

# Impact of Peroxisome Proliferator-Activated Receptor Agonists on Myosteatorsis in the Context of Metabolic Dysfunction-Associated Steatotic Liver Disease

Nathan Boliaki<sup>1</sup>, Guillaume Henin<sup>1,2</sup>, Georgia Bale<sup>1,2</sup>, Nicolas Lanthier<sup>1,2,\*</sup>

<sup>1</sup>Service d'Hépatogastroentérologie, Cliniques universitaires Saint-Luc, UCLouvain, 1200 Brussels, Belgium

<sup>2</sup>Laboratory of Hepatogastroenterology, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain (UCLouvain), 1200 Brussels, Belgium

\*Correspondence: [nicolas.lanthier@saintluc.uclouvain.be](mailto:nicolas.lanthier@saintluc.uclouvain.be) (Nicolas Lanthier)

Published: 20 June 2024

**Background:** Metabolic dysfunction-associated steatotic liver disease (MASLD), and more specifically steatohepatitis may be associated with fat infiltration of skeletal muscles which is known as myosteatorsis. Pan-peroxisome proliferator-activated receptor (PPAR) agonists have been shown to promote metabolic dysfunction-associated steatohepatitis (MASH) remission. However, the effect of PPAR agonists on myosteatorsis remains to be determined. The aim of this review is to evaluate the effect that PPAR agonists alone or in combination, have on myosteatorsis in the context of MASLD.

**Methods:** Original research reports concerning the impact of PPAR agonists on muscle fat in MASLD were screened from PUBMED and EMBASE databases following the PRISMA methodology.

**Results:** Eleven original manuscripts were included in this review. Two preclinical studies assessed the impact of the PPAR $\alpha$  agonist on fat content in the quadriceps muscle and the liver by extracting triglycerides in rats fed a high-fat diet and in insulin-resistant mice. Both models showed muscle and liver triglyceride content reduction using WY14643. Fenofibrate had no significant impact on soleus intramyocellular lipids or liver fat content in insulin-resistant subjects based on proton magnetic resonance spectroscopy. Treatment with PPAR $\delta$  agonists increased the expression of genes involved in fatty acid oxidation in two studies on muscle cell culture. PPAR $\gamma$  agonists were investigated in two preclinical studies and one clinical study using spectroscopy and computed tomography respectively. In the first preclinical study in Zucker diabetic fatty rats, rosiglitazone reduced muscle lipids and hepatic steatorsis. In a second preclinical study using the same animal model, pioglitazone reduced tibialis anterior intramyocellular lipids. In contrast, computed tomography analyses in patients with type 2 diabetes revealed a surface area increase of low-density muscles (suggesting an increase in muscle fat content) after a one-year treatment with rosiglitazone. Varying combinations of PPAR agonists (cevoglitazar, fenofibrate/pioglitazone and muraglitazar) were evaluated in two preclinical studies and one clinical study. In rats, these treatments showed variable results for muscle and liver depending on the combinations studied. In type 2 diabetic patients, treatment with muraglitazar (a PPAR $\alpha/\gamma$  agonist) reduced the intramyocellular lipid content of tibialis anterior as well as liver fat content following spectroscopy assessment.

**Conclusion:** The combination of different PPAR agonists could have a positive impact on reducing myosteatorsis, in addition to their effect on the liver. Some discrepancies could be explained by the different techniques used to assess muscle lipid content, the muscles assessed and the possible adipogenic effect of PPAR $\gamma$  agonists. Further clinical research is needed to fully assess the efficacy of these treatments on both MASLD progression and associated myosteatorsis.

**Keywords:** PPAR; lanifibranor; MASLD; muscle; myosteatorsis; muscle fat; insulin resistance; myokine

## Introduction

The prevalence of obesity, as measured by the World Health Organization growth curves, has nearly tripled worldwide since 1975 [1]. This weight gain forecasts an increased burden of several diseases among Western populations, of which type 2 diabetes mellitus (T2DM), cardiovascular diseases and various cancers [2]. Faced with a growing number of cases of metabolic dysfunction-associated steatotic liver disease (MASLD) [3,4] causing concern in the public health sector, there is a need for it to be properly addressed [5,6].

## MASLD and Metabolic Dysfunction-Associated Steatohepatitis (MASH): General Concepts and Liver Complications

MASLD is defined as the presence of hepatic steatorsis (intrahepatic fat exceeding 5% of the whole liver volume on imaging or biopsy analysis), with the presence of at least one of five cardiometabolic risk factors [3]. The worldwide prevalence of MASLD saw a significant increase recently with 37.8% of the population being affected in 2016 compared to 25.5% in 2005, and is continuing to increase at an alarming rate [7]. The spectrum of MASLD ranges from

isolated liver steatosis to MASH, a severe form of MASLD histologically defined by ballooning hepatocytes and lobular inflammation [8]. This histological pattern favors the subsequent development of progressive fibrosis [8,9], the last stage of which is cirrhosis which can lead to organ failure and hepatocellular carcinoma [10]. The definitive diagnosis of MASH relies on a liver biopsy [11] which is the gold standard for assessing the efficacy of treatments in clinical trials [12–14].

### *MASLD and Extrahepatic Complications*

In addition to the hepatic complications such as cirrhosis and hepatocellular carcinoma, MASLD, and a fortiori MASH, can be associated with extrahepatic complications. These include cardiovascular disease, type 2 diabetes and myosteatosi. Understanding this is key in elaborating the ideal treatment for MASLD which should also have an impact on these extrahepatic parameters.

#### MASLD and Cardiovascular Diseases

Among MASLD patients, the leading causes of death are attributable to cardiovascular diseases [15]. Patients with MASLD are at substantial risk for the development of coronary heart disease, cerebrovascular accidents, cardiomyopathy and cardiac arrhythmias [16]. Furthermore, this cardiovascular risk is strongly associated with the histological severity of MASLD [17]. This can partly be explained by the close ties between MASLD and the cardiometabolic risk factors included in the metabolic syndrome, such as abdominal obesity, atherogenic dyslipidemia, hypertension, and hyperglycemia [18]. Moreover, the association of MASLD with cardiovascular diseases may involve other pathways such as low-grade systemic inflammation, oxidative stress, and perturbations in the gut microbiota [19]. Interestingly, in a large number of investigations, MASLD and more particularly MASH, remains an independent risk factor for atherosclerotic cardiovascular disease even after adjustment for typical risk factor covariates [20], which suggests a role of specific factors originating in the liver. For example, authors recently showed that steatotic hepatocytes are the source of extracellular vesicles that can circulate in the bloodstream and contain microRNA that inhibits a protein that promotes cholesterol efflux from the vesicles thus increasing atheromatosis [21].

#### MASLD and T2DM

A meta-analysis of 33 observational studies showed that patients with MASLD have a doubled risk of T2DM, irrespective of obesity and other metabolic risk factors [22]. Conversely, over 80% of patients with T2DM also suffer from MASLD [23]. The mechanisms linking obesity, MASLD and T2DM are numerous. Firstly, prior to the development of visceral adiposity and obesity, hepatic steatosis can rapidly develop as a result of dietary changes [24,25]. This hepatic steatosis is itself associated with hep-

atic insulin resistance (IR) characterized by increased hepatic gluconeogenesis potentially leading to diabetes [26]. Additionally, the steatotic liver secretes cytokines called hepatokines that can lead to peripheral IR in tissues such as skeletal muscle and adipose tissue [27,28]. Lastly, the joint presence of IR contributes exponentially to the development of liver steatosis with elevated levels of glucose and free fatty acids, meaning more substrate is available for de novo hepatic lipogenesis [29,30]. Accumulation of adipose tissue, and more specifically visceral adipose tissue is associated with altered tissue function whereby adipocyte hypertrophy and a pro-inflammatory phenotype contribute to IR [30,31]. In the same way, hepatic fat accumulation [32,33] compounds the effects previously mentioned, playing a part in a vicious cycle in which MASLD, IR and T2DM are an integral part.

#### Chronic Liver Diseases and Myosteatosi

The beneficial role of healthy skeletal muscle has long been described [34]. In addition to the aforementioned factors, MASLD could be associated with ectopic fat deposition in other organs such as skeletal muscle. Skeletal muscle fat infiltration, also known as “myosteatosi”, is suspected with lowered skeletal muscle radiodensity on computed tomography (CT) [35], but confirmed with precision on quantitative magnetic resonance imaging (e.g., proton density fat fraction). However, only proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) allows for the separate measurement of intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) *in vivo* [35,36]. Intramuscular lipids (including IMCL and EMCL) can also be measured *in vitro* by tissue triglyceride extraction [36,37]. The link between liver disease and muscle was first demonstrated in the context of cirrhosis. Indeed, cirrhotic patients are at higher risk of muscle wasting, functional decay or sarcopenia [38,39], peripheral IR [40] and myosteatosi irrespective of the liver disease etiology [38,39].

This peripheral IR secondary to MASH is frequent and may be linked to muscle fat content [38,39]. Patients with MASLD at earlier disease stages also show greater increases in intramuscular fat content compared with healthy individuals [41,42]. Moreover, recent studies show that muscle fat content is strongly associated with MASLD severity evidenced by transient elastography [43], or more specifically, by histology [44]. Indeed, among MASLD patients, the development of myosteatosi is preferentially the characteristic of patients with MASH [45–48]. As the link between MASLD and myosteatosi has only recently been established, there is as yet no data on the prevalence of myosteatosi worldwide. The mechanisms associated with MASLD and myosteatosi are not yet fully understood. However, it is suspected that IR, mitochondrial dysfunction and diminished lipid storage capacity of subcutaneous adipose tissue, which all characterize MASLD, could contribute to muscle fat development [36]. Myosteatosi

seems to vary according to muscle fiber composition and related oxidative properties, but these data are ambiguous [49,50]. In terms of consequences, muscle fat could potentially decrease muscle strength [51] and is involved in the development of IR by altering insulin signaling pathways [52]. Interestingly, the degree of muscle fat content correlates with patient liver elastography and histology in such a way that a decrease in muscle fat is seen in parallel to an improvement in liver stiffness [44] and liver histology [48], reaffirming the dynamic association between myosteatosis and liver disease. This link highlights the importance of investigating the impact of MASH treatments on myosteatosis.

