion of DAG to phosphatidic acid (PA) activated the mTOR signalling pathway and resulted in hypertrophy in isolated mouse extensor digitorum longus muscle.<sup>105</sup> In skeletal muscle of older humans, there were no observable differences in total ceramide content; however, ceramide species C16:0 was increased with 156% and C20:0-ceramide was up with 30%. In addition, there was a negative correlation between C16:0-ceramide content with lower leg lean mass and an attenuated activation of anabolic signalling molecules such as Akt, FOXO1, and S6K1 after an acute bout of high-intensity resistance exercise.<sup>106</sup> When ageing and obesity in mice occurred in combination, ceramide accumulation was even more pronounced and negatively affected protein synthesis rate.<sup>16</sup> Thus, there seems to be evidence that lipid intermediates in skeletal muscle such as DAG's and ceramides negatively affect insulin sensitivity as well as muscle mass. For completeness, however, it should be mentioned that there are also studies that report conflicting results regarding the role of lipid intermediates in relation to insulin resistance as well as muscle loss. For example, Turpin et al found that apoptosis in skeletal muscle myotubes was induced by ceramides<sup>104</sup> but could not find any signs of apoptosis, autophagy, or proteolysis in high fat diet fed mice, in ob/ob mice, or in mice after an intralipid infusion.<sup>107</sup> Moreover, a number of studies dissociated increased DAG levels from the development of insulin resistance,<sup>108-110</sup> suggesting that this relationship is not quite as straightforward as previously thought. Possible explanations for these inconsistencies include not taking into account the importance of compartmentalization of the lipids, ignoring the structures, chain lengths, and the degree of saturation of the lipids, or bypassing any information on oxidation rates and fluxes. Either way, more research will be needed to establish the exact role of IMCL and lipid intermediates in the development of insulin resistance and muscle loss.

### 3.4 | Mitochondrial dysfunction

Skeletal muscle mitochondrial dysfunction has previously been linked to insulin resistance.<sup>111,112</sup> Kelley et al observed mitochondrial abnormalities with respect to content, size, and morphology in patients with T2D,<sup>113</sup> while Mootha et al showed a lower gene expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha

(PGC1 $\alpha$ ), a key transcriptional cofactor in mitochondrial biogenesis, and a lower gene expression of its target genes encoding key enzymes in oxidative mitochondrial metabolism.<sup>114</sup> Decreased mitochondrial function has also been reported in first degree relatives of patients with T2D,<sup>111,115,116</sup> suggesting that mitochondrial dysfunction may actually be causally related to insulin resistance (Figure 2). It is interesting to note that also age-related muscle loss has also been associated with mitochondrial dysfunction. Specifically, age-related muscle loss has been linked to increased reactive oxygen species (ROS) production, increased mitochondrial apoptotic susceptibility, decline in mitochondrial respiratory chain function,<sup>117</sup> reduced transcriptional drive for mitochondrial biogenesis,<sup>118</sup> and morphological changes in mitochondria<sup>119</sup>; and also, most,<sup>120-124</sup> but not all, studies<sup>125,126</sup> state that the capacity to mobilize and/or oxidize IMCL is substantially impaired in older individuals. In congruence with this, it has recently been suggested that accumulation of dysfunctional mitochondria initiate a signalling cascade leading to motor neuron and muscle fibre death and culminating in muscle loss (Figure 2).<sup>127</sup> Not all studies point in the same direction though, and a number of articles raised doubts concerning mitochondrial dysfunction as a causal factor in the development of insulin resistance and muscle atrophy.<sup>128-132</sup> A study reported normal in vivo mitochondrial function in Zucker diabetic rats throughout the pathogenesis of T2D<sup>130</sup> and comparable in vivo mitochondrial function was found between subjects with normal glycaemia, individuals with impaired glucose tolerance, and individuals with T2D.<sup>131</sup> In addition to that, we showed that although mitochondrial function was decreased in subjects with T2D, it could be completely restored towards the levels of healthy control subjects upon an exercise training programme, whereas insulin sensitivity could only be partially restored.<sup>133</sup> We also recently showed that 5 days of muscle disuse was sufficient to induce substantial loss of both muscle mass and strength in young and older subjects but did not reduce the maximal activity of key mitochondrial enzymes.<sup>129</sup> It seems fair to state that mitochondrial dysfunction is present in many individuals with T2D and may contribute to the development or progression of insulin sensitivity and muscle atrophy. However, considering the multifactorial disease that T2D is, mitochondrial dysfunction cannot be regarded as the main or sole causal factor in general.

