A high fat diet aggravates the age-related decline in skeletal muscle structure and function

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Synopsis

Succinct novel hypothesis: The hypothesis is that a high fat diet leads to an earlier accumulation of intramyocellular lipids (IMCL) and muscle dysfunction in muscles from old than those from younger individuals.

Supporting author publications (list 2-3)**:** Messa GAM, Piasecki M, Hurst J, Hill C, Tallis J, Degens H (2020) The impact of a high-fat diet in mice is dependent on duration and age, and differs between muscles. J Exp Biol 223 (Pt 6) Hurst J, James RS, Cox VM, Hill C, Tallis J (2019) Investigating a dose-response relationship between high-fat diet consumption and the contractile performance of isolated mouse soleus, EDL and diaphragm muscles. Eur J Appl Physiol 119 (1):213-226.

One-page abstract: The age-related decline in muscle function is aggravated by a high-fat diet-induced increase in fat mass. This is at least partly attributable to intramyocelluar lipid (IMCL) accumulation via decreased myofibrillar volume and lipotoxocity. The IMCL accumulation is attenuated in younger organism by an elevated oxidative capacity. Methionine restriction enhances mitochondrial biogenesis and is promising to combat obesity across the ages.

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Key points

- We suggest that the larger adiposity in old than young-adult mice limits the ability to store additional lipids in adipose tissue.
- While in young-adult mice a high fat diet (HFD) induced an increase in oxidative capacity that enhances the fatty acid oxidation capacity, this is not the case in muscles from old animals.
- Thus, the higher need to store excessive lipids in muscle and the lower ability to use fatty acids, as reflected by the absence of a HFD-induced rise in oxidative capacity, results in faster accumulation of intramyocellular lipids and muscle dysfunction in old than young-adult animals.

ABSTRACT

The age-related decline in muscle function is aggravated by a high-fat diet-induced increase in fat mass. This is at least partly attributable to intramyocelluar lipid (IMCL) accumulation via decreased myofibrillar volume and lipotoxocity. The IMCL accumulation is attenuated in young-adult organisms by an elevated oxidative capacity. Methionine restriction enhances mitochondrial biogenesis and is promising to combat obesity across the ages.

Key words: Aging, muscle, methionine restriction, high-fat diet, lipotoxicity, muscle function, obesity

INTRODUCTION

Over the last century, improvements in living conditions and healthcare have resulted in a significant increase in life expectancy in the western world. This increase in life expectancy is not accompanied, however, by a proportional increase (and perhaps even a decrease) in the number of healthy life years (1). The result is a progressively increasing proportion of older adults in the western world suffering from mobility limitations, a poor quality of life and need of healthcare that are to a large extent attributable to muscle weakness (2). While death is ultimately inevitable, healthy life style choices, such as regular physical activity, can result in a 'trajectory of healthy ageing' (3), delaying and reversing mobility limitations even in old age.

Another serious global challenge to health and healthcare systems is that more than 39% of people older than 18 years are overweight and 13% obese (4). Obesity and overweight are risk factors for the development of insulin resistance, cardiovascular diseases and cancer (5). Obesity may also have a negative effect on skeletal muscle function (6), where poor muscle function may act as a catalyst to obesity associated comorbidities. The latter may be a consequence of myosteatosis - the accumulation of fat in the muscle - that is indeed associated with poor fitness and surgical outcome (7). In this review, we discuss the potential synergistic effect of ageing and a high fat diet (HFD) on skeletal muscle structure and function. The working hypothesis of the review is that a HFD leads to a faster accumulation of intramyocellular lipids (IMCL) and an earlier onset of muscle dysfunction in old than in young-adult individuals.

THE EFFECTS OF AGEING ON SKELETAL MUSCLE

In humans, ageing is associated with a decrease in muscle mass (sarcopenia) that is attributable to fiber loss, and atrophy of specifically type II fibers (8). Particularly, postural and locomotor muscles suffer from sarcopenia, with the muscles of the upper body being less

affected (2). In mice, it has even been reported that the diaphragm may, at least transiently, show an age-related hypertrophy (9), perhaps in response to the increased cost of breathing in old age. During ageing, the loss of muscle mass is accompanied with an increase in fat mass and body mass index (BMI) (8), something also seen in rodents (10, 9).