### Current Treatments for MASH

Steatohepatitis has been shown to drive disease progression. Indeed, although liver steatosis may already be associated with extrahepatic complications (such as insulin resistance), it is MASH that appears to be more associated with myosteatosis and that will lead to hepatic complications (fibrosis, cirrhosis, hepatocarcinoma) [36]. Consequently, an indication for pharmacological treatment of MASLD at risk is considered when the histological activity score is high (presence of MASH) and there is fibrosis [53]. Treatment options for MASH are currently under investigation and since no drug treatment has yet been approved by the health authorities, present-day recommendations essentially consist of lifestyle modifications [54]. Physical activity coupled with body weight reduction has been shown to decrease hepatic steatosis, if the body weight reduction exceeds 5% and to contribute to histological remission of MASH if the body weight reduction exceeds 10% [55,56]. Unfortunately, although lifestyle modifications can be a successful intervention for MASH, this strategy has a poor long-term success rate. Multiple pharmacological treatments recently investigated have yielded disappointing results, due to the multifactorial and complex MASH pathophysiology [13,14]. However, recent phase 2 and 3 studies show promising results [14]. Among them, peroxisome proliferator-activated receptor (PPAR) agonists are currently being evaluated as a potential treatment for MASH [57]. PPARs are ligand-activated transcription factors of nuclear hormone receptors which are divided into 3 subtypes (Table 1) [57]. PPAR $\alpha$  is mainly expressed in the liver, skeletal muscles and kidney. It reduces serum triglyceride levels and promotes fatty acid  $\beta$ -oxidation and regulation of energy homeostasis [58]. It also inhibits the expression of cholesterol 7 $\alpha$ -hydroxylase (*CYP7A1*), thereby decreasing hepatic bile acid levels [59]. PPAR $\beta/\delta$  is mainly expressed in skeletal and cardiac muscle as well as in the liver and white and brown adipose tissue. It enhances fatty acid metabolism and has an anti-inflammatory function where liver macrophages take on an anti-inflammatory phenotype [60]. A reduction of *CYP7A1* is also described [61]. Finally, PPAR $\gamma$ , mainly expressed in white and brown adi-

pose tissues, intestines, liver and pancreatic  $\beta$ -cells, stimulates adipocyte differentiation and pancreatic insulin sensitivity [62]. PPAR agonists are artificial ligands that allow the activation of such receptors. Recently, treatment with lanifibranor (pan-PPAR agonist) showed impressive effects on MASH in a phase 2 study [63]. Compared to placebo, lanifibranor induced more frequent MASH resolution, regression of fibrosis, increased insulin sensitivity and decreased liver enzyme levels, lipids and fibrogenesis markers such as cleaved pro-peptide of type III collagen (Pro-C3) [63]. For these reasons, a phase 3 study is currently underway (NATiv3, NCT04849728) [14].

### Objective and Methods

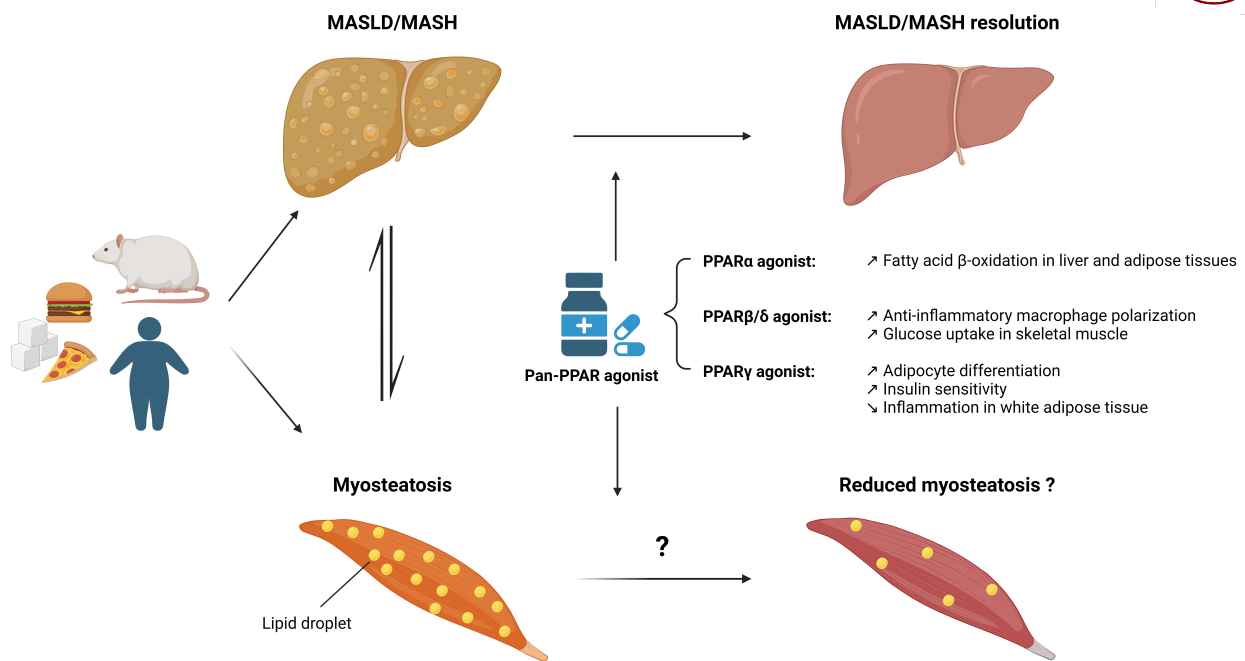
Recent studies showed that patients with myosteatosis were more likely to have early MASH independently of age, sex and metabolic factors [45–48]. This suggests that myosteatosis could not only be a cause but also a consequence of MASH disease (Fig. 1) [36].

Due to the promising results of pan-PPAR agonists regarding MASH resolution [57,63], and considering the existing association between myosteatosis and MASLD severity [45–48,64], it stands to reason that PPAR agonists may also influence the development of myosteatosis (Fig. 1). This could explain the effectiveness of this type of treatment and serve as a marker to monitor treatment response.

Original research reports were screened using PUBMED and EMBASE databases. The review of articles was restricted to those published in English until March 2024. Considering that MASLD is a term recently coined [3,4], terms from the previous nomenclature were used, namely “NAFLD” for “non-alcoholic fatty liver disease”, “NASH” for “non-alcoholic steatohepatitis” and “MAFLD” for “metabolic dysfunction associated fatty liver disease” [65,66]. This review included only original articles. Systematic reviews, book articles and abstracts were excluded. Clinical and pre-clinical model studies were selected. Original articles were not restricted by region, language or period of time. We carefully examined each article and identified relevant results that align with our research topic. Extracted information included: study design, participant characteristics and outcome assessment.

### Results

The first search, combining the words “PPAR agonist and myosteatosis” (N = 0), “PPAR agonist and muscle fat and MAFLD” (N = 0), “PPAR agonist and muscle fat and NAFLD” (N = 9), “PPAR agonist and muscle fat and MASLD” (N = 0), “PPAR agonist and intramyocellular lipids” (N = 15), “PPAR agonist and intramyocellular lipids and liver fat” (N = 10) with the “only articles” and “full text” filters, yielded 34 results (Fig. 2). After reading titles and removing duplicates, 24 articles were assessed for eli-



**Fig. 1. Mechanism of action and effect on metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH) of the pan-peroxisome proliferator-activated receptor (PPAR) agonists, with potential additional effect on skeletal muscle.** The Fig. 1 was created with BioRender at <https://www.biorender.com/>.

gibility according to our inclusion criteria. 13 articles were removed because they did not address the research question. 11 articles were finally kept and reviewed (Fig. 2), including 8 preclinical and 3 clinical studies. In the preclinical studies, skeletal muscle lipid content was assessed by tissue triglyceride extraction (3/8), and  $^1\text{H-MRS}$  (3/8). Fatty acid oxidation (FAO) gene expression levels were measured by real-time quantitative polymerase chain reaction (RT-qPCR) (2/8). In the clinical studies, muscle lipid content was assessed by CT (1/3) and  $^1\text{H-MRS}$  (2/3). In order to answer our research question, we decided to examine the effect each PPAR agonist subtype had on muscle and liver fat content, before looking at the effect of a combination of PPAR agonists.

