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Review

Fat infiltration in skeletal muscle: Influential triggers and regulatory mechanism

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SUMMARY

Fat infiltration in skeletal muscle (also known as myosteatosis) is now recognized as a distinct disease from sarcopenia and is directly related to declining muscle capacity. Hence, understanding the origins and regulatory mechanisms of fat infiltration is vital for maintaining skeletal muscle development and improving human health. In this article, we summarized the triggering factors such as aging, metabolic diseases and metabolic syndromes, nonmetabolic diseases, and muscle injury that all induce fat infiltration in skeletal muscle. We discussed recent advances on the cellular origins of fat infiltration and found several cell types including myogenic cells and non-myogenic cells that contribute to myosteatosis. Furthermore, we reviewed the molecular regulatory mechanism, detection methods, and intervention strategies of fat infiltration in skeletal muscle. Based on the current findings, our review will provide new insight into regulating function and lipid metabolism of skeletal muscle and treating muscle-related diseases.

INTRODUCTION

Skeletal muscle is among one of the largest organs in the human body accounting for approximately 40% of total body weight and playing an indispensable role for locomotion and glucose and lipid homeostasis. Many multinucleated fast and slow myofibers constitute skeletal muscle through the activation of resident myogenic precursor satellite cells (SCs). Activated SCs undergo proliferation and differentiation of the myoblast, fusion of myocytes, and finally maturation of myofibers, during the biological process of myogenesis.[1] Many myogenic transcription factors such as paired box 7 (Pax7), myogenic differentiation (MyoD), myogenic factor 5 (Myf5), and myogenin regulate myogenesis.[2] Besides, adipose tissue can secrete several adipokines and lipokines that regulate skeletal muscle development and homeostasis.[3] Various adipokines (e.g., adiponectin, leptin, chemerin, and resistin) and lipokines (e.g., oxylipins, fatty acid-hydroxy-fatty acids, lysophosphatidic acid, and palmitoleate) play important roles in mediating the crosstalk between adipose tissue and skeletal muscle. After muscle injury, skeletal muscle has a mighty regenerative capacity. The damaged muscle elicits the activation of quiescent SCs and their niche for repair. A number of signaling molecules take part in this process to maintain muscle stem cells homeostasis.[4] However, muscle fiber is replaced by ectopic tissues including fat and fibrous tissue during progressing muscle regeneration. Muscle regeneration ultimately leads to the loss of many muscle functions, this process results from a decreased SCs number.[5] The decline in skeletal muscle mass, strength, and other functions is directly related to human myopathies, including muscular dystrophy, cachexia, and sarcopenia. Hence, how does regulating skeletal muscle development, function, and regeneration benefits human health?

Fat infiltration (also known as fat deposition/accumulation), often exists in non-adipose tissue, such as skeletal muscle, bone, and liver. Myosteatosis, defined as the pathologic fat accumulation in skeletal muscle with poor metabolic and musculoskeletal health, is now considered as a distinct disease from sarcopenia, which is also recognized as a common feature of aging and is related to the decline of muscle strength, muscle architecture, muscle contraction, and muscle capacity.[6] Fat deposition in skeletal muscle has two patterns: (1) fat deposition between skeletal muscle fibers (muscle cells), including the intermuscular adipose tissue (IMAT) between the epimysium of skeletal muscle and intramuscular fat (IMF) in the endomysium and perimysium and (2) fat deposition (lipid droplets) in skeletal muscle fibers, called intramyocellular (IMCL).[7] In this article, IMF content refers to IMF in a broad sense, including IMAT, IMF, and IMCL. However, intramuscular triacylglycerol or lipid droplets also serve as energy storage organelles and the primary goal of lipid droplet biogenesis is to alleviate cellular lipotoxic stress, as well as stresses associated with disturbed endoplasmic reticulum homeostasis, oxidation, and starvation.[8] Recently, Yue et al. have shown lipid droplet dynamics could regulate stem cell fate determination.[9] Hence, lipid droplets play different roles in different diseases and might even have opposite functions during different stages of the same disease.[8] In parallel, animal production research has provided extensive insight into the role of IMF in meat quality. Several muscle disorders and physiological stresses always accompany with fat infiltration in skeletal muscle, which is directly related to insulin resistance and muscular dysfunction, such as aging, sarcopenia, diabetes, and obesity.[10,11] Previous studies found that many triggering factors, including aging, diseases, and muscle injury, can contribute to fat infiltration in skeletal muscle and fat infiltration is modulated by many regulators, genes, and signaling pathways.[12,13] Thus, understanding the

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https://doi.org/10.1016/j.isci.2024.109221

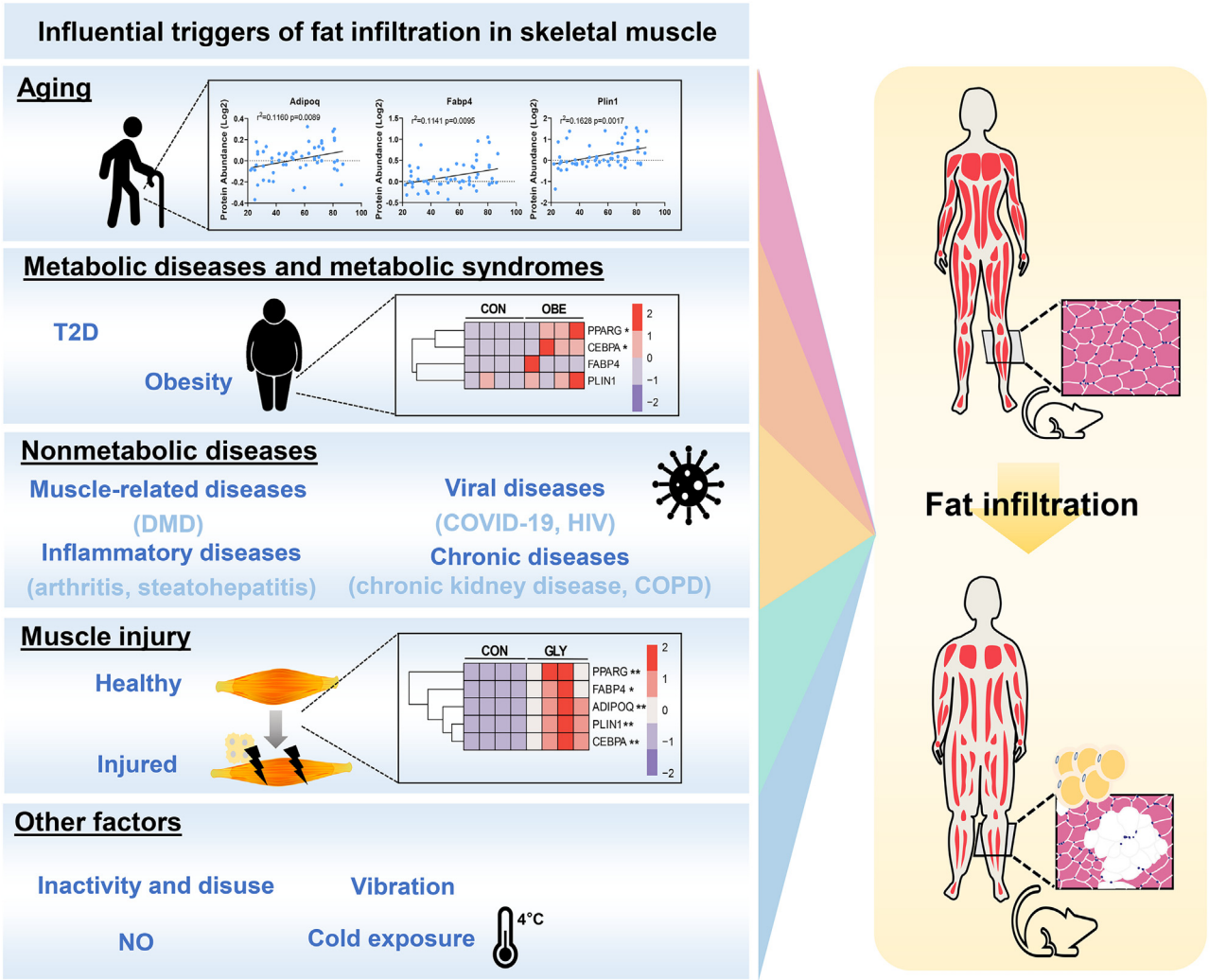

Figure 1. Influential triggers on fat infiltration in skeletal muscle

Several triggering factors induce IMF deposition in skeletal muscle, including aging, diseases, muscle injury, and others. COPD, chronic obstructive pulmonary disease; DMD, duchenne muscular dystrophy; HIV, Human immunodeficiency virus; NO, nitric oxide; T2D, type 2 diabetes.

origins of IMF deposition and the regulatory mechanism of fat infiltration in skeletal muscle are vital for maintaining the development, function and homeostasis of skeletal muscle, and the treatment of muscle-related disease.