## 4 | STRATEGIES TO COUNTERACT INSULIN RESISTANCE AND MUSCLE LOSS

It may be clear that IMCL accumulation is strongly associated with the severity of insulin resistance; however, this is only true for individuals that are sedentary or untrained. Trained athletes are also characterized by elevated fat content in the muscle, but in contrast to their sedentary counterparts, they are highly insulin sensitive.<sup>134-137</sup> The precise mechanism by which trained persons are protected from the insulin desensitizing effects of IMCL is still incompletely understood, but here is substantial evidence that exercise enhances the regulation of lipid droplet degradation and synthesis and increases oxidative capacity, resulting in lower levels of detrimental lipid

intermediates.<sup>138,139</sup> Furthermore, exercise leads to improved insulin sensitivity in adipose tissue, possibly resulting in lower levels of uncontrolled fatty acid release,<sup>140</sup> and thus less fatty acid spillover to skeletal muscle and liver.<sup>141</sup> Considering this, it is no wonder that exercise is fundamental in the treatment of diabetes.<sup>142</sup> Exercise can delay and reduce the incidence of diabetes in persons at high risk by 58%<sup>143</sup> and can improve (or even restore) abnormalities in oxidative capacity, substrate selection (ie, metabolic flexibility),<sup>133</sup> mitochondrial function and density,<sup>133</sup> capillary density, and lipid profile.<sup>144</sup> Interesting from a therapeutic point of view is the evidence that exercise is at least as effective in older participants as it is in younger participants<sup>143</sup> and that also patients on long-standing insulin treatment can benefit from exercise training.<sup>145</sup> For example, in a group of patients with long-standing T2D on insulin therapy, 1 year of exercise training raised *in vivo* skeletal muscle ATP production capacity by 21%, increased expression levels of  $\beta$ -oxidation, Krebs cycle, and oxidative phosphorylation system-related genes, and tended to increase mitochondrial density and complex I activity,<sup>132</sup> underscoring the importance of exercise in the treatment of T2D. Also, nutritional intervention programmes have been shown to be successful in preventing or delaying the onset insulin resistance. Especially weight reduction has been shown to lead to improved insulin sensitivity,<sup>146-148</sup> suggesting that nutritional treatment should primarily focus on achieving weight loss. The underlying mechanism may differ from exercise though; while exercise training leads to an improved mitochondrial function but not to a decreased IMCL content, the opposite observation has been made after weight loss.<sup>149</sup> Importantly, exercise and nutritional interventions are also key in the treatment of muscle loss. Muscle loss is mainly attributed to a decrease in type 2 muscle fibers size,<sup>150-152</sup> and it has been shown that 6 months of strength training can increase type 2 fibers size up to 24%.<sup>150,153</sup> In addition to that, resistance exercise has been shown to prevent muscle atrophy and strength loss in subjects undergoing step reduction<sup>154</sup> and bed rest,<sup>155</sup> while their sedentary counterparts displayed a deterioration in muscle mass and strength. Also, in very old adults in institutions, exercise has shown to be effective<sup>156,157</sup>; a 6-month exercise training programme consisting of twice weekly resistance and balance exercises resulted in a significant increase in grip strength, which may transfer to reduce disability and muscle loss transition.<sup>156</sup> Not only traditional high-load strength exercise has been shown to be effective in the treatment of muscle loss. Low-load, high-volume exercise (30% one repetition maximum [1RM]) to volitional fatigue,<sup>158</sup> low-load blood flow restricted exercise, and walking<sup>154,159</sup> has also shown to stimulate muscle protein synthesis rate. Nutrition is often given in combination with exercise to counter muscle loss, and interestingly, it has been shown that they act synergistically to increase muscle protein synthesis.<sup>160</sup> Importantly though, nutritional interventions should focus on obtaining fat mass loss as well as on ways to improve protein synthesis and gain lean mass. There are indications that older individuals and individuals with obesity have a blunted muscle protein synthesis response upon protein ingestion,<sup>161</sup> and increasing both the quality and the dose of protein might be an effective strategy to maximally stimulate muscle protein synthesis.<sup>162-164</sup> (For informative reviews regarding

the role of exercise and nutrition in relation muscle loss, see other works<sup>165-167</sup>). It must be noted though that exercise as well as weight loss can improve insulin sensitivity in patients with T2D without increasing muscle mass.<sup>139,168</sup> This is not necessarily surprising as it is compatible with the multifactorial character of the disease. Apart from ectopic lipid disposition, insulin sensitivity is also determined by other parameters such as vascularization and oxidative capacity, and these parameters can improve without absolute changes in muscle mass. On a similar note, also, changes in muscle strength do not necessarily go hand in hand with changes in muscle mass; longitudinal studies previously showed that the decline in strength in patients with T2D is much more rapid than the concomitant loss of muscle mass.<sup>169</sup> In fact, changes in lean mass can only explain a small part (5%) of the variability in strength decline, underscoring the importance of muscle quality and the central neural and neuromuscular components determining muscle strength and function.<sup>169,170</sup>