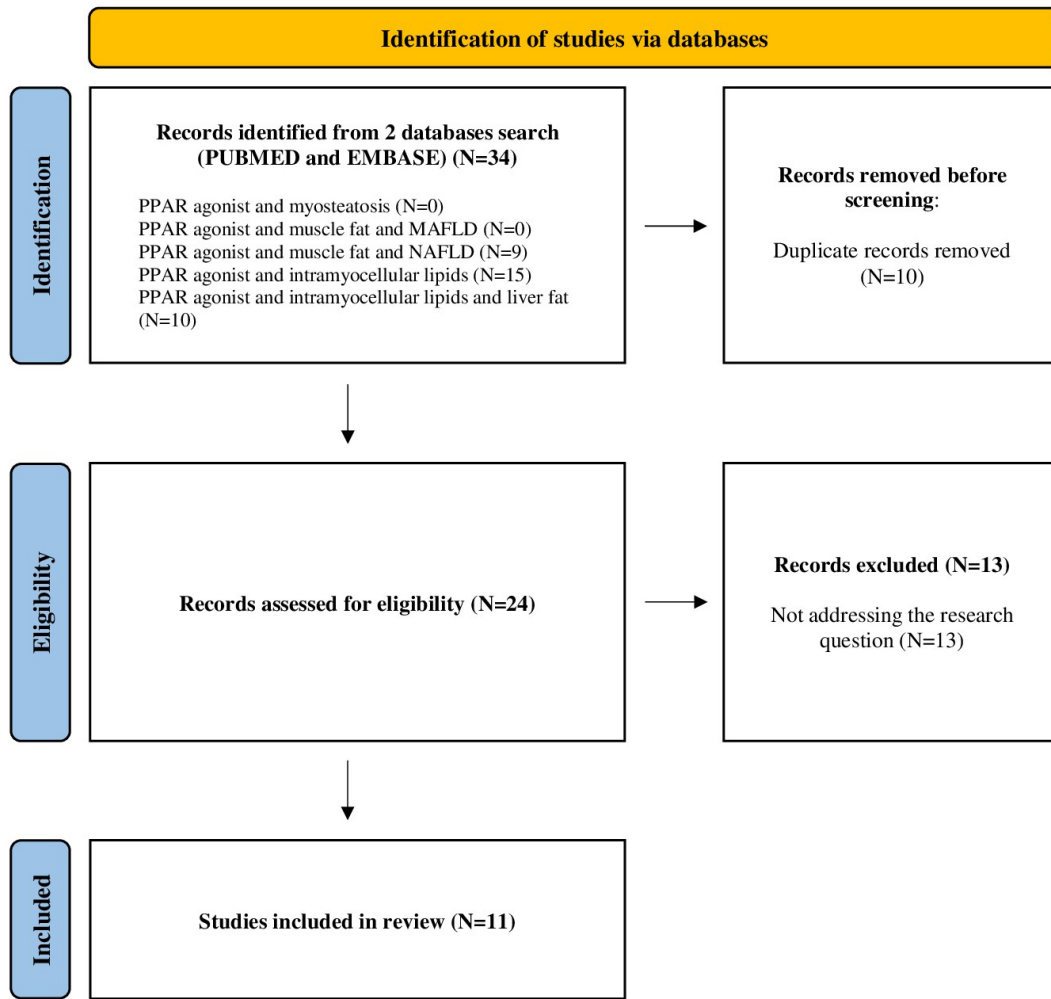
#### *PPAR $\alpha$ Agonists and Myosteatosis*

The impact of two different PPAR $\alpha$  agonists on muscle fat was assessed in two pre-clinical studies and in one clinical study (Table 2, Ref. [67–69]).

In the first pre-clinical study [67], researchers investigated the effect of the PPAR $\alpha$  agonist WY14643 on muscle lipids and insulin sensitivity in Wistar rats. Rats were divided into two groups: control or treated with WY14643 (N = 6–9/group). All rats were fed a high-fat diet (HFD) (59% fat, 350 kJ/day). From the second week of feeding, rats were administered 3 mg/kg/day of WY14643 for a total of two weeks. Tissue triglycerides were extracted from the quadriceps and liver. Treatment significantly decreased triglyceride levels in the quadriceps muscle (–34%) and in the liver (–54%). Moreover, total long-chain acyl-CoAs

(LCACoAs), a lipid subspecies, were substantially lowered in the muscles of rats treated with WY14643 (–41%) compared to HFD-fed control rats. In WY14643-treated rats, most of the measured lipid subspecies (stearoyl 18:0, oleoyl 18:1, and linoleoyl 18:2) were significantly reduced. In the same study, WY14643 substantially decreased muscle IR, a parameter that correlates well with the reduction of lipid accumulation, particularly muscle LCACoAs content. LCA-CoAs have been reported to inhibit the activity of hexokinase, pyruvate dehydrogenase as well as glycogen synthesis in the liver [67]. Similar inhibitory effects may also be present in the muscle, suggesting a direct link between improved insulin action in the muscle caused by PPAR $\alpha$  agonists and the reduction in muscle LCACoAs levels.

In the second pre-clinical study [68], the impact of the same PPAR $\alpha$  agonist, on muscle and liver steatosis was investigated in insulin-resistant lipoatrophic A-ZIP/F-1 mice. Wild-type controls were matched for age and sex. Mice were all fed a standard rodent diet. Mice were either treated for 2 weeks with WY14643 (0.1% of diet) or remained untreated for the same period in the control group (N = 6/group/genotype). Liver and muscle triglyceride levels were assessed by triglyceride extraction. In WY14643 treated A-ZIP/F-1 mice, quadriceps triglycerides were reduced (–44%) compared with untreated A-ZIP/F-1 mice. Unfortunately, due to interspersed adipose tissue, no measurement of muscle triglyceride in wild-type mice was obtained. Additionally, liver triglyceride levels were reduced in treated A-ZIP/F-1 (–40%) and in wild-type mice (–41%), when compared with untreated mice of the same genotype.



**Fig. 2. Flow chart of the selection of manuscripts for inclusion in this systematic review.** PPAR, peroxisome proliferator-activated receptor; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease. Fig. 2 was created by Microsoft Word.

In both wild-type mice and A-ZIP/F-1 mice, treatment led to increased liver weight, probably due to hyperplasia and hepatocyte hypertrophy caused by PPAR $\alpha$  agonists [70]. Additionally, expression of mitochondrial and peroxisomal  $\beta$ -oxidation genes was significantly increased in both liver and muscle tissue of treated mice, another side-effect of PPAR $\alpha$  agonists [71] that may explain the reduced tissue triglyceride content.

In the clinical study [69], fenofibrate, another PPAR $\alpha$  agonist was investigated in elderly people (between 65 and 72 years of age). Following a baseline oral glucose tolerance test, subjects were stratified into one of two categories: healthy (N = 7) or insulin resistant (N = 12). Except for fasting glycemia and insulinemia, the two groups were similar. Patients were treated with 160 mg/day of fenofibrate for a total of 60 days and both soleus and liver fat content were assessed by <sup>1</sup>H-MRS on days 1, 11 and 61. Administration of treatment had no significant impact on IMCL content in both groups. Moreover, liver fat content remained unchanged in either group (Table 2). It is important to note

that IMCL and liver fat content were similar in both groups before treatment, despite the potential relationship between elevated liver and muscle fat content and IR [52]. Unfortunately, no evaluation of the EMCL was carried out despite the possibility of it being affected and the availability of <sup>1</sup>H-MRS to ensure its measurement [36].

#### PPAR $\beta/\delta$ Agonists and Myosteatosi

The literature on the impact of PPAR $\beta/\delta$  agonists on muscle fat content is limited. Only two articles were retained for this review, both of which investigated PPAR $\beta/\delta$  agonists using cell cultures of mouse and human myocytes respectively (Table 3, Ref. [72,73]).

In the first pre-clinical study [72], C2C12 mice myocytes were treated with different compounds, including the PPAR $\beta/\delta$  agonist GW0742 (0.1  $\mu$ M), and compared in terms of oxidative metabolism and secretory activity. FAO gene expression levels were measured using RT-qPCR. Compared to vehicle-treated cells, GW0742 significantly increased expression of the muscle lipid uptake gene,

**Table 1. PPAR subtypes, natural ligands, localization and effects.**

Subtype	Natural activating ligands	Localization	Effects
PPAR $\alpha$	Omega-3 fatty acids	Liver (hepatocytes, stellate cells, Kupffer cells)	↗ Fatty acid $\beta$ -oxidation
		White and brown adipose tissue	↗ Lipid metabolism
		Skeletal and cardiac muscle	↗ Anti-inflammatory
		Kidneys	↘ Hepatic bile acids
PPAR $\beta/\delta$	Unsaturated fatty acids Components of very low-density lipids	Liver (stellate cells, Kupffer cells, hepatocytes)	↗ Anti-inflammatory macrophage polarization
		Skeletal and cardiac muscle	↗ Muscle glucose uptake
		White and brown adipose tissue	↗ Fatty acid $\beta$ -oxidation ↘ Hepatic bile acids
PPAR $\gamma$	Unsaturated fatty acids Prostaglandins	Liver (stellate cells)	↗ Insulin sensitivity
		White and brown adipose tissue	↗ Adipogenic and energy gene expression in adipose tissue
		Pancreas ( $\beta$ -cells)	↗ Adipocyte differentiation
		Skeletal and cardiac muscle	↗ Anti-inflammatory parameters by inhibiting TNF- $\alpha$ , IL-1 $\beta$ and IL-6 production
		Kidneys	in adipose tissue macrophages

↗ : increased; ↘ : decreased; TNF- $\alpha$ , tumor necrosis factor alpha; IL-1 $\beta$ , interleukin 1 beta; IL-6, interleukin 6.

**Table 2. Summary of the impact of PPAR $\alpha$  agonists on muscle and liver fat content.**

Reference	Intervention	Model	Muscles studied	Technique for assessing muscle fat content	Effect of treatment on muscle fat content	Technique for assessing liver fat content	Effect of treatment on liver fat content
Peroxisome proliferator-activated receptor (PPAR)- $\alpha$ Activation Lowers Muscle Lipids and Improves Insulin Sensitivity in High Fat-Fed Rats. (Ye <i>et al.</i> , 2001) [67]	WY14643 3 mg/kg/day for two weeks	Wistar rats (N = 6–9/group)	Quadriceps	Tissue triglyceride extraction	↘	Tissue triglyceride extraction	↘
WY14,643, a Peroxisome-activated Receptor $\alpha$ (PPAR $\alpha$ ) Agonist, Improves Hepatic and Muscle Steatosis and Reverses Insulin Resistance in Lipoatrophic A-ZIP/F-1 Mice. (Chou <i>et al.</i> , 2002) [68]	WY14643 0,1% of the diet for two weeks	A-ZIP/F-1 mice (N = 6/group)	Quadriceps	Tissue triglyceride extraction	↘	Tissue triglyceride extraction	↘
Plasma triglycerides are not related to tissue lipids and insulin sensitivity in the elderly following PPAR $\alpha$ agonist treatment. (Cree <i>et al.</i> , 2007) [69]	Fenofibrate 160 mg/day for 60 days	Insulin-resistant elderly patients (N = 12) Healthy elderly patients (N = 7)	Soleus	<sup>1</sup> H-MRS	↔ (vs baseline) ↔ (vs baseline)	<sup>1</sup> H-MRS	↔ (vs baseline) ↔ (vs baseline)

↗ : increased; ↘ : decreased; ↔ : unchanged; <sup>1</sup>H-MRS: proton magnetic resonance spectroscopy.