In this review, we mainly discuss the triggering factors contributing to fat infiltration, including aging, metabolic diseases, non-metabolic diseases, and muscle injury as well as the cellular origin of fat infiltration. Additionally, recent advances and current discoveries of regulatory mechanisms, detection methods, and intervention strategies (exercise, diet, secreted factors, and gut microbiota) on fat infiltration in skeletal muscle are also discussed.

INFLUENTIAL TRIGGERS OF FAT INFILTRATION IN SKELETAL MUSCLE

Several triggering factors have been reported to induce fat formation and accumulation in skeletal muscle, including aging, metabolic diseases and metabolic syndromes, non-metabolic diseases, muscle injury, and other factors (Figure 1).

Aging

Aging relates to physiological, metabolic, and functional dysfunction, in part through age-related alterations in body composition. Aging is always accompanied by a decreased mass and strength of skeletal muscle, also referred to as "sarcopenia," which is a significant contributor to the emergence of age-related metabolic dysfunction, comorbidities, and premature death.[14] Fat infiltration and IMF deposition often occur in skeletal muscles of elderly people as well as aged animals. In human, a previous study found an age-related remodeling of body composition with a reduction in skeletal muscle and IMAT in older African American women, which suggested that aging-induced fat

infiltration in skeletal muscle is always correlated with sarcopenia.[10] Molecularly, based on the quantitative proteomic analysis in skeletal muscle of human,[15] we performed a correlation analysis and found the levels of adipogenesis-related protein (ADIPOQ, FABP4, and PLIN1) were positive with age (Figure 1). Additionally, fat infiltration in skeletal muscle is often correlated with the emergence of some diseases for the elderly. In Caucasian aged men, skeletal muscle fat infiltration is positively associated with all-cause and cardiovascular mortality.[16] There is a clear linear positive correlation between the greater content of IMF and increasing age in individuals across disease states, including cancer and chronic stroke survivors, total knee arthroplasty, and anterior cruciate deficiency.[17] In older adults, lower muscle mass (also called smaller thigh muscle area) and more IMF content in the muscle are related to the risk of mobility losses.[18] Recently, Perez et al. identified the source of IMF cell types including mesenchymal stem cells (MSCs), fibro/adipogenic progenitors (FAPs), and endothelial cells (ECs) in vastus lateralis of old people by using single-nucleus RNA-seq (snRNA-seq).[19] Similarly, in animals, aging is also related to IMAT and IMF deposition in skeletal muscle. Jing et al. identified adipocytes clusters and other pre-adipocytes clusters (MSCs, FAPs, ECs, and pericytes) in aged muscle of monkeys through snRNA-seq.[20] In brief, aging is always accompanied with fat infiltration in skeletal muscle which might be a significant trigger for muscular dysfunction. However, whether the cell types in IMF of aged skeletal muscle are similar with adipocytes needs to be further explored.

Metabolic diseases and metabolic syndromes

Metabolic diseases are diseases caused by metabolic disorders and metabolic exuberance. Diabetes mellitus (DM) is a common metabolic disease in elderly people. Recent statistics showed that DM is estimated to affect approximately 578 million by 2030 and 700 million by 2045.[21] Importantly, type 2 diabetes (T2D) is the most common form of DM and accounts for about 90% of cases diagnosed. Recently, more and more studies have found IMF content is proportionately higher in men and women with T2D and the metabolic syndrome than in people without these conditions.[22] Greater ectopic fat deposition and lower skeletal muscle mass are associated with T2D and is a heritable trait.[23] Particularly, therefore, lipid accumulation in skeletal muscle in Africans is more prevalent than in Caucasian men.[24] T2D is always accompanied with fat infiltration in skeletal muscle. For example, obese patients with T2D had the highest IMAT and it was strongly associated with insulin resistance.[25] Failure of fat cell proliferation, impairment of fat oxidation, and increased fat intake all lead to ectopic IMF deposition, insulin resistance, and T2D.[26] Myosteatosis contributed to the development of T2D in African men[27] and the degree of myosteatosis was significantly and positively correlated with IMAT content, which can alter skeletal muscle quality and relate to sarcopenia and skeletal muscle aging.[28] A group of complex metabolic disorder syndrome and a risk factor leading to DM, cardiovascular and cerebrovascular diseases, and obesity is one of its main common symptoms. Importantly, obesity is always accompanied by increasing fat deposition between skeletal muscle fibers or surrounding skeletal muscle.[29] Based on the RNA-seq data of primary differentiated human myotubes, we used heatmap and found the expression of adipogenic marker gene (*Pparγ* and *C/ebpα*) was significantly upregulated in obese group[30] (Figure 1). This meant obesity is related to fat deposition in skeletal muscle *in vitro*. Overall, metabolic diseases and metabolic syndromes are always accompanied with IMF deposition but the regulatory mechanism of these processes is still unknown.

Nonmetabolic diseases

Disease is a major risk factor for skeletal muscle mass and strength loss. Emerging evidences implicate that some diseases may be major causes of fat infiltration in skeletal muscle.

Muscle-related diseases

Duchenne muscular dystrophy (DMD) is a degenerative muscle disorder in which muscle cells are damaged and replaced by fibrofatty tissue. Compared with normal muscle, boys suffering from DMD had increased fat infiltration.[31] Specifically, gluteus maximus had the greatest degree of fatty infiltration in boys with DMD.[32] Additionally, in expiratory muscle, Barnard et al. also found fatty infiltration in individuals with DMD.[33] Muscle degeneration in low back pain is characterized by a decrease in cross-sectional area and an increase in fat infiltration in the lumbar paraspinal muscles.[34] These results indicate muscle-related diseases are influential triggers of fat infiltration in skeletal muscle.

Viral diseases

Recently, the COVID-19 pandemic caused an extraordinary global emergency and was threatening human health. Current evidence suggests that skeletal muscle was affected by the COVID-19-related malfunction.[35] COVID-19 leads to the decrease of skeletal muscle strength and mass[36] and is associated with skeletal muscle fat index.[37] Therefore, COVID-19 may impair skeletal muscle function but the special relationship between it and fat deposition in muscle needs further study. Besides, men infected with HIV had lower thigh muscle density and more lipid accumulation in skeletal muscle during aging.[38] Based on these research, viral diseases contribute to fat infiltration in skeletal muscle.

Inflammatory diseases

In patients with rheumatoid arthritis, IMF accumulation has a special association with physical function and physical activity.[39] Similarly, in thigh muscles of people with knee osteoarthritis, increased infiltration of fat was shown both between and within the muscles.[40] Besides, muscle fat content is strongly associated with non-alcoholic steatohepatitis.[41] Kaibori et al. also observed that after partial hepatectomy surgery, patients with hepatocellular carcinoma had even less overall survival and greater IMF accumulation.[42] These data suggest that inflammatory diseases have a powerful role in IMF deposition.

Other diseases

Additionally, other diseases are also correlated with fat deposition in skeletal muscle. Computed tomography images showed that fat accumulation in thigh and calf muscle is increased in patients with chronic obstructive pulmonary disease[43] and it is related to relevant clinical outcomes and comorbidities.[44] Specifically, in patients with lumbar diseases, increased fat infiltration was shown in the multifidus muscle in those patients with lumbar radiculopathy or lumbar degenerative kyphosis.[45] Recently, Avesani et al. reviewed the correlation between muscle fat infiltration and chronic kidney disease, which mainly showed fat infiltration has been associated with a decrease in muscle strength and impaired muscle quality as well as with metabolic abnormalities, cardiovascular disease, and increased mortality.[46] Also, myosteatosis is common in patients with cirrhosis and is associated with higher mortality.[47]

In short, many non-metabolic diseases are also important triggering factors for fat deposition in skeletal muscle accompanying with skeletal muscle mass and strength loss (Figure 1). However, the specific regulatory mechanism is still not clear.

