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Myosteatosis: diagnosis, pathophysiology and consequences in metabolic dysfunction-associated steatotic liver disease

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ABSTRACT

Metabolic dysfunction-associated steatotic liver disease (MASLD) sets an increased risk of multisystemic complications, including muscle changes with sarcopenia and more recently myosteatosis that could reciprocally affect liver function. We conducted a systematic review to highlight innovative assessment tools, pathophysiological mechanisms and metabolic consequences related to myosteatosis in MASLD, based on original articles screened from PUBMED, EMBASE and COCHRANE databases. Forty-six original manuscripts (14 pre-clinical and 32 clinical studies) were included. Microscopy (8/14) and tissue lipid extraction (8/14) are the two main assessment techniques used for muscle lipid content in pre-clinical studies. In clinical studies, imaging is the most used assessment tool and included computed tomography (14/32), magnetic resonance imaging (12/32) and ultrasound (4/32). Assessed muscles varied across studies but mainly included paravertebral (4/14 in pre-clinical; 13/32 in clinical studies) and lower limbs muscles (10/14 in preclinical; 13/32 in clinical studies). Myosteatosis is highly prevalent already in non-cirrhotic stages of MASLD and correlates with disease activity when using muscle density assessed by computed tomography. Numerous pathophysiological mechanisms were found and included: high-fat and high-fructose diet, dysregulation in fatty acids transport and ketogenesis, endocrine disorders and impaired microRNA122 pathway. This review also evidenced several potential consequences related to myosteatosis in MASLD such as insulin resistance, MASLD progression from steatosis to metabolic steatohepatitis and loss of muscle strength. In conclusion, several data on myosteatosis in MASLD are already available. Myosteatosis could appear as a muscle change highly relevant to screen considering its correlation with MASLD activity as well as its related consequences. Recent innovative imaging techniques allow to accurately assess myosteatosis.

Keypoints

- This systematic review highlights the high prevalence of myosteatosis even in non-cirrhotic stages of MASLD.
- Magnetic resonance imaging and spectroscopy are by far the most accurate assessment tool to characterize muscle phenotype.
- Pathophysiological mechanisms included high-fat and high-fructose diet, dysregulation in fatty acids transport and ketogenesis, mitochondrial dysfunction, endocrine disorders and impaired microRNA122 pathway.
- Potential consequences related to myosteatosis are insulin resistance, MASLD progression from steatosis to steatohepatitis and loss of muscle strength.
- Some pre-clinical and clinical data were conflicting and further large sample studies are required to investigate the muscle-liver-adipose tissue axis.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common liver disease worldwide with currently no approved medical treatment. This entity includes hepatic steatosis, defined as a hepatic triglyceride content exceeding 5% of the total liver weight [1], metabolic dysfunction-associated steatohepatitis (MASH), fibrosis and eventually cirrhosis and hepatocellular carcinoma [2] in a metabolic context (overweight, metabolic syndrome, type 2 diabetes) [3]. MASLD prevalence is highly correlated to overweight, insulin resistance (IR) and abdominal obesity [4]. Indeed, MASLD concerns approximately 70% of overweighted adults worldwide [5] and up to 90% in case of morbidly obesity [6]. However, it currently remains difficult to identify MASLD subjects at risk of progressing from isolated steatosis to MASH.

Liver steatosis results from several pathophysiological mechanisms including excessive dietary fatty acids intake [7], increased adipose tissue-related lipolysis [8], lipogenesis [9], altered mitochondrial fatty acid oxidation [10] or even decreased hepatic lipid secretion [8].

Skeletal muscle changes associated with MASLD correlate with all-cause mortality [11]. Those muscle changes include two main entities: sarcopenia and myosteatosis. Sarcopenia is defined by a loss of muscle mass and function while myosteatosis is defined by an excessive muscle lipid content [12,13]. Myosteatosis is frequent in cirrhosis and hepatic encephalopathy [14]. While pathophysiological mechanisms linking hepatic encephalopathy and sarcopenia have been deeply investigated [15], the role of ammonia in myosteatosis pathogenesis is unknown. One hypothesis is the decrease in ammonia detoxification by glutamine synthase in fatty infiltrated skeletal muscles. However, decreasing muscle lipid content in a mouse model of MASH did not improve ammonia metabolism [16]. Therefore, the relationship between ammonia and myosteatosis will not be further discussed in this review considering the current lack of data.

Myosteatosis is also associated with MASLD at earlier disease stages [12,17–19]. It has even been proposed as a key driver in MASLD pathogenesis [20]. However, myosteatosis is a highly

unspecific muscle change seen in physiological (athletes, ageing) and pathological conditions (cachexia, neuromuscular degenerative diseases) [21–24]. Intramyocellular lipid content (IMLC) is also gender-dependent and is physiologically higher in females [25]. Muscle fat increases in athletes due to increased oxidative capacities [21]. Indeed, chronic endurance training increases lipid storage in highly-insulin sensitive muscle cells as energy fuel for oxidative metabolism [21]. This observation is referred as the “athlete’s paradox” considering that myosteatosis is, in contrast, correlated to decreased oxidative capacities in insulin-resistant obese subjects [21,26].

Fat may accumulate outside (intermuscular fat) or inside muscle fascia (intramuscular fat). Intramuscular fat accumulates in the interstitium (extramyocellular) or in myocytes (intramyocellular) [27] (figure 1). It affects preferably some muscles according to their oxidative capacities. IMLC is reported to be higher in oxidative muscles and to be a ready source of substrate for β-oxidation (βOX) [28].This observation is debated [29] as previous studies reported an increased IMLC in glycolytic muscles due to re-esterification of free fatty acids (FFA) as a consequence of decreased mitochondrial oxidative phosphorylation [30].

Myosteatosis has a negative impact on muscle function hypothetically related to lipotoxicity, limited neuromuscular activation, impaired muscle blood flow and increased local inflammation [22,31]. Mechanically, fat infiltration reduces the pennation angle defined by the wrong alignment of muscle fibres and the muscle-force axis [32].

The pathophysiology of myosteatosis in MASLD as well as its related consequences remain unclear. Therefore, we propose here a systematic review on myosteatosis in MASLD with a focus on diagnosis, pathophysiology and consequences related to its presence in MASLD.

2. Criteria and analysis for the systematic review

We combined PUBMED, EMBASE, COCHRANE databases from their inception up to December 31, 2022. The search terms were based on subject terms related to myosteatosis and MASLD. However, MASLD is a definition that emerged from a recent nomenclature

consensus [33]. Search terms used its previous nomenclature, namely nonalcoholic fatty liver disease (NAFLD) and metabolic dysfunction-associated fatty liver disease (MAFLD). Those terms included “NAFLD and muscle fat”, “NAFLD or muscle fat”, “NAFLD and myosteatosis”, “NAFLD or myosteatosis”, “MAFLD and muscle fat”, “MAFLD or muscle fat”, “MAFLD and myosteatosis”, “MAFLD or myosteatosis”.

We did not restrict the search by region, language or period of time. We only included original manuscripts for this systematic review and excluded narrative and systematic reviews, as well as editorials and supplements.

The search of the computerized database turned up a total of 1323 articles (478 on PUBMED, 779 on EMBASE and 66 on COCHRANE). Among these manuscripts, 518 were removed before screening as they were reviews, editorials or abstracts (Figure 2). A total of 805 original manuscripts were retained and screened for inclusion. 694 of these original manuscripts were secondarily excluded as they were considered irrelevant or with the full online version unavailable. Original manuscripts were considered irrelevant if skeletal muscle fat content in MASLD subjects was not specifically assessed or assessed in non-skeletal muscles (e.g myocardium). Hence, 111 entry were retained but included 65 duplicates. In total, 46 original manuscripts were included for this systematic review including 14 pre-clinical and 32 clinical studies. The details of the inclusion process are summarized in the flow chart (Figure 2). The details of included manuscripts are summarized in Table 1 for pre-clinical studies and Table 2 for clinical studies.