**Table 3. Summary of the impact of PPAR $\beta/\delta$  agonists on muscle and liver fat content.**

Reference	Intervention	Model	Muscles studied	Technique for assessing muscle fat content	Effect of treatment on muscle fat content	Technique for assessing liver fat content	Effect of treatment on liver fat content
Retinoic acid increases fatty acid oxidation and Irisin Expression in Skeletal Muscle Cells and Impacts Irisin <i>in vivo</i> (Amen-gual <i>et al.</i> , 2018) [72]	GW0742 (0.1 $\mu$ M) for two days	C2C12 myocytes	/	RT-qPCR of fatty acid oxidation-related genes	<i>CD36</i> $\nearrow$ ; <i>UCP3</i> $\nearrow$ ; <i>CPT1B</i> $\nearrow$ ; <i>FNDC5</i> $\nearrow$	/	/
Triglyceride: High-Density Lipoprotein Cholesterol Effects in Healthy Subjects Administered a Peroxisome Proliferator-Activated Receptor $\delta$ Agonist. (Sprecher <i>et al.</i> , 2007) [73]	GW501516 (10 nmol/L) for two days	Human skeletal muscle cells	/	RT-qPCR of fatty acid oxidation-related genes	<i>CD36</i> $\nearrow$ ; <i>CPT1A</i> $\nearrow$ ; <i>CPT1B</i> $\nearrow$ ; <i>PDK4</i> $\nearrow$	/	/

$\nearrow$  : increased; /: not investigated; RT-qPCR, real-time quantitative polymerase chain reaction; *CD36*, cluster of differentiation 36; *UCP3*, uncoupling protein 3; *CPT1A*, carnitine palmitoyltransferase 1A; *CPT1B*, carnitine palmitoyltransferase 1B; *FNDC5*, fibronectin type III domain-containing protein 5; *PDK4*, pyruvate dehydrogenase kinase 4.

**Table 4. Summary of the impact of PPAR $\gamma$  agonists on muscle and liver fat content.**

Reference	Intervention	Model	Muscles studied	Technique for assessing muscle fat content	Effect of treatment on muscle fat content	Technique for assessing liver fat content	Effect of treatment on liver fat content
Rapid reversal of hepatic steatosis, and reduction of muscle triglyceride, by rosiglitazone: MRI/S studies in Zucker fatty rats. (Hockings <i>et al.</i> , 2003) [79]	Rosiglitazone 3 mg/kg/day for 17 days	Male Zucker fatty rats (N = 8/group)	Tibialis anterior	$^1$ H-MRS	IMCL: $\searrow$ ; EMCL: $\leftrightarrow$	$^1$ H-MRS	$\searrow$
Pioglitazone treatment restores <i>in vivo</i> muscle oxidative capacity in a rat model of diabetes. (Wessels <i>et al.</i> , 2015) [80]	Pioglitazone 30 mg/kg/day for 15 days	Zucker diabetic fatty rats (N = 6/group) Zucker lean rats (N = 6/group)	Tibialis anterior	$^1$ H-MRS	IMCL: $\searrow$ ; EMCL: / IMCL: $\leftrightarrow$ ; EMCL: /	/	/
Effect of PPAR $\gamma$ agonist on aerobic exercise capacity in relation to body fat distribution in men with type 2 diabetes mellitus and coronary artery disease: a 1-yr randomized study. (Bastien <i>et al.</i> , 2019) [81]	Rosiglitazone 8 mg/day for one year	Type 2 diabetes male patients (N = 51–53/group)	Mid-thigh skeletal muscles	CT – <i>surface of low-density muscles</i>	$\nearrow$	/	/

$\nearrow$  : increased;  $\searrow$  : decreased;  $\leftrightarrow$  : unchanged; /: not investigated. IMCL, intramyocellular lipids; EMCL, extramyocellular lipids;  $^1$ H-MRS, proton magnetic resonance spectroscopy; CT, computed tomography.

**Table 5. Summary of the impact of a combination of PPAR agonists on muscle and liver fat content.**

Reference	Intervention	Model	Muscles studied	Technique for assessing muscle fat content	Effect of treatment on muscle fat content	Technique for assessing liver fat content	Effect of treatment on liver fat content
Effects of cevoglitazar, a dual PPAR $\alpha$ /gamma agonist, on ectopic fat deposition in fatty Zucker rats. (Laurent <i>et al.</i> , 2009) [86]	Fenofibrate 150 mg/kg/day for 21 days	Male Zucker fatty rats (N = 10/group)	Tibialis anterior	$^1\text{H-MRS}$	IMCL: $\searrow$ ; EMCL: /	$^1\text{H-MRS}$	$\searrow$
	Pioglitazone 30 mg/kg/day for 21 days				IMCL: $\searrow$ ; EMCL: /		$\leftrightarrow$
	Cevoglitazar 5 mg/kg/day for 21 days				IMCL: $\searrow$ ; EMCL: /		$\searrow$
Discordance between intramuscular triglyceride and insulin sensitivity in skeletal muscle of Zucker diabetic rats after treatment with fenofibrate and rosiglitazone. (Nadeau <i>et al.</i> , 2007) [87]	Fenofibrate 0.1% for 25 weeks	Male Zucker diabetic fatty rats (N = 6/group)	Gastrocnemius	Tissue triglyceride extraction	$\searrow$	/	/
	Rosiglitazone 0.005% for 25 weeks				$\nearrow$		
	Fenofibrate 0.1% + rosiglitazone 0.005% for 25 weeks				$\leftrightarrow$		
Metabolic effects of muraglitazar in type 2 diabetic subjects. (Fernandez <i>et al.</i> , 2011) [88]	Muraglitazar 5 mg/day for 4 months	Type 2 diabetes patients (N = 20)	Tibialis anterior	$^1\text{H-MRS}$	$\searrow$ (vs baseline)	$^1\text{H-MRS}$	$\searrow$ (vs baseline)

$\nearrow$  : increased;  $\searrow$  : decreased;  $\leftrightarrow$  : unchanged; /: not investigated. IMCL, intramyocellular lipids; EMCL, extramyocellular lipids;  $^1\text{H-MRS}$ , proton magnetic resonance spectroscopy.

cluster of differentiation 36 (*CD36*), the carnitine palmitoyltransferase 1B (*CPT1B*) and the uncoupling protein 3 (*UCP3*) genes, which are all involved in lipid catabolism, and an increased expression of *CD36*, a protein that transports fatty acids from the bloodstream to the muscles where they are used for energy [74]. Therefore, GW0742 increases muscle lipid content as a readily available substrate for FAO. Its activation could therefore have the effect of increasing the lipid pool in the muscle participating in increasing myosteatosis. However, the simultaneous activation of this gene with genes such as *CPT1*, whose role is to transport fatty acids into the mitochondria to use them as a fuel source instead of glucose [75], could have a positive impact on muscle fat content. Finally, GW0742 treatment doubled the expression of the fibronectin type III domain-containing protein 5 (*FNDC5*), precursor of irisin, a myokine normally expressed during physical exercise, eventually stimulating lipolysis and adipose tissue browning [76,77].

In the second study [73], cultured human myocytes were treated with 10 nmol/L of the PPAR $\delta$  agonist GW501516 for two days. No cell toxicity occurred since no reduction in cell viability was noted throughout the entire study. Thanks to RT-qPCR an analysis of the expression of genes implicated in FAO such as *CD36*, *CPT1A*, *CPT1B*, and pyruvate dehydrogenase kinase 4 (*PDK4*) was carried out. GW501516 increased the expression of mRNA of *CD36*, *CPT1A*, and *CPT1B* by approximately 2-fold, 5-fold and 3-fold respectively, compared to the vehicle treatment. The *PDK4* gene, which promotes the use of fatty acids as a major source of energy instead of glucose [78], is roughly 200 times more active in response to GW501516 and was barely detectable in vehicle-treated cells. An FAO assay confirmed the use of fatty acids as the principal source of energy with GW501516 upregulating oleate oxidation by a factor of 7 compared to the untreated cells. This suggests that the PPAR $\delta$  agonist will not only ensure the transport of lipids from the circulation to the muscles for storage but will also upregulate fatty acid metabolism.

### PPAR $\gamma$ Agonists and Myosteatosis

Two PPAR $\gamma$  agonists (rosiglitazone and pioglitazone) have been investigated in pre-clinical studies. The impact of rosiglitazone on muscle fat content was also assessed in one clinical study in a cohort of T2DM patients (Table 4, Ref. [79–81]).