Muscle injury

After various types of injuries and diseases, skeletal muscle has a striking regenerative capacity. Several studies demonstrated that muscle injury is always accompanied with degenerative changes such as fat infiltration in recent years.[48] A recent study found that 1-week post motor vehicle collision, muscle attenuation values were significantly related to IMF content in magnetic resonance imaging.[49] Compared with individuals with healthy controls and mild/moderate symptoms, patients with persistent whiplash injury-associated disorders (WAD) had increased muscle fat infiltration within the cervical multifidus muscle after 2 weeks and 3 months.[50,51] Within the severe WAD group and the healthy group, there was a relevance observed between local and distal fat infiltration in skeletal muscle but not in the mild/moderate group.[52] Similarly, people with persistent pain following whiplash injury always have varying levels of fat infiltration in their cervical extensor muscle.[53] However, a meta-analysis suggests that there is still deficient evidence to confirm whether fat infiltration in muscle increases right after whiplash injury.[54] Hence, the crosstalk between WAD and fat infiltration is still controversial. Besides, after chronic spinal cord injury, patients always suffer from IMF infiltration leading to metabolic disease and related mortality.[55]

In murine models, injecting glycerol in skeletal muscle has been used as a new animal model system for inducing muscle damage and regeneration. Our previous data showed the mRNA levels of adipogenesis-related genes (*Fabp4*, *Adipoq*, *Plin1*, *Pparγ*, and *C/ebpα*) were significantly increased in muscle of glycerol-injured mice[56] (Figure 1). This result indicates fat deposition always accompanies with muscle injury. In detail, Joe et al. showed that muscle damage could activate resident FAPs that facilitate myogenesis.[57] Additionally, impaired skeletal muscle regeneration is always accompanied with significant fat deposition in diabetic mice,[58] suggesting that diabetes may increase sarcopenia in obesity through enhancing the anomalous differentiation of FAPs. In brief, there is a close correlation between muscle injury and fat infiltration in skeletal muscle and the glycerol-induced injury model could provide a new and feasible mouse model to study fat deposition and muscle regeneration.

Disuse and inactivity

Pagano et al. found that even after a short period of inactivity, intermuscular adipose tissue content is increased in adult men.[59] In the lumbar multifidus and erector spinae muscles in subjects with sway-back posture, disuse can predispose these muscles to atrophy, which is characterized by a reduced cross-sectional area and fat infiltration.[60] Also, fat infiltration in the gluteus minimus muscle is related to disuse following aging in embalmed elder cadavers.[61] These results emphasize that inactivity and disuse is associated with IMF development in muscle.

Other influential factors

Apart from the aforementioned factors, there are other triggering factors that have been reported to induce fat infiltration. In *mdx* mice, nitric oxide treatment reduces the number of PDGFRα$^+$ cells and IMF deposition.[62] In sarcopenic mice, vibration and β-hydroxy-β-methylbutyrate treatment promote the transdifferentiation of adipocytes and thus could inhibit intramuscular lipid accumulation.[63] Recently, our studies have discovered cold exposure could increase fat infiltration and alter lipid metabolism in skeletal muscle of mice[64] and also increased IMF content in longissimus dorsi muscle of pigs.[65] Taken together, fat filtration in skeletal muscle was induced by many influential triggering factors, which is related to skeletal muscle quality and mass.

THE CELLULAR ORIGIN OF FAT INFILTRATION IN SKELETAL MUSCLE

Recently, multiple studies have investigated the cellular origin of fat infiltration and discovered several cell types leading to the formation of ectopic IMF through multiple technologies including single-cell RNA sequencing, snRNA-seq, genetic lineage tracing, *in vitro* cell culture models, and microscopy[5,56] (Figure 2).

Myogenic cells

Early studies demonstrated that some primary muscle SCs could differentiate into adipocytes *in vitro* under different conditions.[68] However, recent studies suggest that the primary myoblast isolation method used in the early stage may be contaminated by other cells; lineage tracing studies used by the Cre-loxP recombinant system found that IMF was derived from Myod$^-$ and Pax3$^-$ cells rather than the muscle SCs

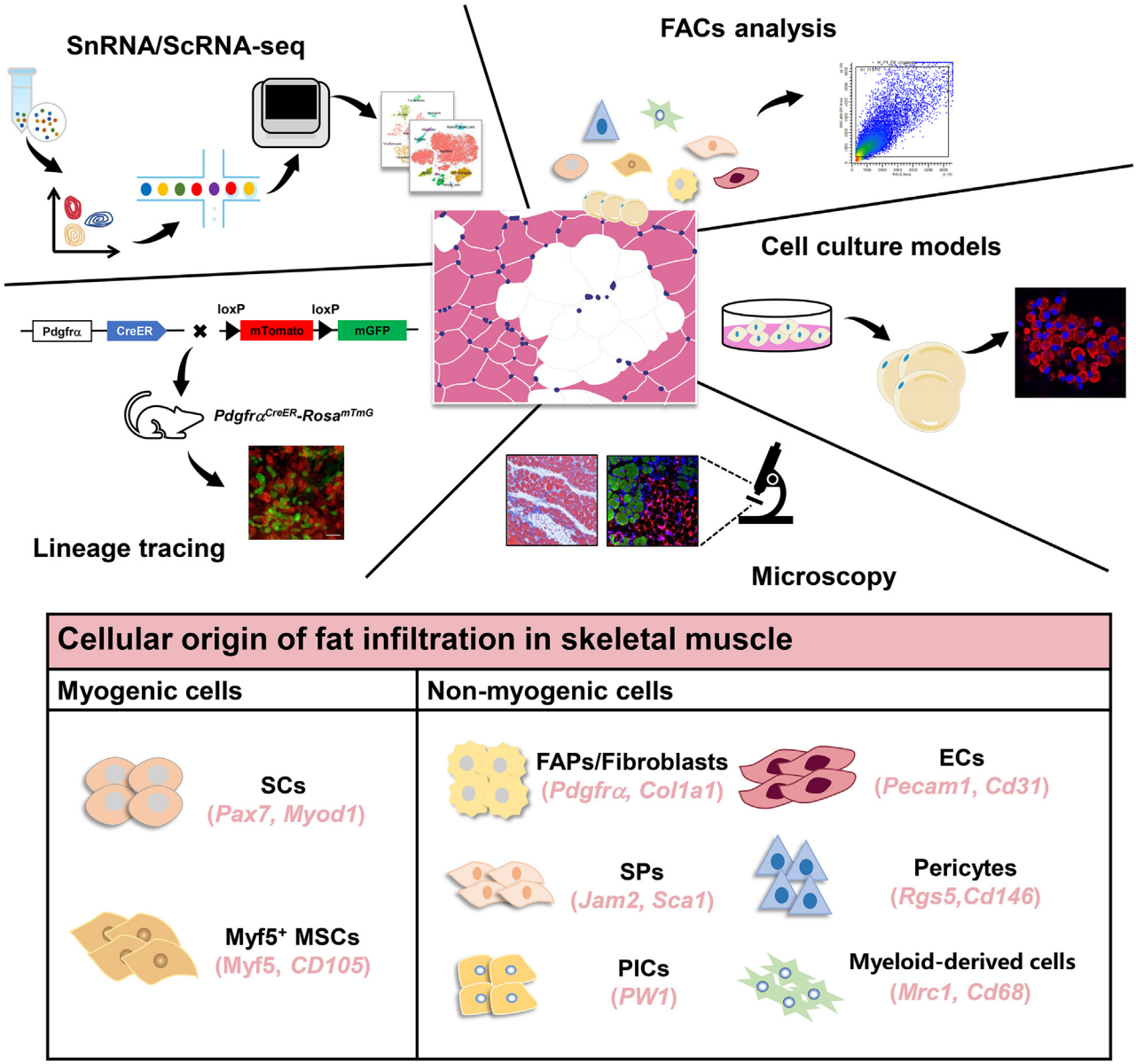

Figure 2. Fat infiltration in skeletal muscle derived from cell types with myogenic or non-myogenic origins