In pre-clinical studies, two main MASLD mouse models were used: transgenic mice on a C57BL/6J background (8/14) and *foz/foz* mice (2/14). Skeletal muscle lipid content was mainly assessed by microscopy (9/14), tissue lipid extraction (8/14), computed tomography (3/14) and magnetic resonance imaging (2/14). In clinical studies, muscle lipid content was assessed by computed tomography (CT - 14/32), magnetic resonance imaging (MRI – 12/32), ultrasound (US – 4/32) and microscopy (4/32). Muscle biopsy was only performed in 3 studies for histology (2/32) and tissue lipid extraction (1/32). Liver biopsy was performed in 12 studies.

Paravertebral (4/14 in pre-clinical and 13/32 in clinical studies) and lower limbs muscles (10/14 in preclinical and 13/32 in clinical studies) were most frequently assessed.

Here we summarize the results of this systematic review by main research questions as followed: the epidemiology of myosteatosis in MASLD, assessment techniques for myosteatosis, its pathophysiology and related consequences. We deliberately do not address the possible treatment of myosteatosis, which is beyond the scope of this work.

3. Myosteatosis prevalence in MASLD: way more frequent than sarcopenia

The prevalence of myosteatosis according to MASLD severity remains poorly known considering the lack of histological assessment of the liver disease. The prevalence of myosteatosis also remains a subject of debate due to the questionable techniques used (see further). In real-life cohorts of MASLD subjects with no liver histology available, myosteatosis is reported as highly prevalent (27.6%) compared to sarcopenia (1.6%). Myosteatosis and sarcopenia even coexists (14%) [34]. Myosteatosis is also reported in pre-clinical and clinical studies with liver histology as an early muscle change linked with disease severity in non-cirrhotic stages [35,36]. However, myosteatosis is also more frequent compared to sarcopenia (83% vs 30%) in cirrhotic MASH subjects waiting for liver transplantation [37].

4. How to assess myosteatosis?

Two types of techniques are used for muscle lipid content assessment: imaging and histology.

4.1. Imaging techniques

The diagnosis is routinely based on imaging. Three imaging techniques have been used to assess myosteatosis: CT, MRI and US (Supplementary table 1).

CT is the most used imaging modality in the topic and highlights muscle fat accumulation though a lower mean muscle radiodensity expressed in Hounsfield units (HU) [18,38]. While HU cut-off values are available from cancer subjects' population as a mortality predictor [39], validated cut-offs are not clearly established for subjects with chronic liver diseases or

cirrhosis, due to an obvious lack of systematic data. A cut-off of 43.14 HU for psoas muscle radiodensity at the level of the fourth to fifth lumbar vertebra was evidenced as a good predictor of 12-months mortality in subjects with cirrhosis [40]. However, it was not the case for predicting post-liver transplant mortality in another study [41]. A decrease in skeletal muscle radiodensity below 41 HU in normal weight subjects (BMI up to 24.9 kg/m²) and 33 HU in overweight subjects (BMI ≥ 25 kg/m²) has been proposed for listed subjects for liver transplant as a criteria for myosteatosis, associated with higher complications rate [42]. Hence, CT only indirectly evaluates muscle fat content by its relative impact on muscle density and does not allow to discriminate its microscopic location (Supplementary table 1).

MRI offers many advantages though qualitative and quantitative sequences to assess muscle phenotype. Quantitative MRI sequences directly measure the relative fat content expressed in percentages by proton density fat fraction (PDFF) on T1-weighted images by suppressing the signal of water [43]. Furthermore, proton magnetic resonance spectroscopy (MRS) allows to measure the IMLC and the extramyocellular lipid content (EMLC) based on their differences in resonant frequencies after excitation of hydrogen nuclei [9,24]. Proton MRS also allows to perform a relative and an absolute quantitation of muscle lipid content respectively expressed in percentages and mass unit (mmol/kg) [44]. Hence, the main advantage of MRI compared to other imaging techniques (US or CT) is spectroscopy as well as the absence of radiation, even if the radiation level of contrast-free CT is very low [18]. Therefore, proton MRS is the first-choice technique for myosteatosis assessment considering that it non-invasively provides both quantitative measures and localization information on muscle lipid content (S

upplementary table 1).

US is a non-radioactive imaging technique semi-quantitatively measuring muscle fat infiltration based on echo intensity variations. In case of myosteatosis, the echo intensity or brightness of skeletal muscle increases compared to normal muscles and is positively correlated to muscle lipid content [45]. However, despite a good correlation between echo intensity and MRI PDFF, US is examiner-dependent, only correlates with EMLC assessed by proton MRS, and provides

inaccurate semi-quantitative measures of myosteatosis like CT [23,45,46] (Supplementary table 1).

4.2. Microscopical assessment of muscle lipid content

Although skeletal muscle biopsy is rarely performed in routine practice, innovative microscopy techniques have been used to assess skeletal muscle lipid content. The most used histological staining for muscle in optical microscopy is haematoxylin and eosin. Haematoxylin and eosin allows to measure muscle fibres diameter and adipocytes easily while immunofluorescence allows to differentiate muscle fibres types [47]. Laser-based nonlinear microscopy offers molecular-specific three-dimensional imaging of potentially large fresh samples. Two nonlinear microscopy techniques have been used to assess lipid content and fibrosis in both liver and skeletal muscles in a mouse model of MASLD : second harmonic generation microscopy for the study of structural proteins such as collagen, and coherent anti-Strokes Raman scattering microscopy for the study of lipids [48]. The correlation between these microscopical techniques and imaging for measuring muscle lipid content has never been studied. As reported in Table 2, muscle lipid content was histologically assessed on rectus abdominis and deltoid in morbidly obese subjects using haematoxylin and eosin staining only in 2 clinical studies [49,50]. An increase in both EMLC and IMLC is reported in that population [49,50].

4.3. Potential biomarkers of muscle damage associated to myosteatosis in MASLD subjects

Biomarkers of skeletal muscle damages such as creatin kinase assess the severity of muscle destruction or rhabdomyolysis [51]. Creatin kinase serum level correlates with lean muscle mass and physiologically decreases with ageing [52]. However, creatin kinase serum level is not specific to skeletal muscle and there is currently no data on the impact of myosteatosis on its serum level in MASLD. Two other biomarkers of muscle damage and body composition are associated to muscle fat content in MASLD in two studies requiring confirmation in larger series [53,54].

The first is titin-N fragment urinary level. Titin is a key structural protein of the sarcomere. The concentration in urine of titin N-terminal fragment is a specific biomarker of muscle damage. A positive correlation between myosteatosis assessed by echo intensity of the rectus femoris and urinary levels of titin-N fragment has been reported [53]. Interestingly, its urinary level was also correlated to forearm muscles strength [53]. The second one is alpha-foetoprotein (AFP) serum level. AFP is a commonly used tumour marker for diagnosis and follow-up of hepatocellular carcinoma and germline cell tumours. Surprisingly, AFP serum level is related to body composition in healthy subjects [54]. Healthy subjects with elevated AFP serum levels have lower liver and muscle fat contents assessed by CT compared to age and sex-matched controls with normal AFP serum levels [54]. Mean psoas density remained an independent factor for elevated AFP serum level even after adjustment for liver steatosis. However, there is no correlation between muscle density and AFP serum level [54].

5. Pathophysiology of myosteatosis in MASLD

Excessive lipid storage in skeletal muscle is mediated by several pathophysiological mechanisms in MASLD. As an excessive lipid storage occurs in hepatocytes in MASLD, the parallel can be established with muscle steatosis characterized by a disbalance between three main physiological pathways: lipid intake, oxidation and export. Only a few data concerning that specific topic have currently been published and almost exclusively in end-stage liver disease [55]. Potentially involved mechanisms are summarized below (Figure 3).