Clearly, the age-related loss of muscle mass is a significant cause of the muscle weakness in old age. It is, however, not the sole explanation as the loss of strength is more than proportional to the loss of mass and results in a lower specific tension (force per muscle cross-sectional area) in muscles from both old humans (8, 11) and rodents (12). Such reductions have also been reported at the single fiber level, although this is not unequivocal, suggesting problems at the myofibrillar level (2). An age-related slowing of the muscle, due to a combination of an increased volume percentage of slow fibers (as there is selective atrophy of type II fibers) and slowing of in particular type I fibers will aggravate the loss of power on top of that incurred by atrophy (2).

The significance of a reduced muscle quality is reflected by the observation that it is not so much loss of muscle mass, but rather the age-related loss of power that is linked with reduced balance, mobility and all-cause mortality (13). One of the potential causes of a reduced specific tension in muscle fibers is the age-related reduction in myosin concentration (2), perhaps accompanied by lipid accumulation. However, the lower specific tension in old mouse muscle (14) was not associated with intramyocellular lipid (IMCL) accumulation in muscle fibers (9). Perhaps other factors, such as oxidative modifications or glycation of the myosin head, underlie the reduced specific tension and/or shortening velocity in old age (2).

THE EFFECTS OF HIGH-FAT DIET AND AGEING ON SKELETAL MUSCLE

Even though the absolute force and power generating capacity of postural and locomotory muscles may be higher in the overweight and obese, they are lower when expressed per body mass (15), or muscle mass (10, 16) in both humans and rodents. These observations indicate that, similar to ageing, overweight and obesity are accompanied with a lower muscle quality (6). Part of the reduced muscle quality may well be attributable to the accumulation of IMCL in both human (17) and mouse muscle, irrespective of fiber type (18, 19).

Life style factors, such as physical activity levels, co-morbidities, genetic differences and diet can have a significant impact on muscle mass and quality in old age (20). Above we discussed that the effects of overweight and a HFD on skeletal muscle are strikingly similar to the effects of ageing, and the question arises whether a HFD and overweight accelerate or aggravate muscle ageing.

As discussed above, the increased loading of the anti-gravity muscles in overweight and obese inidviduals induces an increase in their force and power generating capacity (6). In older adults, however, such an increase in force and power generating capacity occurs less frequently (21, 16) and some studies even report a lower absolute force producing capacity (21). The disparity in response between young-adult and old obese groups may in part be explained by an age-related reduction in myogenesis (2), limiting the adaptions to elevated loading imposed by the higher body mass in overweight or obese individuals.

Undoubtedly, the lower force to body mass or regional lean mass ratio in obese than non-obese older adults (21, 16) contributes to the compromised ability to perform daily life activities (22). Even in the absence of overweight or obesity, a high level of intermuscular adipose tissue was associated with lower muscle specific power and mobility limitations in older people (11). Not only do older women have a lower muscle force to body mass ratio, but they may also suffer from an accelerated decline in strength and muscle volume compared to non-obese women (16). This further accentuates the poorer muscle function with increasing age and puts them at risk to cross a disability threshold earlier than non-obese women. It is interesting to note, however, that the age-related reduction in specific force was slower than in non-obese women (16). We have no explanation for this attenuated age-related decline in muscle quality, but it corresponds with the observation of a HFD-induced decline in specific power and force in the soleus and extensor digitorum longus (EDL) muscles in young-adult (23), but not old mice (10). Perhaps IMCL accumulation, as seen in mice muscle fibers (19), results in a rapid decline in muscle quality via mechanisms discussed below that subsequently diminishes when the muscle storage capacity of IMCL is saturated in young-adult mice. In old mice, the age-related reduction in specific tension caused by other mechanisms (2) may mask those induced by a HFD.

The accelerated age-related decline in muscle volume and strength (but slower decline in specific force as discussed above) in obese women (16) may indicate that the detrimental effects of overweight and a HFD are more pronounced in old than young-adult age. A first indication that this may be the case comes from the observation in mice where HFD-induced increases in body mass, BMI and IMCL accumulation occur earlier in old than in younger animals (19), which may cause an earlier onset of muscle dysfunction in old animals. Indeed, a HFD caused diaphragm dysfunction, but as discussed above, surprisingly not so in the soleus and EDL muscles of old mice (10), even though all muscles had a similar degree of IMCL accumulation (19).

Longer duration of HFD (20 months) led to a lower specific tension than that seen in age-matched lean mice, suggesting that HFD aggravates muscle dysfunction also in old age, probably partly attributable to IMCL accumulation (18). These observations in mice suggest

that the effects of HFD and ageing are synergistic. The potential significance of an elevated susceptibility of IMCL accumulation for older people is illustrated by the observation that the lower specific power of type I fibers in obese than non-obese older people was related to a higher IMCL content (24).