ScRNA-seq, snRNA-seq, genetic lineage tracing, *in vitro* cell culture models, and microscopy represent classical tools for studying the formation of fat infiltration in skeletal muscle and have found myogenic cells and non-myogenic cells types lead to fat infiltration. ECs, endothelial cells; FAPs, fibro/adipogenic progenitors; MSCs, mesenchymal stem cells; PICs, PW1-expressing cells; SCs, satellite cells; scRNA-seq, single-cell RNA sequencing; snRNA-seq, single-nucleus RNA sequencing; SPs, side population cells. Reprinted from[56] Copyright 2020, Wiley Online Library[66]; Copyright 2023, Springer nature[67]; Copyright 2023, Wiley Online Library; under the Creative Commons CC BY License.

spectrum.[69,70] However, it was also found that the primary muscle SCs achieved by fluorescence-activated cell sorting had lipid droplet deposition under certain culture conditions, but did not activate the adipogenesis process, and did not express the terminal differentiation marker genes of mature adipocytes. Fat accumulation in muscle SCs increased under some special conditions such as increased insulin resistance, decreased oxygen supply, and changed local metabolic environment.[71] Additionally, studies have reported the existence of brown adipose tissue (BAT) derived from Myf5^{+} MSCs in skeletal muscle, and Pax7-Cre^{ER} linage tracing studies have found that muscle SCs *in vivo* can differentiate into BAT regulated by Prdm16 (a transcription factor of BAT differentiation).[72] In addition, MSCs are pluripotent stem cells; studies have reported that MSCs isolated from skeletal muscle of adult and mouse can proliferate and differentiate into mesenchymal tissue and non-mesenchymal tissue *in vitro* and *in vivo*.[73]

Non-myogenic cells

FAPs

Different from previous studies on the potential of adipogenic differentiation *in vitro*, Uezumi et al.[74,75] verified *in vivo* transplantation of different cell populations in skeletal muscle and found that a subpopulation of non-myogenic MSCs is the main source of intramuscular adipocytes during muscle regeneration in mice and humans which is different from muscle SCs. This subpopulation of progenitor cells is PDGFRα$^+$ or CD140α$^+$ and has the biological potential of differentiating into lipid-rich adipocytes and expressing collagen I fibroblasts, which are defined as FAPs.[74] Unlike muscle SCs, FAPs are located in the space between muscle fibers and muscle bundles in mice and humans, which is similar to IMF.[74,75] FAPs play an important role in homeostasis and disease of skeletal muscle.[76] Furthermore, interstitial muscle connective tissue cells expressing the transcription factor Osr1 which is found in mouse embryos skeletal muscle are embryo-like FAPs that can differentiate into fibroblasts and adipocytes *in vivo* and *in vitro*. Part of FAPs in adult skeletal muscle are derived from Osr1$^+$ cells,[77] and Osr1 has a low expression level of silent FAPs in skeletal muscle after birth.[78] Uezumi et al. believed the proliferation of FAPs in adult skeletal muscle mainly came from PDGFRα$^+$ cells rather than PDGFRα$^-$ cells or stem cells in the circulatory system.[79] Historically, FAPs are the main source of IMF[57,74] and recent study have found that MME$^+$ FAPs are highly adipogenic and are reduced in fatty infiltrated human muscle.[80] In our previous study, we found FAPs could differentiate into adipocytes in 2D and 3D cultured conditions; specially, FAPs could differentiate into PDE4D$^+$/PDE7B$^+$ adipocytes.[66] In conclusion, FAPs are the main sources of IMF.

Fibroblasts

Fibroblasts can directly differentiate into adipocytes, such as 3T3 cell line. Fibroblasts that activate and produce fibers expressing vimentin and α-SMA are also known as myofibroblasts. Due to the fact that myoblasts also express fibroblast marker proteins vimentin and α-SMA, it is difficult to identify fibroblasts in skeletal muscle; studies on the fate and function of fibroblasts in skeletal muscle are very limited. Recently, TE-7 has been found to be a fibroblast-specific protein in humans; TE-7$^+$/CD56$^-$ fibroblasts isolated from human skeletal muscle can be induced into adipocytes *in vitro*.[81]

ECs

ECs in the human fetal placenta have adipogenic differentiation potential, and CD34$^+$/CD31$^+$ ECs from human omental and subcutaneous adipose tissue can be induced into adipocytes.[82,83] Based on our previous cell-to-cell communication, we found ECs may also have potential capacity to differentiate into adipocytes.[66]

Side population cells and pericytes

In early studies, the heterogeneous cells that express Sca-1 and c-Kit, are located in the muscle fiber space (outside the basement membrane) in skeletal muscle, are referred to as side population cells (SPs). Some of these CD34$^+$/SCA-1$^+$/CD45$^-$ SPs are also named myoendothelial progenitors that could differentiate into adipocytes *in vitro*.[84] Besides, some CD31$^-$/CD45$^-$ SPs also have the potential of adipogenic differentiation *in vitro*.[85] Pericytes expressing NG2, α-SMA, CD146, and PDGFRβ surrounding ECs in capillaries and microvessels can express peroxisome proliferator-activated receptors γ2 (PPARγ2) and form into lipid droplets when cultured in adipogenic induction medium.[86] The mesodermal tissue-derived pluripotent progenitors (mesoangioblasts) expressing Sca-1 and CD34 in skeletal muscle blood vessels may be a subpopulation of pericytes that can also differentiate into adipocytes under appropriate culture conditions.[87]

PW1$^+$/Pax7$^-$ interstitial cells

Mitchell et al. found a subpopulation of MSCs which expressed PW1 but not Pax7 in the muscle stroma in the early postnatal period of mice, called PW1$^+$/Pax7$^-$ interstitial cell (PIC).[88] PIC cells express different level of PW1 and Sca-1 and have different differentiation potential, among which PW1$^+$ cells expressing medium level of Sca-1 had adipogenic potential. Some of PIC cells express PDGFRα, so some PDGFRα$^+$/PW1$^+$ cells may overlap with some FAPs. All the PIC cells expressed NG2, suggesting that these cells may also overlap with muscle pericytes.

Others

In our previous study, we revealed that myeloid-derived cells may be involved in regulating fat infiltration in skeletal muscle by single-cell RNA sequencing.[56] Heterogeneous myeloid-derived cells in muscle tissue were divided into 10 subclusters; some of them express abundant adipose-related genes (*DLK1*, *CD38*, *ZFP423*, and *CD34*) and adipogenesis-regulated genes (*C/EBPα* and *PPARγ*).[56] However, whether there are other sources of IMF needs to be further investigated.

Taken together, numerous kinds of cell types contribute to fat infiltration because skeletal muscle tissues are highly heterogeneous. Specifically, skeletal muscle contains several cell types including myogenic cells (SCs and Myf5$^+$ MSCs) and non-myogenic cells (FAPs, fibroblasts, ECs, pericytes, SPs, PICs, and myeloid-derived cells) (Figure 2). However, FAPs are the main source of IMF, but the mechanism of FAPs committed differentiate into IMF and whether there are other sources of IMF needs to be further investigated.