5.1. Fat and fructose enriched diets increase liver and muscle lipid contents

The same way diet causes steatosis, consumption of a high-fructose (HFrD) or a high-fat diet (HFD) is associated with myosteatosis [56–63]. According to tracer experiments, the most important source of lipid in the steatotic liver is the insulin-resistant adipose tissue [64]. It is therefore logical to imagine that free fatty acids from adipose tissue contribute also to myosteatosis.

Fructose has been incriminated in the growing pandemic of MASLD worldwide considering its frequent use in the food industry notably in the form of high-fructose corn syrup and its well-known toxicity [63]. Indeed, fructose is a substrate for de novo hepatic lipogenesis though fructolysis in glyceraldehyde-3-phosphate eventually increasing circulating FFA. Furthermore, HFrD induces a drop in the ATP/AMP ratio by consumption of inorganic phosphate by the increased cellular respiration flux increasing the risk for MASLD development or progression [56]. Fructose has also a direct mitochondrial toxicity by generating reactive oxygen species damaging mitochondrial DNA and impairing mitochondrial biogenesis [57]. This oxidative stress-mediated mitochondrial toxicity was reported in hepatocytes but also in myocytes [57,58]. Finally, a decreased lipolysis in liver secondary to a HFrD was also highlighted [59]. Hence, HFrD increases liver lipid content by de novo lipogenesis as well as excessive lipid storage by reducing lipolysis, mitochondrial content and fatty acids βOX in both liver and skeletal muscle.

HFrD induced more liver inflammatory degeneration and hence further promoted MASH compared to HFD which induced more liver steatosis [60]. This pro-inflammatory role of fructose was reinforced by a reported higher IL-1α muscle level induced by HFrD compared to HFD [61]. Both HFrD and HFD promote insulin resistance in liver and skeletal muscle by impairing insulin signalling pathway. However, previous data tend to demonstrate a deeper impact of HFD on skeletal muscle insulin sensitivity promoting the accumulation of other energetic substrates such as lipids [60,61].

Furthermore, the impact of HFD on muscle lipid content but also on skeletal muscle insulin sensitivity is reported as highly dependent on the subtype of dietary lipid intake. Indeed, a high intake of saturated fat worsens IR while long-chain n-3 fatty acids enhances insulin sensitivity by promoting muscle mitochondrial oxidative capacities [62].

5.2. Dysregulation in intramyocellular fatty acids transport

The regulation of intramyocellular fat content is highly similar to that of muscle glucose content. Indeed, IMLC are increased by insulin though the PI3K-PKB-Akt signalling pathway and by muscle contraction though increasing adenosine monophosphate-activated protein kinase (AMPK) cytosolic level [65]. It results in increased translocation of fatty acid transporters such as fatty acid translocase/CD36 and fatty acid transport protein [65]. Previous studies reported increased expression of CD36 and fatty acid transport protein in skeletal muscle of obese and type 2 diabetes subjects arguing in favour of an excessive fatty acid uptake promoting myosteatosis in addition of excessive diet intake [66].

5.3. Dysregulation of myocytes mitochondria

When switching to a HFD, skeletal muscle rapidly increases lipid uptake to reduce serum level of FFA [65]. As a response, mitochondrial βOX increases preferably in oxidative muscle [30]. However, mitochondrial capacity is limited, especially in glycolytic muscles due to lower mitochondrial oxidative phosphorylation compared to oxidative fibres [30]. Glycolytic muscles are therefore at higher risk of myosteatosis compared to oxidative muscles [30]. Unfortunately, when excessive IMLC is chronic, myocyte-related mitochondrial lipid oxidation capacity decreases, eventually amplifying myosteatosis [67]. Expressions of key-regulators of lipid oxidation (peroxisome proliferator-activated receptor gamma coactivator 1α and carnitine palmitoyltransferase 1) are also decreased in juvenile iberian pigs fed with high-fructose, high fat diet resulting in an increased muscle lipid content [61]. Conversely, promoting βOX in skeletal muscle of mice decreases the impact of HFD on muscle lipid content. Indeed, downregulating the acyl-CoA thioestherase 2, a mitochondrial enzyme decreasing the availability of βOX substrates by decreasing the hydrolysis of long-chain fatty acyl-CoA in skeletal muscle, protects against HFD-induced myosteatosis [68].

However, the pathophysiological role of mitochondrial dysfunction in myosteatosis remains uncertain due to contradictory data. Indeed, recent studies do not associate IMLC with

mitochondrial oxidative capacity. Mitochondrial function of skeletal muscle of MASLD subjects was indirectly studied and compared to that of healthy subjects by phosphorus MRS assessing mitochondrial ATP production [69,70]. In parallel, IMLC in lower limb muscles was assessed by proton MRS [69,70]. MASLD subjects showed a higher level of liver steatosis but no difference in terms of mitochondrial function as assessed by ATP measured by phosphorus MRS and intramyocellular lipid content evaluated by proton MRS [69,70].

5.4. Impaired ketogenesis

Impaired ketogenesis is reported as associated to increased hepatic steatosis in mice [71] while inducing ketogenesis reduces its content in humans [72]. Impaired ketogenesis might then play a role in MASLD pathophysiology but also in IR. However, it remains currently unclear due to conflicting data whether hepatic ketogenesis in MASLD is impaired or not [73]. Interestingly, the administration of β-hydroxybutyrate induces in vitro a dose-dependent decrease in mitochondrial respiration of human myocytes assessed by real-time respirometry [73]. This observation highlighted a potential link between hepatic ketogenesis and peripheral insulin sensitivity by impairing skeletal muscle mitochondrial respiration [73]. However, this relationship has only been reported in one study and therefore further investigations focusing on this topic are required.

5.5. Endocrine disorders

Oestrogen deficiency and hyperandrogenism are endocrine disorders promoting the onset of myosteatosis in MASLD.

5.5.1. Oestrogen deficiency

Oestrogen is a sexual hormone involved in glucose and lipid metabolisms but also in insulin sensitivity. Indeed post-menopausal women frequently face an increase in global fat mass exposing them to a higher risk for MASLD and related complications such as myosteatosis [74]. Conversely, oestrogen supplementation is reported on a male mouse model to significantly reduce liver and muscle fat leading to an insulin sensitivity enhancement by

decreasing the protein kinase C theta activation [75]. Furthermore, oestrogen supplementation has anti-inflammatory properties by decreasing IL6, IL1β and TNFα levels in plasma and white adipose tissue [75].

5.5.2. Hyperandrogenism

MASLD prevalence is increased in the polycystic ovary syndrome often associated with increased visceral fat content, IR and other features of metabolic syndrome [76]. IR in this syndrome is associated with hyperandrogenism itself. Indeed, liver fat content is increased in hyperandrogenic polycystic ovary syndrome woman compared to normoandrogenic ones [77]. Hyperandrogenism is also positively correlated to IMLC [77]. This association between hyperandrogenism and MASLD is also observed in a gender comparative study. Male gender is an independent risk factor for MASLD potentially due to increased testosterone level [78]. Moreover, testosterone serum level is also reported as a risk factor for MASH development in type 2 diabetes already in physiological values [79]. Hence, androgens might increase muscle lipid content by promoting MASH development though pro-inflammatory properties, but clinical data are contradictory and further investigations are required [80,81].

5.6. Impaired microRNA-122 pathway

MicroRNA-122 (miR-122) is a non-coding RNA expressed in the liver and potentially secreted in the bloodstream with local and systemic effects [82]. Indeed, miR-122 is involved in lipid metabolism and several pathological conditions such as hepatitis C [83]. MiR-122 expression and active secretion by hepatocytes are induced in response to increased free and hepatic fatty acids by activating the retinoic acid-related orphan receptor alpha. MiR-122 decreases FFA synthesis and lipid storage by suppressing triglyceride synthesis in the liver and muscles [84]. MiR-122 also increases white adipose tissue lipolysis and βOX [84]. The expression and secretion of this physiological brake for triglyceride synthesis and storage are decreased in MASH [85]. However, the exact role of miR-122 as lipid metabolism regulator remains debated due to contradictory data possibly secondary to its circadian expression profile [86,87]. To our

knowledge, the direct role of miR-122 in the pathophysiology of myosteatosis in MASLD has not been previously explored.