A (non-exhaustive) summary of the impact of HFD in rodents in given in Table 1. Overall, the evidence suggests that HFD consumption 1) exacerbates the age-related decline in muscle function and 2) that there is an age-related increase in susceptibility of HFD-induced IMCL accumulation and rise in body mass and BMI. Such a synergistic effect of ageing and obesity is likely to contribute to poor health outcomes, reduced physical function and poorer quality of life. Indeed, compared to normal weight counterparts, sarcopenic obese people are at a greater risk of metabolic syndrome, cardiovascular disease, diabetes and all-cause mortality (25).

CAUSES AND MECHANISMS OF SKELETAL MUSCLE DYSFUNCTION DURING HFD AND OBESITY

Ageing is associated with reduced levels of physical activity in all sorts of organisms, including humans, which contributes to the age-related decrements in skeletal muscle mass and function (1). In fact, when adjusted for height, level of physical activity, pain, depression and muscle mass, obesity-induced increases in hand-grip strength, peak isometric force of the elbow extensors and peak isometric force of the knee extensors in older women, were no longer apparent (26). This suggests that a significant part of the problems in overweight is attributable to lower levels of physical activity.

While reduced physical activity apparently contributes significantly to the muscle dysfunction in obesity, a HFD itself may also play an important role. As discussed above, the IMCL content in type I fibers was negatively related to the specific power of human type I fibers (24) and IMCL occurred earlier in old than young-adult HFD fed mice (19). The earlier accumulation of IMCL during a HFD in old than in younger muscles (19) is perhaps due to a substantially greater adiposity in normal old (10) than that seen in normal young-adult mice (23). The larger adiposity may in turn result in the diversion of storage of the excessive fatty acids from a HFD in skeletal muscle fibers rather than in adipose tissue.

Another explanation for earlier accumulation of IMCL in old than young-adult mice on a HFD may be due to a compensatory increase in oxidative capacity in the muscle fibers of young-adult (27), but not old animals (19) that would enhance the capacity for fatty acid oxidation. In support of this, it has been observed in mice that a HFD increases the expression of fatty acid binding protein (FABP) and the rate-limiting enzyme of fatty acid oxidation, m-carnitine

palmitoyl transferase (m-CPT-I) (28). Such an increased capacity for fatty acid oxidation may at least transiently stave off the accumulation of IMCL and the associated muscle dysfunction. Indeed, in a mouse model of lipotoxic cardiomyopathy the uptake of fatty acids by cardiomyocytes exceeded the use of fatty acids and led to cardiac dysfunction (29). In addition, CD36 (the membrane-bound fatty acid transporter) deficiency diminished IMCL accumulation and the increase in body mass during a HFD, but led to enhanced liver steatosis (28). Therefore, the absence of a significant HFD-induced increase in oxidative capacity may well be a factor underlying the earlier rise in body mass, BMI and IMCL accumulation in old than young-adult animals (19).

In addition to IMCL accumulation, in mice a HFD has also been associated with oxidative stress that was mitigated by CD36 deficiency (28). This suggests that perhaps some of the lipotoxocity is a consequence of oxidative stress resulting from the accumulation of IMCL. If so, IMCL accumulation may mediate a reduction in the HFD-induced reduction in specific tension and specific power via both a reduced myofbirillar volume and increased oxidation of myofibrillar proteins.

The HFD-induced increase in muscle oxidative capacity (19, 27) was not accompanied by a commensurate capillary proliferation, resulting in a morphological mismatch between the oxygen supply -reflected by the capillarization- and demand -reflected by the oxidative capacity- in muscle fibers from mice on a HFD (19). While the increased resting red blood cell flux after 8 weeks HFD in mice (27) perhaps at least initially compensated for the reduced morphological mismatch between supply and demand at rest, the morphological mismatch may well limit the oxygen supply during exercise. In obese Zucker rats, capillary rarefaction has even been observed (30). As capillarization is important for muscle function (31), and such a reduction in muscle capillarization also occurs in humans this undoubtedly contributes to the impaired exercise tolerance in obese individuals.

Figure 1 illustrates our working hypothesis. We speculate that IMCL accumulation during a HFD may be accelerated in older people as a consequence of diversion of lipid storage from already loaded adipose tissue to skeletal muscle. The accumulation of IMCL as a consequence of a HFD may cause skeletal muscle dysfunction via lipotoxicity that is to some extent staved off by increasing the capacity for fatty acid oxidation in young-adult but not old muscles. The impact on exercise capacity is aggravated by capillary rarefaction that results in a mismatch between oxygen supply and demand.