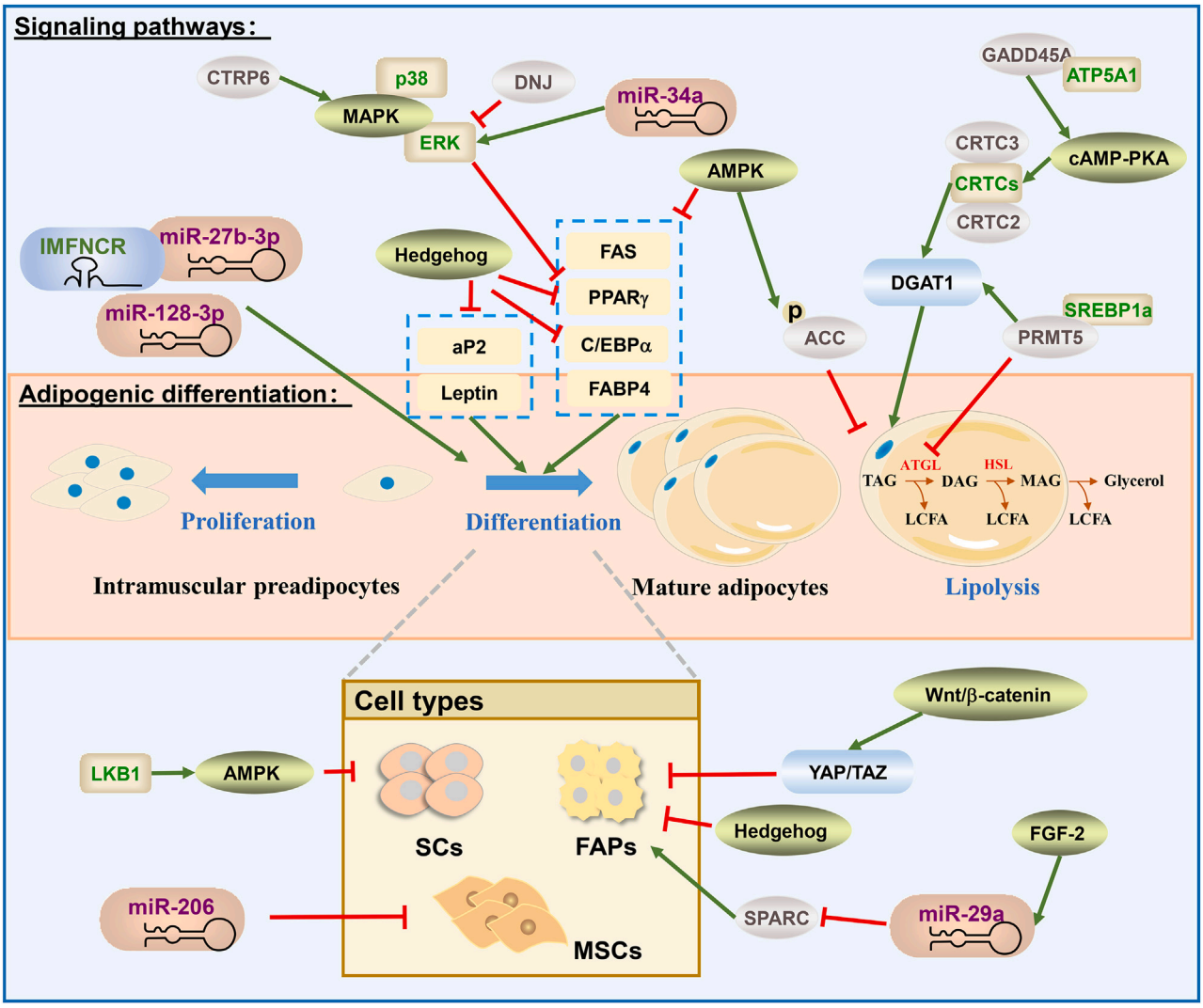

Figure 3. Molecular mechanism of fat infiltration in skeletal muscle

Regulation of IMF deposition involves in many genes and signaling pathways including cAMP-PKA, hedgehog, Wnt/β-catenin, AMPK, MAPK, miRNAs, and lncRNAs. ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; aP2, adipocyte fatty acid-binding protein; ATGL, adipose triglyceride lipase; cAMP-PKA, cAMP-protein kinase A; CASR, calcium-sensing receptor; CREB, cAMP response element-binding protein; CRTCs, CREB-regulated transcription coactivators; CTRP6, C1q/tumor necrosis factor-related protein 6; DGAT1, diacylglycerol acyltransferase 1; DNJ, 1-deoxynojirimycin; ERK, e extracellular signal-regulated kinases; FABP4, fatty acid-binding protein 4; GADD45A, growth arrest and DNA damage-inducible alpha; IMF, intramuscular fat; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; miRNAs, microRNAs; PPARγ, peroxisome proliferator-activated receptors γ; PRMT5, protein arginine methyl transferase 5; SREBP1a, sterol regulatory element-binding transcription factor 1a; TAZ, transcriptional co-activator with PDZ-binding motif; YAP, Yes-associated protein.

FAT INFILTRATION REGULATORY MECHANISM IN SKELETAL MUSCLE

The regulatory mechanism of fat infiltration in skeletal muscle is intricate and involves the regulation of signaling pathways. Genes and regulators regulate fat filtration in skeletal muscle through related signaling pathways in the animal organism (Figure 3).

Lkb1-AMPK

AMP-activated protein kinase (AMPK) exerts a significant effect on regulating metabolism and energy balance in the animals' body. Adiposity is associated with the AMPK signaling pathway in aged skeletal muscle.[89] AMPK regulated the expression of precursor of adipocyte differentiation and maturity-related genes (*Pparγ*, *C/ebpα*, and *Fas*).[90] These studies suggested that AMPK might regulate MSCs and intramuscular preadipocyte differentiation. In skeletal muscle, AMPK promoted lipid oxidation and reduced triglyceride synthesis via acetyl-CoA carboxylase 2 phosphorylation.[91] Liver kinase B1 (Lkb1) is a serine/threonine protein kinase which can be phosphorylated to activate

AMPK activity. Recent studies found Lkb1 affected SCs proliferation and differentiation through the AMPK signaling pathway and regulated lipid metabolism and ectopic fat deposition in muscle progenitor cells and mature muscles.[92] Overall, these observations suggest that the Lkb1-AMPK signaling pathway inhibits intramuscular preadipocyte differentiation in skeletal muscle.

MAPK

Mitogen-activated protein kinase (MAPK) signaling pathway participates in physiological process such as cell proliferation and differentiation and including extracellular signal-regulated kinases 1 and 2 (ERK1/2), ERK5, p38, and C-Jun amino-terminal kinase, this pathway regulates adipogenesis from stem cells to adipocytes. After rotator cuff repair, inhibition of p38 resulted in a reduction of muscle fat infiltration.[93] In porcine intramuscular adipocytes, C1q/tumor necrosis factor-related protein 6 inhibited intramuscular adipocytes proliferation and promoted differentiation by the AdipoR1/MAPK signaling pathway[94] and 1-deoxynojirimycin inhibited lipid accumulation by repressing of the ERK/PPARγ signaling pathway[95] (Figure 3). These results indicate that MAPK regulates adipogenesis from stem cells to intramuscular adipocytes in animal models.

Wnt/β-catenin

The Wnt/β-catenin signaling pathway exerts a vital function on regulating adipocyte differentiation and lipid metabolism. Reggio et al. found the WNT5a/GSK3/β-catenin axis could affect adipogenesis of skeletal muscle FAPs.[96] After muscle injury, inhibition of Wnt10b signaling could activate the adipogenic potential during muscle regeneration.[97] Recently, Fu et al. found WNT7A inhibited adipogenesis of skeletal muscle FAPs and fat infiltration through Wnt-Rho-Yes-associated protein/transcriptional co-activator with PDZ-binding motif signaling axis.[98] Besides, in sarcopenic mice, vibration and β-hydroxy-β-methylbutyrate treatment inhibited IMF accumulation and adipogenic differentiation through the Wnt/β-catenin pathway.[63] These findings demonstrate that Wnt/β-catenin pathway occupies an important position in inhibiting fat deposition in skeletal muscle.

cAMP/PKA

The cAMP-protein kinase A (cAMP-PKA) signaling pathway acts downstream the cAMP response element-binding protein and its transcriptional coactivators (CRTCs) and cAMP-PKA signaling pathway affects lipolysis and lipid metabolism.[99] Overexpression of CRTC2 could increase IMF content and the cross-sectional area of muscle fibers in skeletal muscle.[99] CRTC3 is a member of the CRTC family and significantly affects energy metabolism. Overexpression of CRTC3 increases triglyceride deposition in skeletal muscle by upregulating diacylglycerol acyltransferase 1[100] (Figure 3). Our previous study showed that CRTC3 regulates lipid and energy homeostasis and the adipogenic differentiation of porcine intramuscular adipocytes through activating the calcium pathway.[101] These data suggest that cAMP-PKA signaling pathway could enhance IMF content in skeletal muscle.