6. Three possible consequences of myosteatosis

Myosteatosis in MASLD has three potential metabolic and functional consequences that are reviewed below: IR, MASH and impaired skeletal muscle function (Figure 1).

6.1. Myosteatosis and insulin resistance in humans: is EMLC or IMLC the culprit?

The impact of muscle fat on insulin sensitivity in human is a burning question in the topic currently difficult to answer considering the very low data available and the lack of consistency across studies in terms of assessment techniques. Concerning intramuscular fat, total intramuscular fat assessed by CT positively correlates with IR in obese diabetic subjects [26]. Intramuscular fat also mechanically impairs insulin sensitivity by reducing muscle blood flow and so insulin bioavailability [26]. However, this association is not reported in clinical studies assessing liver histology, IR and muscle density (Table 4).

Separately measured IMLC and EMLC by MRS do not have the same impact on insulin sensitivity [88]. Indeed, an association between IMLC assessed by proton MRS and IR is reported in one cohort of lean type 2 diabetic subjects matched for age, sex and BMI [89]. On the opposite, a drop in IMLC induced by physical exercise is reported as correlated to insulin sensitivity improvement in MASLD subjects independently of body weight and liver steatosis decrease [90,91]. However, this association is not reported in other diabetic cohorts focusing on the topic as well as in healthy athletes with increased IMLC [21,70,92]. On the contrary, no association between EMLC, total adiposity and IR is observed [89]. Therefore, reducing the link between myosteatosis and IR to this lipotoxicity alone appears simplistic.

Intermuscular fat also impacts insulin sensitivity [93]. Indeed, an association between intermuscular fat invasively collected from vastus lateralis of both diabetic and non-diabetic obese subjects and IR is reported [94]. Interestingly, in that same study, exposing cell-cultured myotubes to this intermuscular fat from non-diabetic obese subjects increases the IMLC

specifically in DAG [94]. Hence, intramuscular fat is here reported as a consequence of intermuscular fat exposure [94]. One hypothesis linking inter and intramuscular fat is firstly a decrease in intermuscular fat insulin sensitivity secondarily decreasing lipolysis in intermuscular adipose tissue [94]. Therefore, intermuscular fat releases FFA in myocytes microenvironment promoting EMLC and secondarily IMLC expansion [94].

6.2. Muscle density correlates with MASH

Concerning the correlation between liver histology and muscle fat, previous data reported a positive correlation between muscle lipid content, MASLD activity (ballooning and lobular inflammation) [19,50,95–98] and liver fibrosis [19,50,96–98] (Table 3). Interestingly, the only study with liver and muscle histology reported a positive correlation exclusively between lobular inflammation and IMLC, ballooning being not assessed [50]. EMLC did not correlate with liver histology [50]. Data on the correlation between liver and muscle lipid contents are more conflicting [19,50,96–99] (Table 3). Interestingly, no correlation between BMI and muscle fat assessed by CT was reported in those studies.

Conversely, a decrease in muscle fat after weight loss by diet changes or bariatric surgery and by physical exercise is associated with a histological regression of liver steatosis and ballooning [19,95]. However, in all those studies comparing muscle and liver phenotypes, muscle lipid content was exclusively semi-quantitatively assessed by CT. Indeed, no study with liver histology assessed muscle lipid content by proton MRS in MASLD subjects (Table 3). Therefore, the association between histological features of MASLD and each intramuscular lipid compartment (IMLC and EMLC) is a question currently unanswered. Furthermore, muscle density was often normalized by different parameters across studies (height, subcutaneous adipose tissue density) making an inter-study comparison hazardous (Table 3).

6.3. Myosteatosis might impact skeletal muscle function

Muscle function decay, defined by a decrease in muscle strength or performance, has been deeply investigated in sarcopenia with well-defined assessment tools [100]. Muscle function has been way less studied in myosteatosis with no assessment tools currently validated.

One retrospective study assessed the impact of myosteatosis on muscle function in a real-life cohort of MASLD subjects and reported a similar decrease in muscle strength for both myosteatosis and sarcopenia [34]. Mixed muscle changes combining a high muscle lipid content and a low muscle volume have a synergic effect on muscle function with a greater loss of muscle strength [34]. However, volume and lipid content of anterior thighs muscles were assessed by MRI-PDFF [34]. Hence, there is no available data on the specific impact of IMLC and EMLC on muscle function. Furthermore, muscle function was assessed by questionnaires on walking pace and stair climbing habits which are poor assessment methods.

Other studies assessed the impact of muscle fat on muscle function. However, muscle function was either retrospectively poorly assessed by a deficit index with no impact of muscle fat in terms of frailty [37], or accurately assessed but not on the investigated muscles (muscle function measured on forearm muscles and lipid content by proton MRS on rectus femoris) [53]. Further investigations accurately investigating the correlation between muscle lipid content assessed preferably by proton MRS and muscle function are highly required.

7. What promotes myosteatosis related consequences in MASLD?

Two pathophysiological mechanisms linking myosteatosis to its potential complications cited above have been highlighted with this systematic review: lipotoxicity and disturbances in the muscle-liver-adipose tissue axis notably mediated by inflammation (Figure 1).

7.1. Cytotoxicity related to intracellular lipids: the lipotoxicity concept

Many lipid species in MASLD cause hepatocyte toxicity and involved in MASLD progression, such as saturated fatty acids and ceramide bodies [101]. The lipid species accumulating the most in myocyte cytosol as lipid droplets in a mouse model of MASLD is by far diacylglycerol (DAG) [102]. Indeed, cytosolic droplets of DAG are reported to increase by five times in case

of diet-induced MASLD [102]. Cytosolic droplets of ceramide bodies also increase in these mice but in smaller proportions [102]. However, we do not know at present the more or less toxic role of each of these lipids on muscle. Lipotoxicity has 2 main consequences: IR and inflammation.

Skeletal muscle is a key organ in glucose homeostasis removing up to 75% of all blood glucose content and is therefore extremely insulin sensitive [103]. FFA can modulate muscle insulin sensitivity by several mechanisms.

Skeletal muscle mitochondrial βOX is decreased in overweight diabetic subjects [104,105]. Intramyocellular lipids are oxidized in acetyl-CoA to produce adenosine triphosphate via the tricarboxylic acid cycle and oxidative phosphorylation. In case of acetyl-CoA accumulation secondary to increased βOX, glycolysis and pyruvic dehydrogenase are inhibited by negative feedback [106]. This leads to allosteric inhibition of hexokinase and eventually reduced myocyte glucose uptake, lower respiration rates and IR [107] (Figure 4). Interestingly, restoring muscle βOX by administering an NAD+ precursor in overweight diabetic subjects significantly improves insulin sensitivity [105].

Furthermore, some lipid molecules directly promote IR by interfering with glucose uptake [108,109]. Indeed, DAG and ceramide bodies, both products of triacylglycerol oxidation, halt the translocation of glucose transporter 4 (GLUT4) into the myocyte membrane [108,109]. In addition, DAG decreases the insulin-mediated activation of insulin receptor substrate-1 (IRS-1) up to 30% by activating the protein kinase C theta [110]. This protein kinase increases IRS-1 Ser^{307} phosphorylation which decreases IRS-1 tyrosine phosphorylation and IRS-1-associated PI3-kinase activation. This eventually decreases GLUT4 membrane expression [110] (Figure 4).

However, abnormal structural proteins of lipid droplets are also involved in this lipotoxicity. Perilipin 5 is a lipid droplet-associated protein expressed at the surface of cytosolic lipid droplets regulating lipid hydrolysis and thus intracellular DAG and ceramide bodies levels

[111]. It is also involved in fatty acids addressing to myocyte mitochondria to promote βOX and hence prevent myosteatosis [111]. Indeed, perilipin 5 knockout mice develop IR secondary to ceramide bodies and triacylglycerol accumulation [112] (Figure 4).