METHIONINE RESTRICTION TO REDUCE HFD-INDUCED SKELETAL MUSCLE DYSFUNCTION

Dietary interventions are often used in combination with exercise to combat the negative effects of HFD-induced obesity. In old obese individuals, it is especially important to focus on reducing adiposity and increasing skeletal muscle mass to improve quality of life.

Calorie restriction or dietary restriction attenuates sarcopenia (32), and a combination of calorie restriction and exercise increased *m. plantaris* cross-sectional area in aged rodents (33) compared to those on an *ad-libitum* diet. While calorie restriction yields desirable results, limiting calorific intake over a long time is challenging and not sustainable (34).

Methionine restriction has been described as a calorie restriction mimetic, and has been found to extend lifespan and reduce age-related inflammation in rats (35). Recent research has demonstrated that restricting methionine to 0.17-0.25% from the normal 0.86% is the ideal range to elicit metabolic benefits without stunting growth in young-adult mice (36). In practical terms, methionine restriction can be achieved by switching to a vegan diet as foods like fruits, vegetables, legumes, nuts and soy contain relatively low quantities of methionine compared to animal products (37).

Increased adiposity, insulin resistance and mitochondrial degradation are some of the metabolic outcomes of sarcopenic obesity. Although hyperphagia has been reported in mice on methionine restriction, it nevertheless induced a 30-50% decrease in body mass and fat mass (38, 35). This reduction in fat mass despite hyperphagia was related to an increase in energy expenditure and fat oxidation, and decreased insulin resistance (36, 34). Part of this is attributable to a shift in fuel utilization to lipids as seen after 16 weeks of methionine restriction in obese people (34).

One of the mechanisms underlying the increased energy expenditure during methionine restriction may well be a rapid and persistent increase in the expression of uncoupling protein 1 (UCP1) as seen in adipose tissue of rats and mice, and increased circulating adiponectin and decreased leptin levels (39, 34). The elevated levels of circulating adiponectin preserve or restore insulin sensitivity by lowering muscle triglycerides by oxidation of free fatty acids (39). While these observations were made in non-obese old rats (39), they bear promise to combat obesity in old age.

The increase in plasma adiponectin, decrease in leptin and upregulation of fibroblast growth factor 21 (FGF21) in mice fed a combination of methionine restriction and HFD (38), and obese mice on methionine restriction (40) was associated with protection against obesity and insulin resistance. The latter study (40) also reported elevated levels of circulating FGF21 in humans on a vegan or vegetarian diet, typically low in methionine. In addition, it was shown in

adiponectin, FGF21 and double knock-out mice (41) that the methionine-restriction-induced reduction of adipose tissue in obese mice was associated with elevated lipolysis, apoptosis and autophagy, and can occur independent of circulating levels of adiponectin and FGF21.

The methionine-restriction-induced lypolysis and shift to fatty acid oxidation may be related to the increased secretion of adiponectin by adipose tissue and skeletal muscle that has been linked to enhanced transcription of PGC1α in skeletal muscle (42). PGC1α promotes mitochondrial biogenesis and enhances thereby the capacity for fatty acid oxidation (43). This is significant, as age-associated mitochondrial dysfunction is an important factor in sarcopenia (44). Furthermore, the accumulation of intramuscular fat reduces phosphorylation of targets in the mTOR - AMPK pathways that can lead to anabolic resistance (45) and hence the risk of sarcopenia (21). Amino acid restriction has been found to enhance production of H_2S via the transsulfuration pathway that activates AMPK in the skeletal muscle (46) and upregulates SIRT1 (47) that both increase expression of PGC1α (43). In addition, the increased expression of PPARδ in skeletal muscle of young-adult methionine restricted rats (48) can induce transcription of PGC1α in skeletal muscle (49) and hence further enhance mitochondrial biogenesis. In addition to stimulating mitochondrial biogenesis, methionine restriction has also been shown to reduce mitochondrial oxidative DNA damage and to lower membrane unsaturation in rat brain (50). Although further research is required to fully elucidate the effect of methionine restriction on IMCL and skeletal muscle lipid metabolism, methionine restriction shows promise in being able to enhance lipolysis in skeletal muscle via PGC1α-controlled mitochondrial biogenesis that is prompted by upregulation of AMPK and SIRT1.