Hedgehog pathway

The hedgehog signaling pathway regulates the differentiation fates of precursor cells and influences adipocyte development. Activation of hedgehog downregulated the expression of adipogenic marker genes (*C/ebpα* and *Pparγ*) and mature adipocyte marker proteins (leptin and adipocyte fatty acid-binding protein [aP2]), following inhibited adipogenic differentiation of preadipocyte as well as suppressed adipocytes development.[102] During skeletal muscle regeneration, hedgehog inhibited the transformation of FAPs to adipocytes and suppressed adipogenesis in skeletal muscle.[103] Overall, hedgehog pathway inhibits adipogenesis and the transformation of FAPs to adipocytes in muscle.

miRNAs

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression in skeletal muscle and are associated with aging.[104] Deeply, miRNAs affect lipid accumulation through regulating the expression of adipogenesis-related genes in many animal models. In mice, Michael et al. found miR-206 mimicry *in vivo* limits adipogenic conversion of skeletal muscle MSCs.[13] MiR-34a enhanced fat deposition by regulating PDGFRα to promote the adipogenic differentiation of porcine intramuscular preadipocytes via the ERK signaling pathway.[105] In the aged skeletal muscle, FGF-2 signaling increased miR-29a expression and enhanced miR-29a levels stimulated differentiation of FAPs into adipocytes via the reduction of SPARC, promoting IMAT formation.[106] Taken together, miRNAs could affect IMF deposition in many animal models but different miRNAs have different function. These differences may be due to the cell lines and the dosage used being different.

lncRNAs

In addition to common signaling pathways, more and more long-chain non-coding RNAs (lncRNAs) have been considered to be involved in the regulation of adipogenesis.[107] lncRNAs are a class of non-coding RNAs with a length of over 200 nucleotides and recent studies suggested that lncRNAs function on regulating fat accumulation in skeletal muscle. Zhang et al. discovered an lncRNA, which they named IMF-associated long non-coding RNA, could promote the differentiation of intramuscular adipocyte by sponging miR-128-3p and miR-27b-3p.[108] Recent study has found lncRNAs contribute to fat infiltration by regulating the expression of nuclear receptor subfamily 4 group A member 3.[109] These findings indicate that lncRNAs plays a crucial role in regulating adipogenesis in skeletal muscle through sponging miRNAs.

Others

Previous study has found that adipose triglyceride lipase (ATGL) is associated with lipid droplets biogenesis in skeletal muscle and muscle SCs.[9] Recently, Kim et al. discovered protein arginine methyl transferase 5 methylated and stabilized sterol regulatory element-binding transcription factor 1a to promote lipid droplets biogenesis in myofibers; it also increased the repressive H4R3Me2s modification to suppress ATGL gene expression and lipolysis.[110]

Overall, the molecular and regulatory mechanism of fat infiltration is manifold, complicated, and still challenging; a further studying of interventions combined with these signaling pathways will provide a basis for discovering novel potentially effective therapeutic approaches.

DETECTION METHODS OF FAT INFILTRATION IN SKELETAL MUSCLE

There are many imaging approaches to detect myosteatosis including computed tomography (CT), peripheral quantitative computed tomography (pQCT), MRI, and quantitative ultrasound (QUS).[111] CT is one of the most extensively employed imaging modalities for indirect myosteatosis assessment; it could differentiate SAT and IMAT and allows 3D reconstruction. However, its major disadvantages include high cost and limited access, and it cannot directly measure the location of fat storage or lipid droplets within the muscle. Compared with CT, pQCT has the advantage of lower cost and significantly reduced ionizing radiation emission but it cannot distinguish between IMAT and IMF.[111,112] MRI can be utilized to detect myosteatosis, presenting a distinct advantage over CT devices due to the absence of ionizing radiation; however, a quantitative assessment of muscle density is currently unavailable and it also has high cost and limited access limitations.[111] Besides, QUS is increasingly recognized as an affordable, convenient, and practical device for detecting myosteatosis; it can yield trustworthy measurements of muscle thickness and tissue echogenicity in both appendicular and axial skeletal muscles;[113] however, it also cannot distinguish between IMAT and IMF. IMAT and IMF can be identified using CT and MRI, whereas IMCL necessitates specialized imaging techniques for visualization and quantification, such as magnetic resonance spectroscopy or muscle biopsies. Consequently, an increasing number of potential imaging methods are accessible for detecting fat infiltration in skeletal muscle, yet current challenges persist.

INTERVENTIONS OF FAT INFILTRATION IN SKELETAL MUSCLE

In recent years, many researchers have focused on intervention strategies of fat infiltration in skeletal muscle via randomized controlled trials and animal models.

Exercise

Regular exercise and physical activity can help people to maintain health-promoting physical fitness. For humans, exercise is the key intervention to counteract sarcopenia. The recent study concluded that persistent exercise can prevent muscle fat infiltration and can also improve muscle mass and strength among the elderly.[114] A randomized controlled trial showed that IMAT was significantly reduced in older adults after a 12-week, low-intensity, short-interval, slow-jogging.[115] Similarly, Ryan et al. found that older women could have significantly decreased lipid storages in the spinal and abdominal muscles of the trunk region after an aerobic exercise training.[116] In elderly men with osteosarcopenia, long-term high-intensity resistance training prevented a further increase of muscle fat infiltration of the thigh muscle.[117] Apart from the elderly, exercise also affects fat infiltration in young people. For young girls, a lower physical activity leads to higher IMF content of the calf and thigh.[118] Additionally, more and more studies have been testing whether persistent exercise could decrease fat infiltration in people suffering from different conditions and diseases. Goodpaster et al. found that in older adults having moderate functional impairments, the age-related loss of skeletal muscle strength can be prevented by regular physical activity and increased IMF deposition.[119] Marcus et al. concluded that persistent exercise decreases IMAT contents in skeletal muscle in older patients with a variety of comorbidities.[120] However, Jacobs et al. demonstrated that there is no reduction in thigh IMAT following a three-month exercise intervention in older adults when considering the risk for falling.[121] Hence, the function of exercise on fat deposition in muscle depends on methods of exercise and physical conditions (Figure 4) and whether exercise alone can reduce fat infiltration in patients suffering from different diseases remains to be further explored.

Nutritional interventions

Apart from exercise, in recent years, nutritional interventions are the important strategies to regulate fat deposition in muscle based on clinic trials and many animal models. The main nutrients to regulate IMF deposition are vitamin D, vitamin A and retinoic acid, conjugated linoleic acid (CLA), linseed, amino acid, betaine, and so on (Table 1).

Vitamin D

Vitamin D is a pro-steroid hormone which has been reported as the earliest hormone to arise on earth. The expression of the vitamin D receptor in human skeletal muscle declines with age. Low vitamin D levels are associated with the emergence of many diseases especially myopathies such as sarcopenia, cachexia, type 2 diabetes mellitus, osteoporosis, and cancer, and it also plays a vital role in the immune system that has become a global public health issue.[141] Clinical trials and animal dietary intervention studies have demonstrated that vitamin D treatments exert positive function on muscle mass and the deletion of vitamin D receptor leads to impaired muscle function and sarcopenia.[142,143] However, recent study showed that vitamin D may have adverse effects on muscle health in humans (except athletes)[144]; this systematic review and meta-analysis showed that vitamin D was associated with a significantly longer time spent performing the time up and go test and a significant reduction in maximum knee flexion strength. Many studies have found that vitamin D levels were associated with lipid deposition in