In the first pre-clinical study [79], the authors aimed to determine the effects of rosiglitazone, a PPAR $\gamma$  agonist, on hepatic steatosis and IMCL content in Zucker fatty (ZF) rats. Rosiglitazone is a member of the thiazolidinedione family of drugs, which improves whole-body insulin sensitivity and blood glucose levels in patients with T2DM by activating the transcription factor PPAR $\gamma$ . A ZF rat is an animal model in which there is a missense mutation in the leptin receptor gene, simulating obesity without diabetes and various features of the metabolic syndrome such as IR

and increased liver and muscle fat content [82]. The control model in the study is called “Zucker lean” (ZL). Rats aged 26–28 weeks were divided into 3 groups: ZL control, ZF treated rats and ZF control (N = 8 males/group). No maintenance diet restriction was imposed on ZL control and ZF control. However, the treated ZF rats were fed the amount the ZF control rats had eaten the day before, in order to avoid bias caused by diet differences. Rats received either 3 mg/kg/day of rosiglitazone or vehicle treatment for 17 days. Tibialis anterior (TA) IMCL and EMCL contents were assessed by <sup>1</sup>H-MRS at day 17 but not at baseline. Liver steatosis was assessed by <sup>1</sup>H-MRS at baseline and day 17. Both ZF rat groups exhibited similar liver fat content at baseline. As expected, the amount of IMCL, EMCL and liver fat content was much lower in the ZL control group than in both ZF groups. Rosiglitazone decreased at least 40% of IMCL content compared to control ZF rats, but EMCL content remained unchanged. Additionally, rosiglitazone treatment resulted in a marked reduction of hepatic steatosis at day 17, compared to the ZF control group. Authors suggest that these results are consistent with the known adipogenic action of rosiglitazone [83] which leads to increased fat storage capacity and a reduction in circulating free fatty acids and triglycerides, resulting in extrahepatic triglyceride storage and diminished integration of lipid droplets in the cytoplasm of skeletal muscle fibers.

The second pre-clinical study [80] aimed to determine the effect of pioglitazone, another thiazolidinedione-based PPAR $\gamma$  agonist, on *in vivo* and *ex vivo* muscle mitochondrial function in a rat model of diabetes. In this paper, the authors selected 12-week-old Zucker diabetic fatty (ZDF) rats and their lean controls. ZDF rats are a breed derived from the ZF strain and are widely used for studying T2DM associated with obesity since the onset of diabetes in these rats starts as early as 10 weeks of age with 100% of rats being affected at 20 weeks old [84]. Both groups of rats were either administered 30 mg/kg/day of pioglitazone (N = 6/genotype) or were on a strict water-only diet (N = 6/genotype) for 15 days. Once treatment was completed, *in vivo* muscle oxidative capacity was measured in TA muscle by performing a phosphorus MRS, while IMCL levels were assessed by <sup>1</sup>H-MRS. EMCL and liver fat content were not assessed. After 15 days, IMCL content was 14 times higher in the water-treated ZDF rats than in the water-treated lean controls. In ZDF rats, compared to water only, treatment with pioglitazone significantly decreased IMCL (–43%) and remained eight-fold higher than in the water-treated lean controls. In contrast, pioglitazone had no impact on TA IMCL content in lean rats. Finally, phosphorus MRS measurement showed that administration of pioglitazone improved *in vivo* muscle oxidative capacity in ZDF rats, so much so that it was comparable to levels seen in lean rats. Interestingly, this improvement correlated with low plasma triglycerides, low TA IMCL and a normalization of TA  $\beta$ -oxidation which were all initially higher in ZDF rats.

Finally, in a randomized, double-blind, placebo-controlled study [81] researchers investigated the effect of PPAR $\gamma$  agonist rosiglitazone on aerobic exercise capacity in T2DM patients with stable cardiovascular disease. 104 men aged from 45 to 75 years old were randomized into two groups: rosiglitazone (N = 53) or placebo (N = 51). Rosiglitazone was administered at a dosage of 8 mg/day for a period of one year. Mid-thigh muscle fat content was assessed by CT. Measurements were divided into two categories: low-density muscle with hypothetically more abundant fat content, and normal-density muscle with a pre-defined normal fat content. However, this evaluation method did not allow for differentiation between IMCL and EMCL [36]. Liver fat content was not measured. In contrast to the placebo group where no change in the surface of low-density muscles occurred, subjects having received rosiglitazone for one year exhibited a significant increase in the surface of muscles of low-density muscles suggesting an increase in muscle fat content. Moreover, normal-density muscles noted a significant surface decrease. In addition, these changes were associated with improved insulin sensitivity as well as weight gain and marked volume of abdominal subcutaneous fat, a common effect of PPAR $\gamma$  agonist [85]. Low-density muscle surface, suggestive of increased fat infiltration caused by long-term treatment with rosiglitazone could therefore be explained by its adipogenic effect [83], which promotes fat storage not only in the liver but also in skeletal muscles.

### *Combination of PPAR Agonists and Myosteatosis*

Combining PPAR agonists appears to be a promising treatment for MASH [63]. In this section, we will investigate its effect on myosteatosis. The combination of multiple PPAR agonists was investigated in 2 pre-clinical studies and one clinical study (Table 5, Ref. [86–88]).

In the first pre-clinical study [86], authors compared cevoglitazar, a dual PPAR $\alpha/\gamma$  agonist, with isolated administration of a PPAR $\alpha$  agonist (fenofibrate) and a PPAR $\gamma$  agonist (pioglitazone) alone on ectopic fat deposition in ZF rats. Six-week-old male ZF rats were placed on HFD. At eight weeks, rats were either treated with 5 mg/kg of cevoglitazar, 150 mg/kg of fenofibrate, 30 mg/kg of pioglitazone, or with only a vehicle treatment for the control group (N = 10/group). TA IMCL and liver fat contents were assessed by  $^1\text{H}$ -MRS on days 0 and 21. EMCL levels were not assessed. After 21 days, compared to the vehicle treatment, all three PPAR agonists significantly reduced IMCL levels, and to the same extent. Only fenofibrate and cevoglitazar reduced liver fat content, with cevoglitazar being the most effective.

In another pre-clinical study [87], researchers worked on the basis that a correlation between muscle fat content and IR was found in the previous study [89] in order to study the effect of fenofibrate, rosiglitazone and their combination on muscle triglyceride levels in insulin-resistant ZDF

rats. To do so, they selected male ZDF rats (N = 24) and their lean controls (N = 6) of the same age. From the age of six weeks, rats were exposed to a mixture of standard food with either 0.1% fenofibrate, 0.005% rosiglitazone, a combination of both drugs or no treatment at all for 25 weeks (N = 6 per group). After the treatment period, gastrocnemius triglyceride content was measured by triglyceride extraction. Liver fat content was not recorded. As expected, muscle triglycerides were lower in lean control rats than in ZDF control rats. In this study, only isolated treatment with fenofibrate resulted in a significant reduction in gastrocnemius muscle triglyceride content in ZDF rats, compared to untreated ZDF rats. The association of fenofibrate and rosiglitazone did not significantly lower gastrocnemius triglyceride content in ZDF rats. Moreover, muscle triglyceride levels increased in ZDF rats treated with rosiglitazone alone when compared to untreated ZDF. Interestingly, when combined with fenofibrate, the increase in muscle triglycerides caused by rosiglitazone was limited. All three treatment groups saw a decrease in serum glucose and insulin levels, despite an increase in muscle triglyceride levels due to rosiglitazone, suggesting that muscle fat content and insulin sensitivity are not correlated. Authors suggest that a combination of PPAR $\alpha$  and PPAR $\gamma$  could be an ideal treatment for T2DM, by limiting the increase in weight and triglyceride content in adipose tissue and muscle caused by PPAR $\gamma$  alone, while decreasing insulin resistance.

In this last double-blind, placebo-controlled clinical study [88], authors investigated the effect of the PPAR $\alpha/\gamma$  agonist, muraglitazar, on T2DM patients. They selected 27 patients with T2DM, with good general health and without evidence of other chronic diseases. All patients had stable body weight for a period of 3 months, took 6 months of antidiabetic treatment, and none participated in any exercise program on a regular basis. Patients were randomized into 2 groups: 5 mg/day of muraglitazar (N = 20) or placebo (N = 7) for 4 months. TA muscle and liver fat content were assessed by  $^1\text{H}$ -MRS. The results showed that TA IMCL and liver fat content significantly decreased in the muraglitazar group but not in the placebo group. These changes were also accompanied by an increase in the hepatic insulin sensitivity index, as well as a reduction in liver transaminases.

## Discussion

The findings of this research provide insight into how PPAR agonists could affect muscle fat content even though only a limited number (N = 11) of relevant articles pertaining to our research question were identified. Furthermore, among these original manuscripts, only three were clinical studies. This highlights a gap in the literature when it comes to the interaction of PPAR agonists with muscle fat infiltration, despite the existence of a variety of treatments that have been used for several years. This could be explained by the relatively recent emergence of the concept

of myosteatorosis and its even more recent association with MASH. Indeed, myosteatorosis is a recent term, generating a growing interest in the field of MASLD [36,46].

Collectively, the results show that PPAR agonists, isolated and in combination, are promising treatments for MASH-associated disorders such as myosteatorosis. Alone, each PPAR agonist allowed a significant reduction of both myosteatorosis [67,68,79,80,86–88] and liver steatorosis [67,68,79,86,88] or activation of  $\beta$ -oxidation genes in the muscle in the case of PPAR $\beta/\delta$  agonists [72,73]. These findings give a clue into whether PPAR agonists might have an effect on myosteatorosis by mechanisms we would not have initially suspected. The few discrepancies observed appear to be associated with long-term treatment with PPAR $\gamma$  agonists [81,87] and could be explained by the differences in assessment method (CT for example) [81] and/or by the possible adipogenic effect of these treatments [83]. More importantly, this adipogenic effect can be countered with the concomitant administration of PPAR $\alpha$  agonists [87]. In humans, the net effect of a PPAR $\alpha$  agonist may be less marked if the initial muscle fat content is low [69]. It should also be borne in mind that, on average, PPAR $\alpha$  expression in human hepatocytes is estimated to be 5–10% of the levels found in rodent hepatocytes [90]. Finally, a drop in density on CT with PPAR $\gamma$  agonists [81] is not necessarily a sign of fat overload and could possibly be linked to sodium and water retention, a common characteristic of this pharmacological therapy [91].