CONCLUSION

The age-related decrement in muscle function is aggravated by high fat diet (HFD) resulting in overweight or obesity. It is suggested that older organisms are more susceptible to an accelerated accumulation of intramyocellular lipids (IMCL) as adipose tissue stores are already loaded and in contrast to young-adult organism do not show a HFD-induced increase in muscle oxidative capacity. The IMCL accumulation not only potentially causes a deterioration of muscle function by a larger volume fraction of the muscle fiber, but also via lipotoxic effects. Rodent studies suggest that calorie restriction or methionine restriction is a promising tool to combat obesity.

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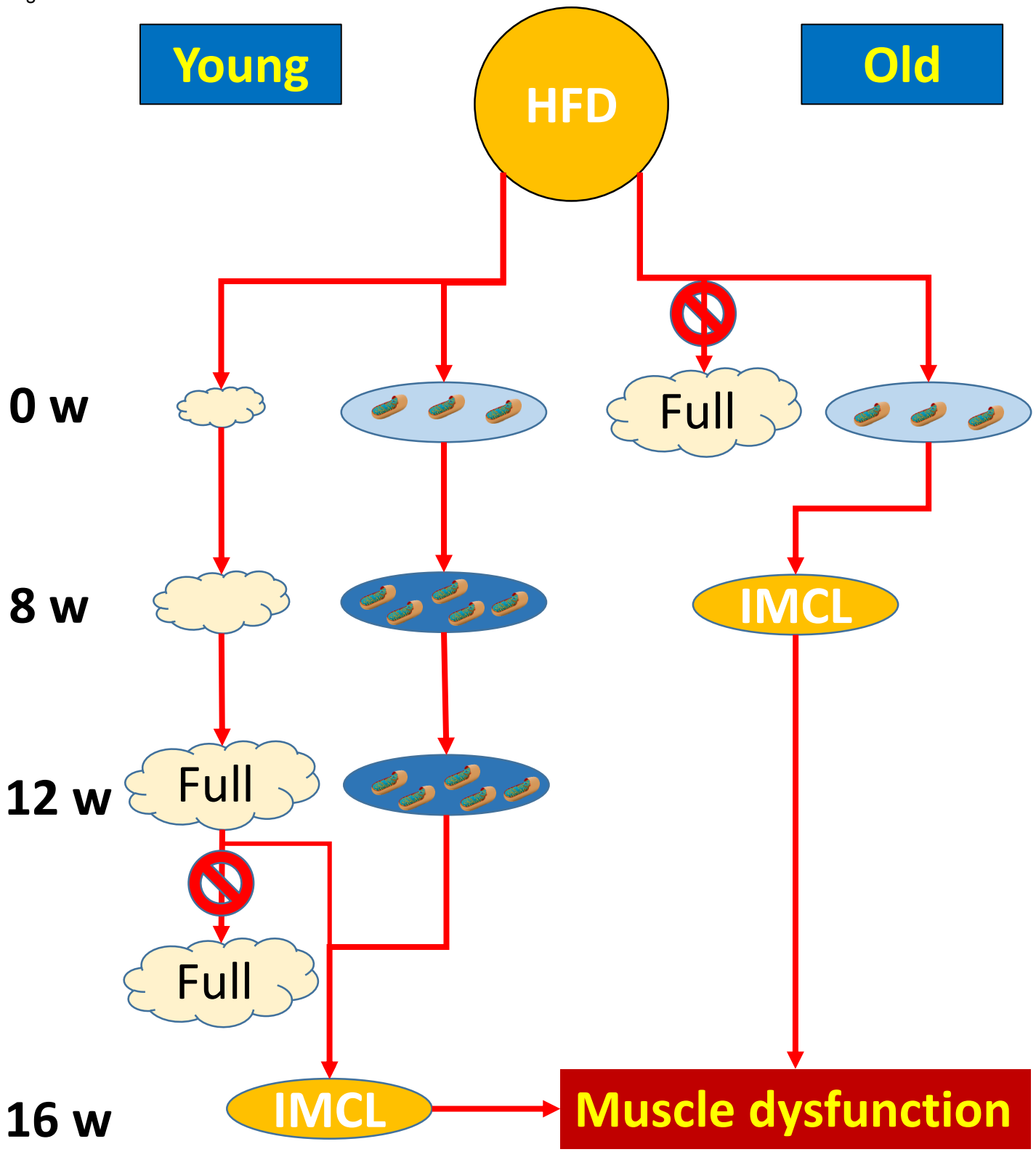
Table 1. Summary of studies examining the effect of HFD on contractile function, myosteatosis and metabolic characteristics of skeletal muscle