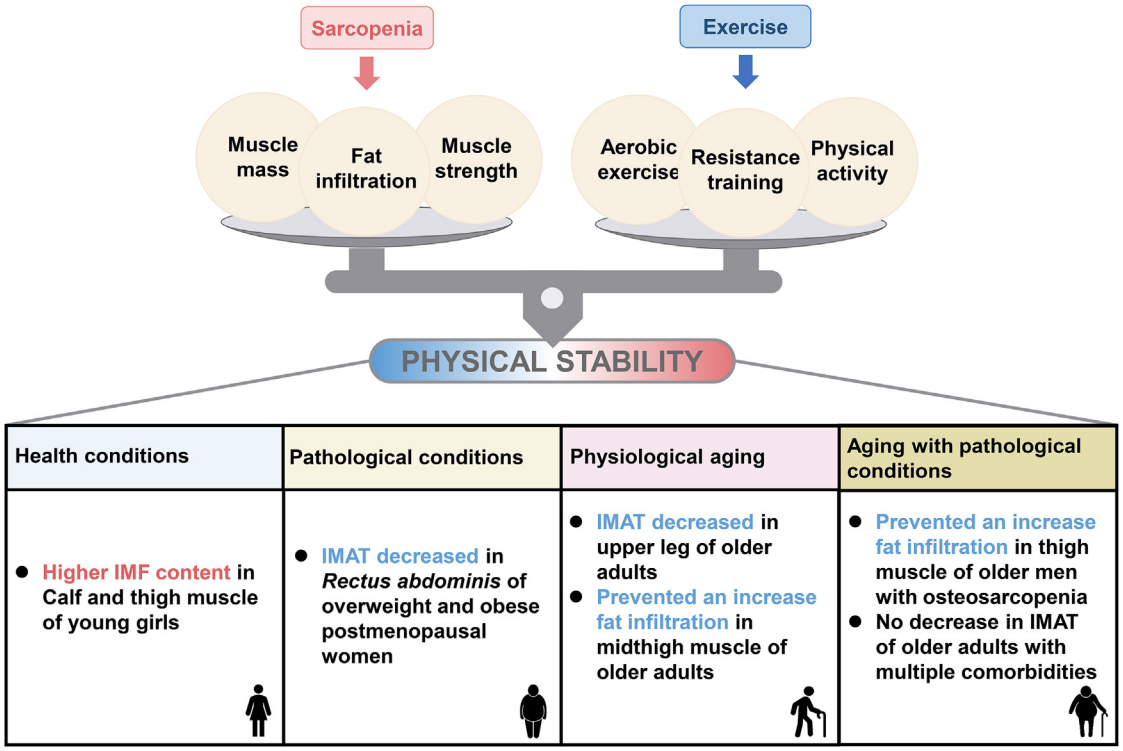

Figure 4. The balance between sarcopenia and exercise under different conditions

The effects of exercise on regulating fat infiltration in skeletal muscle under health, pathological, physiological aging, and aging with pathological conditions.

skeletal muscle for young/older and healthy/unhealthy people. Gilsanz et al. also showed that vitamin D insufficiency was significantly associated with increased muscle fat infiltration in healthy post pubertal females.[145] In older adults, vitamin D status could affect gastrocnemius intramyocellular lipid content independently of body mass and physical activity[146] and in older adults with sarcopenia, fat mass was significantly lower in nutrition supplementation (whey protein, fish oil, and vitamin D) group.[122] In post-stroke convalescent rehabilitation patients, vitamin D supplement decreases lipid accumulation into the thighs muscle.[123] Additionally, reduced limb muscle strength is correlated to increased IMF levels and vitamin D deficiency in some patients with metabolic diseases, such as T2D and impaired glucose tolerance.[147] Hence, the aforementioned findings confirmed that vitamin D can influence IMF deposition in human but the results need to be elaborated further and the regulatory mechanism also needs to be investigated further.

Vitamin A and retinoic acid

Vitamin A (also known as retinol), an essential micronutrient for human health, whose dietary supply is always in the form of retinyl esters, originates from animal sources. Vitamin A has important influences on regulating the metabolism and homeostasis of glucose and lipid in skeletal muscle. An increasing number of studies have shown that vitamin A inhibits adipocyte differentiation and restrictive vitamin A intake could increase IMF deposition in skeletal muscle in many animal models.[124] Retinoic acid, a metabolite of vitamin A, regulates the roles of vitamin A that are required in growth and development. Retinoic acid plays increasingly important roles in adipogenesis and terminal maturation of adipocytes. In skeletal muscle, retinoic acid can restrict myoblast development and regulate the immature state of human skeletal muscle progenitor cells *in vitro*.[148] In conclusion, vitamin A and retinoic acid play major roles in regulating muscle fat infiltration. However, the functions of vitamin A on skeletal muscle development and regeneration especially the effects of retinoic acid on IMF content in human are rarely reported.

CLA

CLA is a class of positional and geometric isomers of linoleic acid with a conjugated double bond which is generally found in ruminant animals and dairy products. CLA has anti-adipogenic, anti-cancer, anti-diabetic, and anti-hypertension effects. For Chinese adults with elevated body fat percentage, 3.2 g/day CLA supplementation significantly increased trunk muscle mass.[125] For student athletes, after administering 0.9 g/day CLA intake, body weight variation significantly increased, amount of body fat percentage variation tended to decrease, and muscle mass increased.[126] Pinkoski et al. found 5 g/day CLA combined with resistance training could increase lean tissue mass and decrease fat mass in participants.[127] Liquid chromatography-mass spectrometry metabolomics showed CLA altered 57 metabolites enriched in lipids/lipid-like molecules including glycerophospholipids, fatty acyls, and sphingolipids in plasma.[149] For old adults, 5 g/d creatine monohydrate + 6 g/d CLA for 6 months following resistance exercise training improved muscular endurance, isokinetic knee extension

Table 1. Nutritional interventions of fat infiltration in skeletal muscle

Means[a]	Subjects/Animals	Measures	Duration	Effects	Muscle	Reference
Vitamin D	Sarcopenic elderly	Whey protein, fish oil, vitamin D	12 weeks	Fat mass was lower	/	Li et al. [122]
	Subacute post-stroke rehabilitation patients	Whey protein and vitamin D	16 weeks	Fat infiltration was lower	Thigh muscle	Honaga et al. [123]
Vitamin A	Iberian pigs	Vitamin A restriction	At early growing and at finishing	Increased the preadipocyte number	Longissimus thoracis muscle	Ayuso et al. [124]
CLA	Chinese adults	3.2 g/d CLA supplementation	12 weeks	Increased muscle mass	Trunk muscle	Chang et al. [125]
	Student athletes	0.9 g/day intake	14 days	Increased muscle mass	/	Terasawa et al.[126]
	Female and male participants	5 g/d CLA	7 weeks	Increased in lean tissue mass and greater losses of fat mass	Lean tissue	Pinkoski et al. [127]
	Older adults	5 g/d creatine monohydrate + 6 g/d CLA	6 mouths	Improved muscular endurance and lower fat mass	/	Tarnopolsky et al.[128]
	Sedentary older adults	4000 mg/d CLA	8 weeks	No significant muscle anabolic effects	Vastus lateralis	van Vliet et al.[129]
	Mice	0.7 g/kg or 0.5% CLA with endurance training	6 weeks	induced a fiber-type-specific hypertrophy	Plantaris muscle	Barone et al. [130]
	Pigs	1% CLA	35 days	Increased IMF content	LDM	Wang et al. [131]
Linseed	Patients with non-alcoholic fatty liver disease	20 g/d flaxseed oil	12 weeks	reduced fat mass	/	Rezaei et al. [132]
	Dystrophic hamsters	30% flaxseed	/	Promote regeneration of injured skeletal muscle	Biceps femoris muscles	Carotenuto et al.[133]
	Barrows	10% linseed	90 days	IMF content increased	Longissimus muscle	Luo et al. [134]
Herbal extract ALS-L1023	Obese rats	0.4% or 0.8% (w/w) of ALS-L1023	4 weeks	Reduced fat deposition	Gastrocnemius muscle	Shin et al.[135]
Chronic sugar	C57BL/6J male mice	15% fructose or 15% glucose	7 months	Intramyocellular lipids accumulated fed with fructose	Gastrocnemius muscle	De Stefanis et al.[136]
Betaine	*In vitro* (C2C12 cells)	10 mM betaine	24 and 48 h	Elevated lipid accumulation	/	Wu et al.[137]
EGCG	Mice	100 mg/kg EGCG	14 days	Decreased IMF deposition	Tibialis anterior	You et al.[67]
Gut microbiota	Patients with metabolic syndrome and C57BL/6J mice	*Porphyromonas gingivalis*	6 weeks	Increased fat infiltration	Lumbar muscle	Watanabe et al.[138]
	SMAP8 mice	*Lactobacillus casei* Shirota	12 weeks	Delayed age-related muscle mass deposition	Gastrocnemius muscle	Chen et al.[139]
	SPF C57BL/6J mice	bacterial consortium	4 weeks	Increased IMF content	Quadricep muscle	Xie et al.[140]

[a]CLA, conjugated linoleic acid; EGCG, epigallocatechin-3-gallate; FAPs, fibro/adipogenic progenitors; HFD, high-fat diet; IMAT, intermuscular adipose tissue; IMCL, intramyocellular lipid; IMF, intramuscular fat; LDM, longissimus dorsi muscle.

strength, and had lower fat mass.[128] However, van Vliet S et al. discovered CLA supplementation does not have muscle anabolic effects in sedentary older adults.[129] Hence, the specific effects of CLA on muscle function are still controversial. In mice, CLA has been identified to prevent sarcopenia by maintaining redox balance during aging, positively regulate mitochondrial adaptation, improve muscle metabolism, and induce hypertrophy in type IIx muscle fibers after endurance exercise.[130,150] Besides, in porcine models, our previous studies have demonstrated that adding CLA into the diet could improve IMF contents in *longissimus dorsi* muscle of lean pig breeds and Chinese indigenous pig breeds.[131,151] However, CLA supplementation positively affects IMF content in porcine models, but the effects on fat infiltration and deposition in human skeletal muscle as well as in rodents need to be further explored.