Impaired AMPK signalling also plays a key role in MASLD by several mechanisms [113]. AMPK is an ubiquitous energy sensor involved in many homeostatic pathways depending on the tissue studied. It is composed by 3 subunits (α, β, γ) and is activated in case of low energy stores. In MASLD, hepatic AMPK is downregulated. Promoting pharmacologically AMPK signalling results in reduced serum inflammation by stimulating anti-inflammatory function of macrophages [114,115]. This anti-inflammatory property is mediated by suppressing key pro-inflammatory mediators expression such as NFκB [116,117], IL6 or even TNFα [118]. Furthermore, experimental studies on mouse models demonstrated that upregulation of AMPK signalling fights against IR specifically in skeletal muscle cells by promoting glucose uptake via increasing the GLUT4 translocation [119,120] (Figure 4).

Eventually, FFA have direct pro-inflammatory properties by activating innate immunity via the myeloid differentiation factor 2/toll-like receptor 4 pathway [121]. Myeloid differentiation factor 2/toll-like receptor 4 recognizes lipopolysaccharides present on bacterial wall but also metabolism-associated molecular patterns leading to the recruitment of intracellular adaptor protein myeloid differentiation factor 88. Myeloid differentiation factor 88 increases pro-inflammatory cytokines expression and release via the mitogen-activated protein kinase and nuclear factor-κB pathways [122]. The increase in the intracellular level of NF-κB activates the NOD-like receptor family, pyrin domain containing 3 inflammasome promoting IL1β and IL18 extracellular release. Interestingly, NOD-like receptor family, pyrin domain containing 3 participates in hepatic inflammation and hence MASLD pathogenesis and progression on mouse models [123] (Figure 4).

7.2. The muscle-liver-adipose tissue axis is disrupted in MASLD subjects with myosteatosis

The muscle-liver-adipose tissue axis is an inter-organ crosstalk involved in the metabolic regulation of all three organs involved [124]. Circulating mediators specifically involved in this crosstalk might be originate from each tissue respectively : hepatokines from the liver [125,126], myokines from the skeletal muscle [127] and adipokines from the adipose tissue with autocrine, paracrine and endocrine properties [128]. The expression and function of these mediators are disturbed in MASLD promoting metabolic dysfunctions including myosteatosis, eventually leading to liver and muscle function decay [128–130]. However, even if those mediators have been highly studied in MASLD these last years, there is only a few data available concerning the impact of myosteatosis on those mediators.

In the following section, we present the tissue-specific mediators whose expression levels are reported as specifically disturbed in MASLD subjects presenting myosteatosis (Figure 5).

7.2.1. Myosteatosis in MASLD subjects impacts tissue-specific mediators of the muscle-liver-adipose tissue axis

7.2.1.1. Hepatokines

Hepatokines are proteins secreted into the circulation by hepatocytes with autocrine, paracrine and endocrine functions [125]. These mediators can interact with skeletal muscle and modify positively or negatively its metabolic functions [126]. Hepatokines secretions profile is altered in MASLD promoting inflammation, IR and potentially myosteatosis [129] (Figure 5).

7.2.1.1.1. Selenoprotein P

Selenoprotein P (SeP) is a glycoprotein mainly involved in the transport of selenium from the liver to peripheral tissues [131]. SeP is increased in MASLD and promotes IR by altering insulin signaling [132]. SeP increases phosphorylation of IRS-1 decreasing insulin receptor substrate 1 tyrosine phosphorylation and altering distal insulin signal transduction [133]. SeP also inhibits the AMPK/acetyl-CoA carboxylase pathway [133]. This inhibition results in increased intracellular lipid accumulation in hepatocytes and myocytes [133]. Furthermore, SeP might also promote IR by decreasing the expression of adiponectin (see point 7.2.1.3.) considering

a negative correlation between SeP serum level and adiponectin reported in type 2 diabetes (Figure 5) [134].

7.2.1.1.2. Cathepsin D

Cathepsin D is a lysosomal enzyme mainly produced by hepatocytes. Cathepsin D is released from hepatocytes in case of liver steatosis by the resulting lysosomal dysfunction resulting in an increased serum level in MASLD [135]. It activates the TLR4 pathway increasing systemic and liver inflammation by upregulating the expression of pro-inflammatory cytokines (TNFα, CCL2) [136–138]. Hence, cathepsin D might be involved in MASLD progression to MASH [139]. Furthermore, cathepsin D serum level is reported as positively correlated to IR and myosteatosis potentially by promoting this inflammation (Figure 5) but further investigations are required to determine its precise role in myosteatosis pathogenesis in MASLD [140].

7.2.1.2. Musclin (myokine)

Myokines are proteins secreted by the skeletal muscle with potential metabolic effects mediated by endocrine, paracrine or autocrine features. Serum levels of myokines vary according to physical activity degree [127]. Myokines secretion profile is also disrupted in MASLD and is reported to negatively modulate the liver-muscle axis. Musclin is a myokine reported as decreased in MASLD with a direct impact on muscle lipid content (Figure 5). Indeed, downregulating musclin expression by G9a, a histone methyltransferase recruiting a repressive marker (H3K9me2), increases muscle fat content [130,141]. On the contrary, upregulating muscle expression by suppressing G9a decreases muscle and liver fat contents via the FOXO1 pathway [130]. However, the recruited pathways mediating its positive impact on liver and skeletal muscle remain unknown.

7.2.1.3. Adiponectin (adipokine)

Adipokines are mediators produced by adipocytes previously described as the main actors of IR in MASLD. Those mediators are reported as involved in several metabolic pathways though a highly regulated dynamic balance disturbed in MASLD though adipose tissue expansion

[128] (Figure 5). Adiponectin is a key-regulator of energy metabolism in liver and skeletal muscle by activating AMPK and peroxisome proliferator-activated receptor α signalling pathways increasing fatty acid β- and ω-oxidations [142]. This leads to an insulin sensitivity enhancement by reducing liver and muscle lipid content [143]. Adiponectin serum level is significantly decreased in obese subjects and is negatively correlated to IR and type 2 diabetes [144,145]. However, this decrease in adiponectin serum level is not reported as a key mechanism sufficient for IR development in MASLD [146].

7.2.2. Inflammation increases with myosteatosis and highly disrupts the muscle-liver-adipose tissue axis

The link between inflammation and myosteatosis in MASLD has been reported by several studies highlighting increased serum and tissue levels of inflammatory biomarkers in obese and non-obese subjects with increased muscle lipid content (Figure 5). Indeed, serum level of interferon-alpha 2 (IFNα2), a cytokine involved in the early immune response to viral infections, is increased in obese subjects with myosteatosis compared to healthy subjects [147]. Other members of the interferon family are not overexpressed. Furthermore, IFNα2 serum level is inversely correlated to muscle lipid content assessed by ultrasound in these subjects and positively correlated to visceral adipose tissue [147]. However, the pathophysiological mechanism linking IFNα2 and muscle fat needs further investigations. Beside IFNα2 in obese subjects, serum levels of other inflammatory mediators are increased in overweight MASLD subjects with myosteatosis, such as TNFα, IL1α and TGFβ [61,148–150].

Furthermore, inflammatory markers histologically assessed are highly expressed in adipose tissue, liver and skeletal muscle in obese subjects undergoing bariatric surgery. This obesity-related systemic inflammation is highly correlated to MASH and IMLC [49]. This inflammatory infiltration is also reported in that population as significantly higher in visceral adipose tissue compared to subcutaneous adipose tissue, confirming that inflammation in adipose tissue has a different impact on MASLD depending on its location [49]. However, the most relevant observation reported on that population is the strong correlation between muscle inflammation,

EMLC and liver fibrosis highlighting muscle inflammation as a potential driver of liver fibrosis [49].