Apart from exercise and nutrition, also, pharmacological interventions are applied to treat patients with T2D. The use of metformin is widespread among patients with T2D. Metformin ameliorates hyperglycaemia via suppression of hepatic glucose production and via improvements in peripheral glucose uptake, and although the exact mode of action is not entirely clear, metformin is known for its weight-loss properties.<sup>171</sup> Some mechanistic studies also demonstrated a positive effect on lipid accumulation in the liver. However, other studies show that the effect on the liver was no longer present after correction for weight loss or that it could be attributed to the use of suprapharmacological dosages of metformin.<sup>172</sup> Also, multiple meta-analysis studies suggested that metformin has no effect on histological responses in the liver.<sup>171,173,174</sup> Nevertheless, due to its safe use and its weight-losing and insulin-sensitizing properties, the use of metformin remains the first-line agent to treat insulin resistance in patients with T2D. Another well-known class of medication that improves insulin sensitivity in individuals with T2D are the peroxisome proliferator-activated receptors gamma (PPAR $\gamma$ ) agonists thiazolidinediones (TZDs).<sup>175,176</sup> Interestingly enough, TZD use leads to an increase in extramyocellular lipid content and sometimes even weight gain.<sup>177,178</sup>

On the other hand, TZDs ameliorate ectopic fat storage and improve lipid storage and metabolism in adipocytes,<sup>177</sup> and this finding supports the notion that it is not the total amount of fat mass that is important, but rather where and how the fat is stored. Unfortunately, not all patients respond to TZDs, and its clinical use is also limited due to adverse events,<sup>176</sup> and therefore, other pharmacological therapies should be explored. In the past few years, a lot of research has been directed towards identifying new molecular mechanisms to target for the treatment of nonalcoholic fatty liver disease (NAFLD). Potential drug targets include microRNAs, incretin analogues/antagonists, liver-specific thymomimetics, AMP-activated protein kinase activators, farnesoid X receptor (FXR) and pregnane X receptor (PXR) agonists, fibroblasts growth factor 19 (FGF-19) and fibroblasts growth factor 21 (FGF-21) analogues, and antilipemic agents.<sup>179</sup> There is evidence that some of these drugs improve liver steatosis and fibrosis, and ameliorate lipid metabolism and insulin sensitivity in cellular and animal models. However, (long-term) efficiency has not yet been proven in

humans, and some drugs come with severe side effects.<sup>179-181</sup> Therefore, more basic research and more randomized controlled trials of adequate size and duration are needed in the search for novel treatments. Also, pharmacological targets to treat muscle loss are sparse. In 2014, a review summarized the impact of different therapeutic interventions in relation to body composition changes in men and females that were older and affected by obesity. The authors concluded that weight loss based on diet combined with exercise—and not drugs—represents the best strategy to treat phenotypic aspects of sarcopenic obesity.<sup>182</sup> A very recent study investigated systematic reviews and meta-analyses. The study included vitamin D, combined oestrogen-progesterone, dehydroepiandrosterone, growth hormone, growth hormone-releasing hormone, combined testosterone-growth hormone, insulin-like growth factor-1, pioglitazone, testosterone, and angiotensin-converting enzyme inhibitors. While the authors found that testosterone and vitamin D mildly increased muscle mass in older males and females respectively, this was only the case in individuals with low starting values.<sup>183</sup> Up to now, there is insufficient evidence to recommend pharmacological interventions for the treatment of muscle loss, and more research is needed in this field.<sup>183</sup>

## 5 | CONCLUSION

Many patients with T2D are characterized by peripheral insulin resistance and muscle atrophy. Derangements in lipid storage have been identified as a hallmark of the development and progression of insulin resistance, and an increasing amount of evidence suggest that it also plays an important role in the development of muscle atrophy. The exact mechanisms have not been established yet, but there is evidence that it may be via the accumulation of harmful lipid intermediates in skeletal muscle, decreased mitochondrial function, lipid accumulation in other organs, or a combination of those. Unfortunately, research is often hampered by the fact that many patients with T2D have decreased physical activity levels compared with the rest of the population. In many studies, it is therefore difficult to distinguish whether insulin resistance and muscle loss are caused by the mechanisms mentioned in this review and/or by a decrease in exercise per se. Furthermore, since insulin signalling plays a permissive role in skeletal muscle amino acids delivery and metabolism, it is possible that insulin resistance may aggravate skeletal muscle anabolic resistance. Future research will be needed to investigate the underlying mechanisms of muscle atrophy. This is important not only in the setting of T2D but also in other conditions of lipid-induced insulin resistance, including ageing, disuse, and critical illness. With regard to drug therapy, there are currently no well-established guideline recommendations to reduce lipid accumulation in the liver or other organs. Results of basic research over the past decade have been encouraging, and a number of new molecular mechanisms have been explored. However, most drugs pose long-term safety issues, and so the biggest challenge lies in the translation into the clinical setting. Up till now, nutritional and exercise interventions are key in the treatment of T2D and muscle loss, and if possible, both should be applied to achieve optimal results.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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