Linseed

Linseed is the ripe seed of flax which is rich in n-3 PUFAs and has many physiological functions including anti-adipogenic, anti-inflammatory, and anti-cancerous while regulating the metabolism and homeostasis of glucose and lipid metabolism. Previous studies have found that patients with non-alcoholic fatty liver disease after 20 g/d flaxseed oil had significantly reduced fat mass, after 20 g/d sunflower oil had significantly reduced muscle mass.[132] However, Babajafari et al. found that there is no significant difference in triglyceride and cholesterol content in serum between patients who received isolated soy protein with or without flaxseed oil.[152] Like CLA, the function of flaxseed oil on fat deposition in muscle is still unknown and the effects of fat infiltration in human skeletal muscle also need to be studied in the future. In animal models, linseed could promote regeneration of injured skeletal muscle *in vivo* and *in vitro* in dystrophic hamsters.[133] In porcine models, linseed, flax, or flaxseed oil affect fatty acid composition, gene expression in skeletal muscle, and sensory quality through affecting the expression of adipogenesis-related genes (PPARγ and aP2).[134,153] In a word, based on these studies, linseed and linseed oil are considered to affect IMF content but their specific function needs to be further elucidated.

Plant extract

After treatment with the herbal extract ALS-L1023, which was isolated from *Melissa officinalis*, the reduction of fat deposition was observed in the skeletal muscle of obese rats.[135] In addition, Wu et al. described that 10 mM betaine treatment promoted lipid accumulation and regulated lipid metabolism in C2C12 cells through activating the ERK/PPAR signaling pathway.[137] Recently, You et al. found dietary factor epigallocatechin-3-gallate protects against fat infiltration via repressing GADD45A expression in muscle of mice.[67] These data suggest that plant extract could regulate fat deposition in skeletal muscle in mice and *in vitro*.

Muscle-adipose crosstalk

Skeletal muscle and adipose tissue both can serve as secretory organs that act in an endocrine, paracrine, or autocrine manner to release myokines and adipokines to facilitate tissue-to-tissue communication, work together to improve overall metabolic health, and regulate IMF deposition in muscles.[154,155]

Myokines

Myostatin is one of the best-characterized myokines that negatively regulates skeletal muscle growth and development.[156] In animal models, myostatin mutation leads to higher lean mass, reduced body fat content, and lower IMF in *M. longissimus* and *M. quadriceps*.[157] Leukemia inhibitory factor transplantation could reduce the number of FAPs and inhibit fibrogenesis of muscle cells, further diminishing pathology through transforming growth factor β signaling. Besides, fibroblast growth factor 21 significantly inhibits the differentiation of porcine intramuscular preadipocytes and goat intramuscular preadipocytes.[158,159] Myonectin has shown beneficial effects on systemic lipid homeostasis;[160] however, Petro et al. have found serum levels of myonectin are lower in adults with metabolic syndrome and serum myonectin was negatively correlated with the android/gynoid fat mass ratio but not with the IMF content.[161]

Adipokines and lipokines

Apart from lipid storage and metabolism, adipose tissue serves as an endocrine organ and secretes regulatory factors such as adipokines and lipokines, which can regulate lipid metabolism in skeletal muscle.[3] In goat intramuscular preadipocytes, knockdown of adiponectin promoted goat IMF deposition[162] and FGF10 promoted the adipogenesis during adipogenic differentiation of intramuscular preadipocyte.[163] Another adipokine, chemerin, could promote lipolysis and induce adipogenesis during preadipocyte differentiation in bovine intramuscular adipocytes.[164] Lipokines are described as important fatty acid-derived products that emanate from adipose tissue. Palmitoleic acid is an adipose tissue-derived lipokine and is catalyzed by stearoyl-CoA desaturase-1,[165] which is reported to reduce intramuscular lipid and restores insulin sensitivity in obese sheep.[166]

Overall, these data confirmed the association between adipose tissue and skeletal muscle through myokines and adipokines, which also regulate fat infiltration in skeletal muscle, but the underlying mechanisms remain to be elucidated.

Gut microbiota

Gut microbiota play important roles in skeletal muscle metabolism and function. Gut microbiota are associated with muscle mass, muscle function, physical performance, and muscle fat infiltration in animal models and humans.[167] In patients with metabolic syndrome, the anti-*Porphyromonas gingivalis* (Pg) IgG antibody titers positively correlated with intramuscular adipose tissue content and Pg

administration increased fat infiltration in murine skeletal muscle.[138] In the SAMP8 mice, *Lactobacillus casei* Shirota delayed the appearance of senescence and age-related muscle mass deposition.[139] Compared with control mice, inoculating the bacterial consortium into mice increased IMF content in SPF C57BL/6J mice.[140] Deeply, alteration of gut microbiota may lead to skeletal muscle atrophy via a bile acid-farnesoid X receptor pathway.[168] Furthermore, PPARs may regulate fat deposition in skeletal muscle along the gut-muscle axis in both health and disease.[169] Besides, a systematic review has shown that potential mechanisms of microbiome modulating muscle mainly include lipid and glucose metabolism and mitochondrial function.[170] Short-chain fatty acids (SCFAs) have been effective in enhancing muscle mass and host function,[171] and increase fatty acid uptake and oxidation while preventing lipid accumulation in skeletal muscle.[172] In children, total body fat content mediated the associations of gut microbiota and SCFAs with skeletal muscle quality.[173] These evidences showed gut microbiota are tightly related to lipid metabolism in skeletal muscle and could regulate the lipid deposition in the muscle of pigs and mice. However, little studies have targeted the human microbiome associated with fat infiltration in skeletal muscle.

CONCLUDING REMARKS

Summarizing the previously presented data, we concluded the triggering factors such as aging, diseases, muscle injury, etc. and we detailed the cellular origin of muscle fat infiltration is at the beginning of being understood. We also discussed regulatory mechanisms, detection methods, and interventions related to fat infiltration in skeletal muscle, including exercise, several nutritional interventions, muscle-adipose crosstalk, and gut microbiota. Full understanding of affected factors and the cellular origins of fat infiltration may prove useful for the building up of skeletal muscle, for regeneration of injured muscle, and for ultimately improving human health. Regulating genes in farmed animals, persistent exercise, and several nutritional interventions may be a great and safe way to alleviate fat infiltration in skeletal muscle and to treat muscle-related diseases. Besides, we could apply these strategies to increase the IMF content in skeletal muscle and to improve meat quality in animal production. However, there are still some questions that need to be further addressed: (1) In addition to the aforementioned cell types, we need to find out whether other cell types are involved in fat infiltration and how the cellular origins of fat infiltration could be better investigated and understood. (2) Influential triggering factors affect fat infiltration in human and animal models, but how these factors are interconnected and interact through molecular mechanisms need to be further studied. (3) The interactions between key genes, regulators, and signaling pathways regulating fat infiltration in skeletal muscle need to be further explored. (4) The effects of some important nutrients, such as CLA, linseed, and vitamin A, on fat infiltration have been mainly studied in animal models or *in vitro* studies so far, but their effects on fat infiltration in skeletal muscle in humans or specifically in individuals with skeletal muscle-related diseases are largely unknown. (5) How to apply exercise and nutrients safely and efficiently to alleviate fat infiltration in skeletal muscle, and treat muscle-related diseases in humans need to be further studied. In a word, our current contribution summarized and discussed cellular origins, influential triggering factors, regulatory mechanisms, detection methods, and intervention strategies relating to fat infiltration in skeletal muscle and we provided useful information for regulating function and lipid metabolism of skeletal muscle, further treating muscle-related diseases by using nutritional interventions.

ACKNOWLEDGMENTS

This work was partially supported by the Natural Science Foundation of Zhejiang Province (LZ22C170003) and the "Hundred Talents Program" funding from Zhejiang University to T.S. and we thank the members of the Shan laboratory for their comments.

AUTHOR CONTRIBUTIONS

L.W.: conceptualization, methodology, investigation, and writing – Ooiginal draft preparation. T.G.V.: writing – reviewing and editing. T.S.: conceptualization, funding acquisition, supervision, validation, and writing – reviewing and editing.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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