These observations reinforce the well-described pro-inflammatory state occurring in obesity secondary to a disbalance between pro and anti-inflammatory cytokines. This resulting chronic low-grade inflammation occurs in several insulin-sensitive tissues such as adipose tissue, liver or skeletal muscle and eventually contributes to IR [151]. However, these data are in favor of inflammation as a driver of myosteatosis in MASLD and MASLD progression independently of obesity itself.

7.2.3. The particular case of interleukin 15: a mediator expressed by several organs

IL15 is a member of the γc cytokine family expressed by several tissues including liver [152]. It is therefore difficult to identify its main source in the overall proinflammatory metabolic context (Figure 5). It is a pro-inflammatory cytokine acting on T lymphocytes [153,154]. IL15 serum level is also increased in MASLD [155] and promotes liver steatosis by inducing PPARγ expression eventually impairing mitochondrial function in liver and adipose tissue [155,156]. It also increases liver inflammatory infiltration by chemotaxis [155]. IL15 is also highly expressed in skeletal muscle and is often defined as a myokine [157,158]. Furthermore, it has a dose-dependent role in muscle glucose and lipid metabolisms [159–161]. Indeed, supraphysiological doses of IL15 on mouse model under HFD enhances insulin sensitivity and decreases adiposity [159–161]. Hence, myosteatosis appears as an inflammatory process not only driven by an excessive serum level of FFA secondary to MASLD but also by pro- and non-inflammatory mediators altering both skeletal muscle and liver metabolisms (Figure 5).

8. Conclusion

MASLD is the most common chronic hepatopathy worldwide with only weight loss and physical exercise as proven effective therapeutic weapons in terms of liver fibrosis regression and

hence improvement in outcomes. Among extra-hepatic complications of MASLD, myosteatosis is highly prevalent even in non-cirrhotic stages. This association is compatible with a pathogenic axis between muscle and liver, the direction of which being unanswered.

In terms of pathophysiology, only a few mechanisms as well as mediators are currently reported as involved in myosteatosis pathogenesis in MASLD (Figure 1). However, many others might also be involved by modulating glucose and lipid metabolisms in both liver and skeletal muscle.

In terms of potential complications, myosteatosis is reported to decrease insulin sensitivity, muscle strength and to correlate with MASH (Figure 1). Therefore, myosteatosis could become an interesting screening tool for MASH in clinical practice. However, it is paramount to notice that the causal link between IR, MASH, muscle function decay and myosteatosis has currently not been well demonstrated as well as the respective contribution of inter and intramuscular fat to those complications. It is important to consider that the association between MASH and myosteatosis was only reported in obese subjects with myosteatosis semi-quantitatively assessed by CT (Table 3). Studies in other population groups, including all MASLD disease stages and using MRI and MRS techniques as valid tool to characterize the muscle compartment will surely shed light in the complex muscle-liver-adipose tissue intricacy anticipated to be determinant for MASLD pathogenesis and evolution. With these results, it will then be interesting to assess the extent to which specific treatment of myosteatosis by dietary changes, weight loss or targeted therapy, could have an impact on liver disease.

List of abbreviations:

AFP : alpha-foetoprotein

ALD : alcoholic-related liver disease

AMPK : adenosine monophosphate-activated protein kinase

BMI : body mass index

βOX : β-oxidation

CT : computed tomography

DAG : diacylglycerol

EMLC : extramyocellular lipid content

FFA : free fatty acids

GLUT4 : glucose transporter 4

HFD : high fat diet

HFrD : high fructose diet

HU : Hounsfield unit

IFNα2 : interferon-α2

IMLC : intramyocellular lipid content

IR : insulin resistance

IRS-1 : insulin receptor substrate-1

MAFLD : metabolic dysfunction-associated fatty liver disease

MASLD : metabolic dysfunction-associated steatotic liver disease

NAFLD : nonalcoholic fatty liver disease

MiR-122 : micro-RNA-122

MRI : magnetic resonance imaging

MRS : magnetic resonance spectroscopy

MASH : metabolic dysfunction-associated steatohepatitis

PDFF : proton density fat fraction

US : ultrasound

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Figure legends

Figure 1. Potential pathophysiological mechanisms, phenotypic description and consequences of myosteatosis in MASLD. MASH: metabolic dysfunction-associated steatohepatitis.

Figure 2. Flow chart of the selection of original manuscripts for inclusion in this systematic review. MASLD: metabolic dysfunction-associated steatotic liver disease.

Figure 3. Pathophysiological mechanisms involved in myosteatosis pathogenesis in metabolic dysfunction-associated steatotic liver disease.

Figure 4. Signalling pathways involved in the cellular lipotoxicity related to myosteatosis in metabolic dysfunction-associated steatotic liver disease. ↑: increased; ↓: decreased; Akt: protein kinase B; AMP: adenosine monophosphate; AMPK: AMP-activated protein kinase; ATP: adenosine triphosphate; β-ox: β-oxidation; CD36: cluster of differentiation 36; CPT1 : carnitine palmitoyltransferase 1; DAG: diacylglycerol; GLUT4: glucose transporter 4; IL: interleukin; IRS: insulin receptor substrate; MAPK: mitogen-activated protein kinase; Myd88: myeloid differentiation factor 88; NFκB: necrosis factor kappa B; NLRP3: NOD-like receptor family, pyrin domain containing 3; PDK1: 3-phosphoinositide-dependent protein kinase 1; PGC1α: peroxisome proliferator-activated receptor gamma 1 α; PLIN5: perilipin 5; PI3K: phosphatidylinositol-3-kinase; PKC: protein kinase C; TLR4: toll-like receptor 4; TNFα: tumor necrosis factor α.

Figure 5. Mediators of the muscle-liver-adipose tissue axis promoting myosteatosis in metabolic dysfunction-associated steatotic liver disease (MASLD). ↑: increased; ↓: decreased; Adiponectin serum level decreases in MASLD, increasing liver and muscle lipid contents. Cathepsin D serum level increases in MASLD, promoting systemic inflammation. Musclin serum level decreases in MASLD, increasing liver lipid content. Seleprotein P serum level increases in MASLD, increasing liver and muscle lipid contents and decreasing adiponectin expression. Systemic inflammation and insulin resistance occurring in

MASLD reciprocally promote an increase in muscle and liver lipid contents as well as adipose tissue expansion. IFNα2: interferon-α2; IL15: interleukin 15; IL1α: interleukin-1α; SeP: selenoprotein P; TGFβ: tumor growth factor β; TNFα: tumor necrosis factor-α.

First author	Manuscript title	Journal	Year	Model	N diet/treatment	Investigated muscles	Investigation techniques of muscle lipid content
Brackmann C. et al.	Nonlinear microscopy of lipid storage and fibrosis in muscle and liver tissues of mice fed high-fat diets	Journal of Biomedical Optics	2010	C57BL/6J mice	6 mice per group HFD versus ND	soleus diaphragm	electron microscopy
Camporez J. et al.	Anti-inflammatory effects of oestrogen mediate the sexual dimorphic response to lipid-induced insulin resistance	Journal of Physiology	2019	C57BL/6J mice	8 mice per group ♂ versus ♀ HFD versus ND matched for age ♂ versus ♀ HFD versus ND matched for body weight ♂ versus ♂ Estradiol versus vehicle	gastrocnemius soleus	^{1}H-MRS tissue lipid extraction
Ceddia R. et al.	The PGE2 EP3 receptor regulates diet-induced adiposity in male mice	Endocrinology	2016	C57BL/6J mice *EP3*$^{+/+}$ versus *EP3*$^{-/-}$	7-10 mice per group *EP3*$^{+/+}$ HFD vs ND *EP3*$^{-/-}$ HFD vs ND	gastrocnemius soleus	tissue lipid extraction