Even though myosteatorosis could be simply defined as fat infiltration in skeletal muscle [92], the diagnostic criteria for myosteatorosis are not well-established and there is currently no consensus on the diagnosis method or the cut-off values above which muscle fat content is abnormal or pathological [36,38]. Muscle fat content differs significantly within a population depending on various determinants such as body composition [93], degree of physical activity and muscle fiber type [94]. Although a high muscle fat infiltration is strongly associated with IR [95] and MASH [36], the limit at which this fat infiltration becomes pathological remains unclear. Furthermore, we cannot investigate myosteatorosis without considering its physiological levels in healthy individuals. For example, higher muscle lipid concentrations can be found in endurance athletes compared to sprinters or untrained patients, probably due to a myocellular adaptation to energy demand [96]. Muscle fiber composition may also play a crucial role in lipid accumulation and metabolism in the muscle. Indeed, muscle fat content is related to muscle fiber type and seems to be greater in fibers that are more oxidative (“slow-twitch” type 1 fibers) compared to more glycolytic ones (“fast-twitch” type 2 fibers-rich muscles) [94]. In humans, type 1 fibers are characterized by a high oxidative capacity and more resistance to fatigue. Type 2A fibers predominantly use oxidative metabolism, while type 2X and 2B fibers use glycolytic metabolism [97]. The composition of these muscles also differs between humans and mice [98,99]. In this

work, the studies include both human and animal models, as well as cell lines. All the previously mentioned characteristics could have influenced muscle lipid levels and their impact on metabolic health. Thus, the results observed in one model may not be predictive of the outcome in another.

This lack of consensus in the diagnosis of myosteatorosis was highlighted in the reviewed manuscripts, as there was a disparity in the assessment methods. At present, the reference technique for assessing myosteatorosis is  $^1\text{H-MRS}$  [36]. Some of the reviewed articles also used tissue triglyceride extraction [67,68,87], which implies post-sacrifice tissue harvesting and therefore makes initial analysis impossible. CT is also used [83] but involves exposure to radiation, does not differentiate between IMCL and EMCL in muscle tissue, and, above all, only measures muscle density [36] a parameter that can vary with the amount of muscle fat, water, perfusion and protein synthesis [100]. CT imaging is therefore not the test of choice for robust assessment of myosteatorosis [36,100]. In addition, PPAR $\gamma$  agonists, for example, have shown benefits on muscle protein synthesis [101] and could therefore explain a direct effect on muscle density, unrelated to myosteatorosis. Interestingly, some researchers have also used phosphorus magnetic resonance spectroscopy to dynamically evaluate muscle mitochondrial function [80]. This is considered the gold standard for assessing *in vivo* muscle oxidative metabolism and certain muscular pathologies in humans [102] and could lead to further understanding of the pathophysiology of steatorotic liver diseases. In the clinical study evaluating lanifibranor in patients with fibrosing MASH [63], body composition and in particular skeletal muscles were not examined. According to the results of our systematic review, it is possible that the beneficial effects seen on the liver are associated with benefits in terms of reduction of myosteatorosis. Compared to individual selective PPAR activators, lanifibranor provides an interesting synergistic effect in preclinical models [103,104], in particular by targeting the activation of hepatic macrophages [104] which play a key role in the pathogenesis of MASH and IR [25,105–107]. The effect this molecule could have on muscle would therefore be interesting to study and would support the hepatic (muscle-liver axis) and extra-hepatic (insulin resistance in particular) benefits associated with the treatment.

The limitations of our analysis are mainly linked to the fact that, in the research manuscript collected, the joint study of the severity of liver damage and fatty infiltration of the muscles were not the initial objectives of the studies. In addition, a precise study of liver histology has not been carried out, although it is known that it is particularly the severe form of MASLD (MASH) that is associated with myosteatorosis and that is only defined histologically. Another factor could be the effect of PPAR agonists via a mechanism of action different from those usually described and evaluated (fatty acid oxidation, anti-inflammation). For example, the reduction in bile acid synthesis obtained with PPAR $\alpha$ - $\delta$  agonists could potentially

be beneficial. Changes in bile acid composition with an increase in systemic bile acids are indeed associated with MASH [108]. The other limitations of this analysis are the usual limitations of systematic reviews, namely the risks of selection bias, publication bias, and selective communication of results, as well as the risks of inconsistency due to the heterogeneity of the models and results. Finally, the presence of an external author with methodological expertise would be an additional asset.

### Conclusion

PPAR agonists, and more specifically pan-PPAR agonists have emerged as a promising treatment for MASLD/MASH. They could also have a positive impact on MASH extrahepatic complications such as myosteatosis, through their action on FAO and inflammation. However, further research on humans is necessary to establish cutoff values for the diagnosis of myosteatosis and use them as a baseline to fully assess the effectiveness of treatments on both MASH and muscle fat infiltration. Given the strong link that exists between MASH and myosteatosis, it follows that a future treatment that is active in MASH would also have a beneficial impact on muscle composition. Precise evaluation of future therapies for MASH, using spectroscopy for example, should be considered, given the muscle-liver axis suspected in the pathogenesis of the disease.

### Availability of Data and Materials

Not applicable.

### Author Contributions

NB and NL conceived and designed the analysis; NB collected the data, performed the analysis, and wrote the first draft of the manuscript; GH and GB were involved in data curation; NL, GH and GB revised and edited the manuscript for intellectual content and interpretation of the data; NL supervised the work; all authors revised final version and gave approval for publication; all authors agreed to be accountable for all aspects of the work.

### Ethics Approval and Consent to Participate

Not applicable.

### Acknowledgment

We would like to thank Charles De Smet from UCLouvain for his advice on bibliographic research and data interpretation.

### Funding

This work was supported by a concerted research action grant “Myo-SLD” 22/27-122. Nicolas Lanthier has a mandate of Clinical Researcher from the Fonds de la Recherche Scientifique (FNRS), Belgium.

### Conflict of Interest

Nicolas Lanthier is serving as Editor-in-Chief of this journal. We declare that NL had no involvement in the peer review of this article and had no access to information regarding its peer review. NL received speaker fees from Gilead Sciences, Fresenius Kabi and Orphalan; received travel grants from Abbvie, Gilead Sciences and Norgine; received grants from Gilead Sciences. Other authors have no conflict of interest to declare.

### References

- [1] Arroyo-Johnson C, Mincey KD. Obesity Epidemiology Worldwide. *Gastroenterology Clinics of North America*. 2016; 45: 571–579.
- [2] Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet*. 2011; 378: 815–825.
- [3] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016; 64: 73–84.
- [4] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, *et al*. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. *Journal of Hepatology*. 2023; 64: 73–84.
- [5] Lazarus JV, Mark HE, Allen AM, Arab JP, Carrieri P, Noureddin M, *et al*. A global research priority agenda to advance public health responses to fatty liver disease. *Journal of Hepatology*. 2023; 79: 618–634.
- [6] Lanthier N, Francque S. NASH: a welfare disease with emerging questions and adequate answer attempts. *Acta Gastroenterologica Belgica*. 2020; 83: 339.
- [7] Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, *et al*. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *The Lancet. Gastroenterology & Hepatology*. 2022; 7: 851–861.
- [8] Brown GT, Kleiner DE. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Metabolism: Clinical and Experimental*. 2016; 65: 1080–1086.
- [9] Lanthier N. La NASH en 2018. *Louvain Med*. 2018; 137: 308–313.
- [10] Kopec KL, Burns D. Nonalcoholic fatty liver disease: a review of the spectrum of disease, diagnosis, and therapy. *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition*. 2011; 26: 565–576.
- [11] Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic Steatohepatitis: A Review. *JAMA*. 2020; 323: 1175–1183.
- [12] Ratziu V, Francque S, Sanyal A. Breakthroughs in therapies for NASH and remaining challenges. *Journal of Hepatology*. 2022; 76: 1263–1278.
- [13] Lanthier N. New therapies in non-alcoholic steatohepatitis | Les nouveaux traitements de la stéatohépatite non-alcoolique. *Nutrition Clinique et Métabolisme*. 2020; 34: 216–222.