Reference	Species	Strain, Sex	Age*	Duration of HFD	Muscle morphology and function	Myosteatosis	Metabolic characteristics
(23)	Mouse	CD-1, ♀	20 weeks	2, 4, 8, 12 weeks	• Mass soleus↑ after 8 and 12 weeks • Mass EDL↑ after 4, 8 and 12 weeks • Force, specific, force soleus and EDL= • Specific force diaphragm↓ after 8 weeks • WL power ($W \cdot kg^{-1}$) soleus and EDL↓ after 8 weeks and 12 weeks • WL power of the diaphragm↓ after 2 weeks • EDL↓ fatigue resistance after 8 and 12 weeks		
(27)	Mouse	C57BL/6J, ♂	14 weeks	8 weeks		• Adipocyte accumulation in skeletal muscle interstitium↓ • Extracellular collagen↓	• Insulin resistance↑ and glucose tolerance↓ after 7 weeks • EDL RBC flux and velocity↑
(28)	Mouse	CD36 KO C57BL/6J	5-6 months	13 weeks	• Mass soleus↑, biceps brachii↑ HFD C57BL/6J	• IMCL, tibialis anterior HFD C57BL/6J ↑	• Type IIb fibres C57BL/6J EDL↓ WT • Oxidative stress HFD C57BL/6J ↑, CD36 KO↓ • Genes involved with fatty acid uptake and utilization HFD CD36 KO↑, HFD C57BL/6J ↑ • Myogenic capacity HFD C57BL/6J ↓, CD36 KO↓,
(18)	Mouse	C57BL/6J, ♂	6 months 22 months	4, 20 months	• Mass EDL unchanged after 4, 20 months of HFD • Force, specific force EDL↓ after 4, 20 months	• IMCL EDL↑ after 4, 20 months	• 4, 20 months: glucose tolerance↓, contraction-stimulated Ca^{2+}↓ • 20 months of HFD: insulin resistance↑
(19)	Mouse	CD-1, ♀	Young-adult: 20 weeks Older: 79 weeks	8, 16 weeks 9 weeks		• Young-adult: IMCL soleus and EDL↑ after 16 but not 8 weeks • Older: IMCL soleus and EDL↑ after 9 weeks.	• Young-adult: SDH in EDL and soleus ↑ after 8 and 16 weeks • Young-adult: C:F↑ in soleus and EDL after 16 but not 8 weeks • Older: C:F↑ after 9 weeks in soleus only
(10)	Mouse	CD-1, ♀	79 weeks	9 weeks	• Mass soleus and EDL↑ • WL power output soleus and EDL↑ • Specific force diaphragm↓ • WL power of the diaphragm↓ • Fatigue resistance soleus, EDL & diaphragm=		

**- age at terminal experiment. HFD: high fat diet; EDL: extensor digitorum longus; SDH: succinate dehydrogenase; C:F: capillary to fibre ratio; WL: work loop; $W \cdot Kg^{-1}$: Watts per kilogram muscle mass; MHC: Myosin Heavy Chain; IMCL: Intramyocelluar lipids; KO: Knockout; WT: Wild type [For more details see (6)].*

Figure 1: A high fat diet (HFD) leads to an earlier accumulation of intramyocellular lipids (IMCL) in muscles from old than young-adult mice. On the left hand side is the duration of HFD in weeks (w). The beige clouds illustrate the progressive increase in adiposity (shown as increasing size of the clouds) up to a point (indicated as full) beyond which further lipid accumulation in adipose tissue is reduced (indicated by the red circle with a diagonal line). Here we suggest that the larger adiposity in old than young mice limits the ability to store additional lipids in adipose tissue In addition, while in young mice a HFD induced an increase in oxidative capacity (reflected by the darker blue stained ovals and the larger number of mitochondria icons), enhancing the ability to perform fatty acid oxidation, this was not the case in muscles from old animals. Thus, the higher need to store excessive lipids in muscle as the adipose tissue is progressively filling up and the lower ability to use fatty acids as reflected by the absence of a HFD-induced rise in oxidative capacity in old muscles, results in an earlier accumulation of IMCL and muscle dysfunction in old than young animals.

Figure


Young
HFD
Old
0 w
Full
8 w
IMCL
12 w
Full
Full
16 w
IMCL
Muscle dysfunction