Chai C. et al.	Metabolic circuit involving free fatty acids, microRNA 122, and triglyceride synthesis in liver and muscle tissues	Gastroenterology	2017	C57BL/6J mice	3-14 mice per group CL316243 vs control CL316243 +/- antagomiR-122 antagomiR-122 vs antagomiR-18	*Not available*	optical microscopy
Hennige A. et al.	Enforced expression of protein kinase C in skeletal muscle causes physical inactivity, fatty liver and insulin resistance in the brain	Journal of Cellular and Molecular Medicine	2010	MLC-PKC-β2 transgenic C57BL/6 mice	4-8 mice per group 6 months old transgenic mice vs WT on ND 3 months old transgenic mice HFD vs ND	tibialis muscles	MRI tissue lipid extraction
Imai N. et al.	Up-regulation of thioesterase superfamily member 2 in skeletal muscle promotes hepatic steatosis and insulin resistance in mice	Hepatology	2022	C57BL/6J *S-Them2-/-*	4 mice per group HFD vs ND	gastrocnemius	optical microscopy tissue lipid extraction
Maj M. et al.	Consumption of high-fructose corn syrup compared with sucrose promotes adiposity and increased triglyceridemia but comparable NAFLD severity in Juvenile Iberian Pigs	Journal of Nutrition	2021	iberian pigs	7 mice per group HS vs HFCS	longissimus dorsi	optical microscopy

Marecki J. et al.	Hyperinsulinemia and ectopic fat deposition can develop in the face of hyperadiponectinemia in young obese rats	Journal of Nutritional Biochemistry	2011	male Sprague-Dawley rats	10 mice per group TEN HFD versus ND	gastrocnemius	CT optical microscopy
Meneses M. et al.	Distinct impacts of fat and fructose on the liver, muscle, and adipose tissue metabolome: An integrated view	Frontiers in Endocrinology	2022	C57BL/6J	7 mice per group ND versus HFD versus HFrD	gastrocnemius	^{1}H-MRS tissue lipid extraction
Nachit M. et al.	Myosteatosis rather than sarcopenia associates with nonalcoholic steatohepatitis in non-alcoholic fatty liver disease preclinical models	Journal of Cachexia, Sarcopenia and Muscle	2020	*foz/foz* mice	48 *foz/foz* - 89 WT ND versus HFD versus HFFD	erector spinae, quadratus lumborum, psoas	CT optical microscopy
Pichon C. et al.	Impact of L-ornithine L-aspartate on nonalcoholic steatohepatitis-associated hyperammonemia and muscle alterations	Frontiers in Nutrition	2022	*foz/foz* mice	6-7 mice per group *foz/foz* versus WT ND versus HFD *foz/foz* HFD +/- LOLA	erector spinae/quadratus lumborum Psoas muscle	CT
						quadriceps	optical microscopy tissue lipid extraction
Preuss C. et al.	A new targeted lipidomics approach reveals lipid droplets in liver, muscle and heart as a repository	Cells	2019	C57BL/6 SREBP-1c Tg mice	6 mice per group C57B16 versus SREBP-1c Tg ND	*Not available*	optical microscopy tissue lipid extraction

	for diacylglycerol and ceramide species in non-alcoholic fatty liver						
Spooner H. et al.	High-fructose, high-fat diet alters muscle composition and fuel utilization in a juvenile iberian pig model of non-alcoholic fatty liver disease	Nutrients	2021	iberian pigs	6-8 pigs per group ND versus ND + probiotics versus HFFD versus HFFD + probiotics	longissimus dorsi	tissue lipid extraction
Zhang W. et al.	Muscular G9a regulates muscle-liver-fat axis by musclin under overnutrition in Female Mice	Diabetes	2020	G9a-/- mice	3 mice per group HFD Mus33 versus vehicle	tibialis anterior	optical microscopy

Table 1. ***Original manuscripts on pre-clinical studies included in this systematic review listed in alphabetical order by first authors.***

♂ : male; ♀: female; antagomiR: microRNA antagonist; CT: computed tomography; HFCS: high fructose corn syrup; HFD: high-fat diet; HFFD: high-fat high-fructose diet; HFrD: high fructose diet; ^{1}H-MRS: proton magnetic resonance spectroscopy; HS: high sucrose; LOLA: L-ornithine L-aspartate; MLC PKC β2: MLC protein kinase C beta-2; MRI: magnetic resonance imaging; mus33: musclin 33; NAFLD: nonalcoholic fatty liver disease; ND: normal diet; PGE2 EP3: prostaglandin E2 E receptor 3; SREBP-1c Tg: sterol regulatory binding protein 1c triglycerides; TEN: total enteral nutrition.

First author	Manuscript title	Journal	Year	N	Investigated muscles	Investigation techniques of muscle lipid content
Bhanji RA. et al.	Differing impact of Sarcopenia and Frailty in Nonalcoholic Steatohepatitis and Alcoholic Liver Disease	Liver Transplantation	2019	136 MASLD/129 ALD	psoas, paraspinal and abdominal wall	CT
Chasapi A. et al.	Can obesity-induced inflammation in skeletal muscle and intramuscular adipose tissue accurately detect liver fibrosis ?	Journal of Musculoskeletal Neuronal Interactions	2018	50	rectus abdominis	optical microscopy
Cree Green M. et al.	Nonalcoholic fatty liver disease in obese adolescent females is associated with multi-tissue insulin resistance and visceral adiposity markers	Metabolism Open	2019	73	soleus	^{1}H-MRS ^{13}C-MRS
Ding L. et al.	Myosteatosis in NAFLD patients correlates with plasma cathepsin D	Biomolecular Concepts	2021	45	multifidus, erector spinae	MRI-PDFF
Han E. et al.	Muscle fat contents rather than muscle mass determines nonalcoholic steatohepatitis and liver fibrosis in patients with severe obesity	Obesity	2022	104	psoas	CT
Hsieh Y. et al.	Muscle alterations are independently associated with significant fibrosis in patients with nonalcoholic fatty liver disease	Liver International	2020	521	psoas, paraspinal and abdominal wall	CT
Hsieh Y. et al.	Myosteatosis, but not sarcopenia, predisposes NAFLD	Clinical Gastroenterology and Hepatology	2022	87 MASH/251 MASLD	psoas, paraspinal and abdominal wall	CT

	subjects to early steatohepatitis and fibrosis progression					
Jang S. et al.	Elevated serum alpha-fetoprotein level in asymptomatic individuals : clinical features, outcome and association with body fat deposition	Hepatology	2021	137	psoas	CT
Jones H. et al.	Polycystic ovary syndrome with hyperandrogenism is characterized by an increased risk of hepatic steatosis compared to nonhyperandrogenic PCOS phenotypes and healthy controls, independent of obesity and insulin resistance	Journal of Clinical Endocrinology and Metabolism	2012	22 PCOS/22 controls	soleus, tibialis anterior	^{1}H-MRS
Jun D. et al.	Association between low thigh fat and non-alcoholic fatty liver disease	Journal of Gastroenterology and Hepatology	2008	408	thigh muscles	CT
Kato K. et al.	Ectopic fat accumulation and distant organ-specific insulin resistance in Japanese people with nonalcoholic fatty liver disease	PLOS One	2014	69	soleus	^{1}H-MRS
Kitajima Y. et al	Age-related fat deposition in multifidus muscle could be a marker for nonalcoholic fatty liver disease	Journal of Gastroenterology	2010	333	multifidus muscle	CT
Kitajima Y. et al.	Severity of non-alcoholic steatohepatitis is associated with substitution of adipose tissue in skeletal muscle	Journal of Gastroenterology and Hepatology	2013	208	multifidus muscle	CT