- [14] Harrison SA, Loomba R, Dubourg J, Ratziu V, Noureddin M. Clinical Trial Landscape in NASH. *Clinical Gastroenterology and Hepatology: the Official Clinical Practice Journal of the American Gastroenterological Association*. 2023; 21: 2001–2014.
- [15] Byrne CD, Targher G. NAFLD: a multisystem disease. *Journal of Hepatology*. 2015; 62: S47–S64.
- [16] Kasper P, Martin A, Lang S, Kütting F, Goeser T, Demir M, *et al.* NAFLD and cardiovascular diseases: a clinical review. *Clinical Research in Cardiology: Official Journal of the German Cardiac Society*. 2021; 110: 921–937.
- [17] Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *European Heart Journal*. 2012; 33: 1190–1200.
- [18] Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *The New England Journal of Medicine*. 2010; 363: 1341–1350.
- [19] Targher G, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut*. 2020; 69: 1691–1705.
- [20] Duell PB, Welty FK, Miller M, Chait A, Hammond G, Ahmad Z, *et al.* Nonalcoholic Fatty Liver Disease and Cardiovascular Risk: A Scientific Statement From the American Heart Association. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2022; 42: e168–e185.
- [21] Chen X, Chen S, Pang J, Huang R, You Y, Zhang H, *et al.* Hepatic steatosis aggravates atherosclerosis via small extracellular vesicle-mediated inhibition of cellular cholesterol efflux. *Journal of Hepatology*. 2023; 79: 1491–1501.
- [22] Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments. *Nature Reviews. Gastroenterology & Hepatology*. 2021; 18: 599–612.
- [23] Binet Q, Loumaye A, Hermans MP, Lanthier N. A Cross-sectional Real-life Study of the Prevalence, Severity, and Determinants of Metabolic Dysfunction-associated Fatty Liver Disease in Type 2 Diabetes Patients. *Journal of Clinical and Translational Hepatology*. 2023; 11: 1377–1386.
- [24] Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis. *Diabetes & Metabolism*. 2008; 34: 643–648.
- [25] Lanthier N, Molendi-Coste O, Horsmans Y, van Rooijen N, Cani PD, Leclercq IA. Kupffer cell activation is a causal factor for hepatic insulin resistance. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2010; 298: G107–G116.
- [26] Seppälä-Lindroos A, Vehkavaara S, Häkkinen AM, Goto T, Westerbacka J, Sovijärvi A, *et al.* Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *The Journal of Clinical Endocrinology and Metabolism*. 2002; 87: 3023–3028.
- [27] Stefan N, Häring HU. The role of hepatokines in metabolism. *Nature Reviews Endocrinology*. 2013; 9: 144–152.
- [28] Lanthier N, Lebrun V, Molendi-Coste O, van Rooijen N, Leclercq IA. Liver Fetuin-A at Initiation of Insulin Resistance. *Metabolites*. 2022; 12: 1023.
- [29] Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients*. 2013; 5: 1544–1560.
- [30] Binet Q, Loumaye A, Preumont V, Thissen JP, Hermans MP, Lanthier N. Non-invasive screening, staging and management of metabolic dysfunction-associated fatty liver disease (MAFLD) in type 2 diabetes mellitus patients: what do we know so far? *Acta Gastro-enterologica Belgica*. 2022; 85: 346–357.
- [31] Verboven K, Wouters K, Gaens K, Hansen D, Bijnen M, Wetzels S, *et al.* Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. *Scientific Reports*. 2018; 8: 4677.
- [32] Yu SJ, Kim W, Kim D, Yoon JH, Lee K, Kim JH, *et al.* Visceral Obesity Predicts Significant Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Medicine*. 2015; 94: e2159.
- [33] Lanthier N, Leclercq IA. Adipose tissues as endocrine target organs. *Best Practice & Research. Clinical Gastroenterology*. 2014; 28: 545–558.
- [34] Lanthier N, Lefere S, De Schepper H, Reenaers C. Clinical nutrition, skeletal muscle and liver disease: linking the dots for good management. *Acta Gastro-enterologica Belgica*. 2023; 86: 267–268.
- [35] Ebadi M, Tsien C, Bhanji RA, Dunichand-Hoedl AR, Rider E, Motamedrad M, *et al.* Myosteatosis in Cirrhosis: A Review of Diagnosis, Pathophysiological Mechanisms and Potential Interventions. *Cells*. 2022; 11: 1216.
- [36] Henin G, Loumaye A, Leclercq IA, Lanthier N. Myosteatosis: Diagnosis, pathophysiology and consequences in metabolic dysfunction-associated steatotic liver disease. *JHEP Reports*. 2023; 6: 100963.
- [37] Collino M, Mastrocola R, Nigro D, Chiazza F, Aragno M, D'Antona G, *et al.* Variability in myosteatosis and insulin resistance induced by high-fat diet in mouse skeletal muscles. *BioMed Research International*. 2014; 2014: 569623.
- [38] Goffaux A, Delorme A, Dahlqvist G, Lanthier N. Improving the prognosis before and after liver transplantation: Is muscle a game changer? *World Journal of Gastroenterology*. 2022; 28: 5807–5817.
- [39] Henin G, Lanthier N, Dahlqvist G. Pathophysiological changes of the liver-muscle axis in end-stage liver disease: what is the right target? *Acta Gastro-enterologica Belgica*. 2022; 85: 611–624.
- [40] Clarembeau F, Bale G, Lanthier N. Cirrhosis and insulin resistance: current knowledge, pathophysiological mechanisms, complications and potential treatments. *Clinical Science (London, England: 1979)*. 2020; 134: 2117–2135.
- [41] Kitajima Y, Hyogo H, Sumida Y, Eguchi Y, Ono N, Kuwashiro T, *et al.* Severity of non-alcoholic steatohepatitis is associated with substitution of adipose tissue in skeletal muscle. *Journal of Gastroenterology and Hepatology*. 2013; 28: 1507–1514.
- [42] Guo W, Zhao X, Cheng D, Liang X, Miao M, Li X, *et al.* Muscle Fat Content Is Associated with Nonalcoholic Fatty Liver Disease and Liver Fibrosis in Chinese Adults. *The Journal of Nutrition, Health & Aging*. 2023; 27: 960–965.
- [43] Lanthier N, Rodriguez J, Nacht M, Hiel S, Trefois P, Neyrinck AM, *et al.* Microbiota analysis and transient elastography reveal new extra-hepatic components of liver steatosis and fibrosis in obese patients. *Scientific Reports*. 2021; 11: 659.
- [44] Nacht M, Lanthier N, Rodriguez J, Neyrinck AM, Cani PD, Bindels LB, *et al.* A dynamic association between myosteatosis and liver stiffness: Results from a prospective interventional study in obese patients. *JHEP Reports*. 2021; 3: 100323.
- [45] Hsieh YC, Joo SK, Koo BK, Lin HC, Lee DH, Chang MS, *et al.* Myosteatosis, but not Sarcopenia, Predisposes NAFLD Subjects to Early Steatohepatitis and Fibrosis Progression. *Clinical Gastroenterology and Hepatology: the Official Clinical Practice Journal of the American Gastroenterological Association*. 2023; 21: 388–397.e10.
- [46] Henin G, Loumaye A, Deldicque L, Leclercq IA, Lanthier N. Unlocking liver health: Can tackling myosteatosis spark remission in metabolic dysfunction-associated steatotic liver disease? *Liver International*. 2024.
- [47] Machado MV, Ferreira DMS, Castro RE, Silvestre AR, Evangelista T, Coutinho J, *et al.* Liver and muscle in morbid obesity: the interplay of fatty liver and insulin resistance. *PloS One*. 2012; 7: e31738.

- [48] Nachit M, Kwanten WJ, Thissen JP, Op De Beeck B, Van Gaal L, Vonghia L, *et al.* Muscle fat content is strongly associated with NASH: A longitudinal study in patients with morbid obesity. *Journal of Hepatology*. 2021; 75: 292–301.
- [49] Herbison GJ, Jaweed MM, Ditunno JF. Muscle fiber types. *Archives of Physical Medicine and Rehabilitation*. 1982; 63: 227–230.
- [50] Umek N, Horvat S, Cvetko E. Skeletal muscle and fiber type-specific intramyocellular lipid accumulation in obese mice. *Bosnian Journal of Basic Medical Sciences*. 2021; 21: 730–738.
- [51] Naimo MA, Varanoske AN, Hughes JM, Pasiakos SM. Skeletal Muscle Quality: A Biomarker for Assessing Physical Performance Capabilities in Young Populations. *Frontiers in Physiology*. 2021; 12: 706699.
- [52] Hausman GJ, Basu U, Du M, Fernyhough-Culver M, Dodson MV. Intermuscular and intramuscular adipose tissues: Bad vs. good adipose tissues. *Adipocyte*. 2014; 3: 242–255.
- [53] Zeng J, Fan JG, Francque SM. Therapeutic management of metabolic dysfunction associated steatotic liver disease. *United European Gastroenterology Journal*. 2024; 12: 177–186.
- [54] André-Dumont SI, Lanthier N. Quelle alimentation proposer aux patients présentant une stéatohépatite non-alcoolique? *Nutrition Clinique et Métabolisme*. 2022; 36: 12–20.
- [55] Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, *et al.* Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology*. 2015; 149: 367–378.e5; quiz e14–5.
- [56] Sung KC, Ryu S, Lee JY, Kim JY, Wild SH, Byrne CD. Effect of exercise on the development of new fatty liver and the resolution of existing fatty liver. *Journal of Hepatology*. 2016; 65: 791–797.
- [57] Staels B, Butruille L, Francque S. Treating NASH by targeting peroxisome proliferator-activated receptors. *Journal of Hepatology*. 2023; 79: 1302–1316.
- [58] Kersten S. Peroxisome proliferator activated receptors and lipoprotein metabolism. *PPAR Research*. 2008; 2008: 132960.
- [59] Marrapodi M, Chiang JY. Peroxisome proliferator-activated receptor alpha (PPARalpha) and agonist inhibit cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription. *Journal of Lipid Research*. 2000; 41: 514–520.
- [60] Chen J, Montagner A, Tan NS, Wahli W. Insights into the Role of PPAR $\beta/\delta$  in NAFLD. *International Journal of Molecular Sciences*. 2018; 19: 1893.
- [61] Kouno T, Liu X, Zhao H, Kisseleva T, Cable EE, Schnabl B. Selective PPAR $\delta$  agonist seladelpar suppresses bile acid synthesis by reducing hepatocyte CYP7A1 via the fibroblast growth factor 21 signaling pathway. *The Journal of Biological Chemistry*. 2022; 298: 102056.
- [62] Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. *Journal of Advanced Pharmaceutical Technology & Research*. 2011; 2: 236–240.
- [63] Francque SM, Bedossa P, Ratziu V, Anstee QM, Bugianesi E, Sanyal AJ, *et al.* A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. *The New England Journal of Medicine*. 2021; 385: 1547–1558.
- [64] Han E, Kim MK, Lee HW, Ryu S, Kim HS, Jang BK, *et al.* Muscle fat contents rather than muscle mass determines nonalcoholic steatohepatitis and liver fibrosis in patients with severe obesity. *Obesity (Silver Spring)*. 2022; 30: 2440–2449.
- [65] Méndez-Sánchez N, Bugianesi E, Gish RG, Lammert F, Tilg H, Nguyen MH, *et al.* Global multi-stakeholder endorsement of the MAFLD definition. *The Lancet. Gastroenterology & Hepatology*. 2022; 7: 388–390.
- [66] Lanthier N, Vanuytsel T. Metabolic dysfunction-associated fatty liver disease: a new clearer nomenclature with positive diagnostic criteria. *Acta Gastro-enterologica Belgica*. 2020; 83: 513–515.
- [67] Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. *Diabetes*. 2001; 50: 411–417.
- [68] Chou CJ, Haluzik M, Gregory C, Dietz KR, Vinson C, Gavrilova O, *et al.* WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice. *The Journal of Biological Chemistry*. 2002; 277: 24484–24489.
- [69] Cree MG, Newcomer BR, Read LK, Sheffield-Moore M, Paddon-Jones D, Chinkes D, *et al.* Plasma triglycerides are not related to tissue lipids and insulin sensitivity in elderly following PPAR-alpha agonist treatment. *Mechanisms of Ageing and Development*. 2007; 128: 558–565.
- [70] Burri L, Thoresen GH, Berge RK. The Role of PPAR $\alpha$  Activation in Liver and Muscle. *PPAR Research*. 2010; 2010: 542359.
- [71] Reddy JK. Nonalcoholic steatosis and steatohepatitis. III. Peroxisomal beta-oxidation, PPAR alpha, and steatohepatitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2001; 281: G1333–G1339.
- [72] Amengual J, García-Carrizo FJ, Arreguín A, Mušinić H, Granados N, Palou A, *et al.* Retinoic Acid Increases Fatty Acid Oxidation and Irisin Expression in Skeletal Muscle Cells and Impacts Irisin In Vivo. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*. 2018; 46: 187–202.
- [73] Sprecher DL, Massien C, Pearce G, Billin AN, Perlstein I, Willson TM, *et al.* Triglyceride:high-density lipoprotein cholesterol effects in healthy subjects administered a peroxisome proliferator activated receptor delta agonist. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007; 27: 359–365.
- [74] Son NH, Basu D, Samovski D, Pietka TA, Peche VS, Willecke F, *et al.* Endothelial cell CD36 optimizes tissue fatty acid uptake. *The Journal of Clinical Investigation*. 2018; 128: 4329–4342.
- [75] Mazzarelli P, Pucci S, Bonanno E, Sesti F, Calvani M, Spagnoli LG. Carnitine palmitoyltransferase I in human carcinomas: a novel role in histone deacetylation? *Cancer Biology & Therapy*. 2007; 6: 1606–1613.
- [76] Xiong XQ, Chen D, Sun HJ, Ding L, Wang JJ, Chen Q, *et al.* FND5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. *Biochimica et Biophysica Acta*. 2015; 1852: 1867–1875.
- [77] Norheim F, Langlete TM, Hjorth M, Hølen T, Kielland A, Stadheim HK, *et al.* The effects of acute and chronic exercise on PGC-1 $\alpha$ , irisin and browning of subcutaneous adipose tissue in humans. *The FEBS Journal*. 2014; 281: 739–749.
- [78] Pettersen IKN, Tusubira D, Ashrafi H, Dyrstad SE, Hansen L, Liu XZ, *et al.* Upregulated PDK4 expression is a sensitive marker of increased fatty acid oxidation. *Mitochondrion*. 2019; 49: 97–110.
- [79] Hockings PD, Changani KK, Saeed N, Reid DG, Birmingham J, O'Brien P, *et al.* Rapid reversal of hepatic steatosis, and reduction of muscle triglyceride, by rosiglitazone: MRI/S studies in Zucker fatty rats. *Diabetes, Obesity & Metabolism*. 2003; 5: 234–243.
- [80] Wessels B, Ciapaite J, van den Broek NMA, Houten SM, Nicolay K, Prompers JJ. Pioglitazone treatment restores in vivo muscle oxidative capacity in a rat model of diabetes. *Diabetes, Obesity & Metabolism*. 2015; 17: 52–60.
- [81] Bastien M, Poirier P, Brassard P, Arsenault BJ, Bertrand OF, Després JP, *et al.* Effect of PPAR $\gamma$  agonist on aerobic exercise capacity in relation to body fat distribution in men with type 2 diabetes mellitus and coronary artery disease: a 1-yr random-