Linge J. et al.	Adverse muscle composition is linked to poor functional performance and metabolic comorbidities in NAFLD	Journal of Hepatology Reports	2021	9545	thigh	MRI PDFF
Machado V. et al.	Liver and muscle in morbid obesity : the interplay of fatty liver and insulin resistance	PLOS One	2012	51	deltoid	optical microscopy
Mey T. et al.	B-hydroxybutyrate is reduced in humans with obesity-related NAFLD and displays a dose-dependent effect on skeletal muscle mitochondrial respiration in vitro	American Journal of Physiology, Endocrinology and Metabolism	2020	1	human primary myoblasts	real-time respirometry
Nachit M., Kwanten W. et al.	Muscle fat content is strongly associated with NASH : A longitudinal study in patients with morbid obesity	Journal of Hepatology	2021	184	psoas, dorsal and abdominal muscles	CT
Nachit M., Lanthier N et al.	A dynamic association between myosteatosis and liver stiffness : Results from a prospective interventional study in obese patients	Journal of Hepatology Reports	2021	48	psoas, dorsal and abdominal muscles	CT
Nakajima T. et al.	Age is a negative, and visceral fat accumulation is a positive, contributor to hepatic steatosis, regardless of the fibrosis progression in non-alcoholic fatty liver disease	Journal of Gastroenterology and Hepatology Research	2012	60 MASLD/26 controls	multifidus muscle	CT
Oh S. et al.	Therapeutic effect of hybrid training of voluntary and electrical muscle contractions in middle-aged obese women with	Therapeutics and Clinical Risk management	2015	15	hamstrings, quadriceps	^{1}H-MRS

	nonalcoholic fatty liver disease: A pilot trial					
Oh S. et al.	Whole-body vibration for patients with nonalcoholic fatty liver disease: a 6-month prospective study	Physiological reports	2019	25	quadriceps	^{1}H-MRS
Oshida N. et al.	Urinary Levels of Titin-N Fragment, a Skeletal Muscle Damage Marker, are Increased in Subjects with Nonalcoholic Fatty Liver Disease	Scientific Reports	2019	153 MASLD/100 controls	rectus femoris	US ^{1}H-MRS
Pasco J. et al.	Fatty Liver Index and Skeletal Muscle Density	Calcified Tissue International	2022	403	radial, tibial	CT
Preuss C. et al	A new targeted lipidomics approach reveals lipid droplets in liver, muscle and heart as a repository for diacylglycerol and ceramide species in nonalcoholic fatty liver	Cells	2019	1	vastus lateralis	optical microscopy
Shida T. et al.	Skeletal muscle mass to visceral fat area ratio is an important determinant affecting hepatic conditions of nonalcoholic fatty liver disease	Journal of Gastroenterology	2018	366	bilateral quadriceps	^{1}H-MRS
Shida T. et al.	Clinical and anthropometric characteristics of non-obese non-alcoholic fatty liver disease subjects in Japan	Hepatology Research	2020	404	thigh	^{1}H-MRS
Shigiyama F. et al.	Characteristics of Hepatic Insulin-Sensitive Nonalcoholic Fatty Liver Disease	Hepatology Communications	2017	26 MASLD/5 controls	tibialis anterior	^{1}H-MRS
Smajis S. et al.	Metabolic effects of a prolonged, very-high-dose	American Journal of Clinical Nutrition	2020	11 MASLD/10 controls	*not available*	^{1}H-MRS

	dietary fructose challenge in healthy subjects					
Tarantino G. et al.	Circulating levels of sirtuin 4, a potential marker of oxidative metabolism, related to coronary artery disease in obese patients suffering from nafld, with normal or slightly increased liver enzymes	Oxidative Medicine and Cellular Longevity	2014	234	left biceps brachii	US
Tarantino G. et al.	Interferon-alpha2 but not interferon-gamma serum levels are associated with intramuscular fat in obese patients with nonalcoholic fatty liver disease	Journal of Translational Medicine	2019	80 MASLD/38 controls	left biceps brachii	US
Zhang W. et al.	Fat accumulation, Liver Fibrosis, and Metabolic Abnormalities in Chinese Patients with Moderate/Severe Versus Mild Hepatic Steatosis	Hepatology communications	2019	160	dorsal muscles	CT
Zhang W. et al.	Metabolic abnormalities , liver and body fat in American versus Chinese patients with non-alcoholic fatty liver disease	Journal of Gastroenterology and Hepatology Open	2022	101 American/160 Chinese	dorsal muscles	CT

Table 2. Original manuscripts on clinical studies included in this systematic review listed in alphabetical order by first authors. ALD: alcohol-related liver disease; ^{13}C-MRS: carbon 13 magnetic resonance spectroscopy; CT: computed tomography; MASH: metabolic dysfunction-associated steatohepatitis; MASLD: metabolic dysfunction-associated steatotic liver disease; MRS: magnetic resonance

spectroscopy; MRI-PDFF: magnetic resonance imaging proton density fat fraction; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; PCOS: polycystic ovary syndrome; SVR: skeletal muscle mass to visceral fat area ratio; US: ultrasound.

Associations investigated (myosteatosis vs liver histology)	Reported associations	References	Assessment technique of myosteatosis	Association between myosteatosis and IR
myosteatosis - liver steatosis	muscle lipid content ↑ when liver lipid content ↑	Han E. Obesity. 2022	CT (SMFI)	/
		Kitajima Y. J Gastroenterol. 2010	CT (IMAC)	/
		Nachit M., Kwanten W. et al. J Hepatol. 2021	CT (SMFI)	/
	muscle lipid content ↔ when liver lipid content ↑	Hsieh Y. Clin Gastroenterol Hepatol. 2022	CT (MA)	no association
		Machado M. PLOS One. 2012	OM	no association with IMLC & EMLC
	muscle lipid content ↓ when liver lipid content ↑	Hsieh Y. Liver Int. 2021	CT (MA)	no association
myosteatosis – total MASLD activity	muscle lipid content ↑ when MASLD activity ↑	Han E. Obesity. 2022	CT (SMFI)	/
		Hsieh Y. Liver Int. 2021	CT (MA)	no association
		Hsieh Y. Clin Gastroenterol Hepatol. 2022	CT (MA)	no association
		Kitajima Y. J Gastroenterol Hepatol. 2013	CT (IMAC)	/
		Machado M. PLOS One. 2012	OM	no association with IMLC & EMLC
		Nachit M., Kwanten W. et al. J Hepatol. 2021	CT (SMFI)	/
• myosteatosis - lobular inflammation	muscle lipid content ↑ when lobular inflammation ↑	Hsieh Y. Liver Int. 2021	CT (MA)	no association
		Hsieh Y. Clin Gastroenterol Hepatol. 2022	CT (MA)	no association
		Machado M. PLOS One. 2012	OM	no association with IMLC & EMLC
		Nachit M., Kwanten W. et al. J Hepatol. 2021	CT (SMFI)	/
• myosteatosis - ballooning	muscle lipid content ↑ when hepatocyte ballooning ↑	Han E. Obesity. 2022	CT (SMFI)	/
		Hsieh Y. Liver Int. 2021	CT (MA)	no association
		Hsieh Y. Clin Gastroenterol Hepatol. 2022	CT (MA)	no association
		Nachit M., Kwanten W. et al. J Hepatol. 2021	CT (SMFI)	/
myosteatosis - fibrosis		Han E. Obesity. 2022	CT (SMFI)	/

	muscle lipid content ↑ when liver fibrosis ↑	Hsieh Y. Liver Int. 2021	CT (MA)	no association
		Hsieh Y. Clin Gastroenterol Hepatol. 2022	CT (MA)	no association
		Machado M. PLOS One. 2012	OM	no association with IMLC & EMLC
		Nachit M., Kwanten W. et al. J Hepatol. 2021	CT (SMFI)	/

Table 3. Associations highlighted between liver phenotype exclusively histologically assessed, muscle lipid content and insulin resistance assessed by the homeostasis model assessment of insulin resistance in clinical studies. ↑: increased; ↓: decreased; ↔: unchanged; /: association not investigated; CT: computed tomography; EMLC: extramyocellular lipid content; IMAC: intramuscular adipose tissue content (density of the region of interest of the muscle/ density of the region of interest of subcutaneous fat); IMLC: intramyocellular lipid content; MA: muscle attenuation; MASLD: metabolic dysfunction-associated steatotic liver disease; MRI: magnetic resonance imaging; OM: optical microscopy; SMFI: skeletal muscle fat index (100*(muscle area/muscle density)).