- ized study. *American Journal of Physiology-Endocrinology and Metabolism*. 2019; 317: E65–E73.
- [82] Zucker LM, Zucker TF. Fatty, a new mutation in the rat. *Journal of Heredity*. 1961; 52: 275–278.
- [83] Teruel T, Hernandez R, Rial E, Martin-Hidalgo A, Lorenzo M. Rosiglitazone up-regulates lipoprotein lipase, hormone-sensitive lipase and uncoupling protein-1, and down-regulates insulin-induced fatty acid synthase gene expression in brown adipocytes of Wistar rats. *Diabetologia*. 2005; 48: 1180–1188.
- [84] Yokoi N, Hoshino M, Hidaka S, Yoshida E, Beppu M, Hoshikawa R, *et al*. A Novel Rat Model of Type 2 Diabetes: The Zucker Fatty Diabetes Mellitus ZFDM Rat. *Journal of Diabetes Research*. 2013; 2013: 103731.
- [85] Fonseca V. Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *The American Journal of Medicine*. 2003; 115: 42S–48S.
- [86] Laurent D, Gounarides JS, Gao J, Boettcher BR. Effects of cevoglitazar, a dual PPAR $\alpha$ /gamma agonist, on ectopic fat deposition in fatty Zucker rats. *Diabetes, Obesity & Metabolism*. 2009; 11: 632–636.
- [87] Nadeau KJ, Ehlers LB, Aguirre LE, Reusch JEB, Draznin B. Discordance between intramuscular triglyceride and insulin sensitivity in skeletal muscle of Zucker diabetic rats after treatment with fenofibrate and rosiglitazone. *Diabetes, Obesity & Metabolism*. 2007; 9: 714–723.
- [88] Fernandez M, Gastaldelli A, Triplitt C, Hardies J, Casolaro A, Petz R, *et al*. Metabolic effects of muraglitazar in type 2 diabetic subjects. *Diabetes, Obesity & Metabolism*. 2011; 13: 893–902.
- [89] Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, *et al*. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a <sup>1</sup>H NMR spectroscopy study. *Diabetologia*. 1999; 42: 113–116.
- [90] Holden PR, Tugwood JD. Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. *Journal of Molecular Endocrinology*. 1999; 22: 1–8.
- [91] Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, *et al*. PPAR $\gamma$  signaling and metabolism: the good, the bad and the future. *Nature Medicine*. 2013; 19: 557–566.
- [92] Miljkovic I, Zmuda JM. Epidemiology of myosteatosis. *Current Opinion in Clinical Nutrition and Metabolic Care*. 2010; 13: 260–264.
- [93] Moro C, Galgani JE, Luu L, Pasarica M, Mairal A, Bajpeyi S, *et al*. Influence of gender, obesity, and muscle lipase activity on intramyocellular lipids in sedentary individuals. *The Journal of Clinical Endocrinology and Metabolism*. 2009; 94: 3440–3447.
- [94] Shaw CS, Swinton C, Morales-Scholz MG, McRae N, Erfte-meyer T, Aldous A, *et al*. Impact of exercise training status on the fiber type-specific abundance of proteins regulating intramuscular lipid metabolism. *Journal of Applied Physiology*. 2020; 128: 379–389.
- [95] Li Y, Xu S, Zhang X, Yi Z, Cichello S. Skeletal intramyocellular lipid metabolism and insulin resistance. *Biophysics Reports*. 2015; 1: 90–98.
- [96] Nakagawa Y, Hattori M. Intramyocellular lipids of muscle type in athletes of different sport disciplines. *Open Access Journal of Sports Medicine*. 2017; 8: 161–166.
- [97] Plotkin DL, Roberts MD, Haun CT, Schoenfeld BJ. Muscle Fiber Type Transitions with Exercise Training: Shifting Perspectives. *Sports*. 2021; 9: 127.
- [98] Augusto V, Padovani CR, Campos GER. Skeletal muscle fiber types in C57BL6J mice. *Journal of Morphological Sciences*. 2004; 21: 2004.
- [99] Scott W, Stevens J, Binder-Macleod SA. Human skeletal muscle fiber type classifications. *Physical Therapy*. 2001; 81: 1810–1816.
- [100] Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *Journal of Applied Physiology*. 2000; 89: 104–110.
- [101] Crossland H, Constantin-Teodosiu D, Greenhaff PL. The Regulatory Roles of PPARs in Skeletal Muscle Fuel Metabolism and Inflammation: Impact of PPAR Agonism on Muscle in Chronic Disease, Contraction and Sepsis. *International Journal of Molecular Sciences*. 2021; 22: 9775.
- [102] Meyerspeer M, Boesch C, Cameron D, Dezortová M, Forbes SC, Heerschap A, *et al*. <sup>31</sup>P magnetic resonance spectroscopy in skeletal muscle: Experts’ consensus recommendations. *NMR in Biomedicine*. 2020; 34: e4246.
- [103] Wettstein G, Luccarini JM, Poekes L, Faye P, Kupkowski F, Adarbes V, *et al*. The new-generation pan-peroxisome proliferator-activated receptor agonist IVA337 protects the liver from metabolic disorders and fibrosis. *Hepatology Communications*. 2017; 1: 524–537.
- [104] Lefere S, Puengel T, Hundertmark J, Penners C, Frank AK, Guillot A, *et al*. Differential effects of selective- and pan-PPAR agonists on experimental steatohepatitis and hepatic macrophages. *Journal of Hepatology*. 2020; 73: 757–770.
- [105] Gadd VL, Skoien R, Powell EE, Fagan KJ, Winterford C, Horsfall L, *et al*. The portal inflammatory infiltrate and ductular reaction in human nonalcoholic fatty liver disease. *Hepatology*. 2014; 59: 1393–1405.
- [106] Lanthier N. Targeting Kupffer cells in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Why and how? *World Journal of Hepatology*. 2015; 7: 2184–2188.
- [107] Etienne Q, Lebrun V, Komuta M, Navez B, Thissen JP, Leclercq IA, *et al*. Fetuin-A in Activated Liver Macrophages Is a Key Feature of Non-Alcoholic Steatohepatitis. *Metabolites*. 2022; 12: 625.
- [108] Ferslew BC, Xie G, Johnston CK, Su M, Stewart PW, Jia W, *et al*. Altered Bile Acid Metabolome in Patients with Nonalcoholic Steatohepatitis. *Digestive Diseases and Sciences*. 2015; 60: 3318–3328.