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Exercise counteracts lipotoxicity by improving lipid turnover and lipid droplet quality

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Abstract. Zacharewicz E, Hesselink MKC, Schrauwen P (Maastricht University Medical Center, Maastricht). Exercise counteracts lipotoxicity by improving lipid turnover and lipid droplet quality (Key Symposium). *J Intern Med* 2018; **284**: 505–518.

The incidence of obesity and metabolic disease, such as type 2 diabetes mellitus (T2D), is rising globally. Dietary lipid over supply leads to lipid accumulation at ectopic sites, such as skeletal muscle. Ectopic lipid storage is highly correlated with insulin resistance and T2D, likely due to a loss of metabolic flexibility – the capacity to switch between fat and glucose oxidation upon insulin stimulation – and cellular dysfunction because of lipotoxicity. However, muscle lipid levels are also elevated in endurance-trained athletes, presenting a paradoxical phenotype of increased intramuscular lipids along with high insulin sensitivity – the 'athletes' paradox'. This review focuses on recent human data to characterize intramuscular lipid species in order to elucidate some of the underlying mechanisms driving skeletal muscle lipotoxicity. There is evidence that lipotoxicity is characterized by an increase in bioactive lipid species, such as ceramide. The athletes' paradox supports the notion that regular physical exercise has health benefits that might originate from the alleviation of lipotoxicity. Indeed, exercise training alleviates intramuscular ceramide content in obese individuals without a necessary decrease in ectopic lipid storage. Furthermore, evidence shows that exercise training elevates markers of lipid droplet dynamics such as the PLIN proteins, and triglyceride lipases ATGL and HSL, as well as mitochondrial efficiency, potentially explaining the improved lipid turnover and a reduction in the accumulation of lipotoxic intermediates observed with the athelets' paradox.

Keywords: athletes' paradox, exercise, lipotoxicity, obesity, skeletal muscle, type 2 diabetes mellitus (T2D).

Introduction

Global levels of individuals' body weights are rising at epidemic proportions. The World Health Organization (WHO) reported a doubling in world obesity levels in 2016 since 1980 [1]. In 2016, 39% of adults were overweight (BMI $\geq$ 25), and 13% of adults were obese (BMI $\geq$ 30) [1]. The fundamental cause of excessive weight is an imbalance between energy consumption and energy expenditure. Overweight and obesity are risk factors for metabolic diseases including insulin resistance (IR) and type 2 diabetes mellitus (T2D), heart disease and various cancers. Although the underlying biological mechanisms that link obesity to metabolic disease are not completely understood, it is obvious that the flexibility in substrate utilization for energy provision becomes progressively impaired in overweight and obese individuals.

Metabolic flexibility is the degree to which metabolic tissues, such as muscle, liver and the heart, can switch between substrates for essential ATP production based on acute changes to nutrient availability and energy demands. Impaired metabolic flexibility is highly correlated with insulin resistance [2] and interventions that result in improved metabolic flexibility also tend to alleviate insulin resistance and vice versa. During fasting conditions, such as between meals, plasma insulin levels are low, and therefore, tissue glucose oxidation is low. Under these conditions, most energy is

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derived from the oxidation of fatty acids. Thus, plasma glucose levels can be maintained and serve to fuel several organs and tissues, such as the brain that mainly relies on glucose as a source for energy production. In the fasted condition, adipose tissue lipolysis is stimulated, liberating free fatty acids into the blood stream as a consequence. The free fatty acids are taken up by metabolic tissues and utilized for energy production via fat oxidation.

On the other hand, following a mixed meal there is a surge in plasma glucose followed by a release of insulin into the plasma. Insulin-sensitive tissues respond to the insulin by taking up glucose from the circulation. The glucose taken up by these tissues is either stored within the tissues or oxidized to produce ATP. When tissues become insulin resistant a state of metabolic inflexibility occurs resulting in a reduced clearance of glucose from the circulation postprandially. Indeed, it has been shown that obese and T2D patients have a markedly reduced metabolic flexibility, which could be largely attributed to a reduced metabolic flexibility at the level of the skeletal muscle [3]. Therefore, understanding the link between overweight or obesity and metabolic inflexibility may be important in the development of strategies to fight metabolic diseases. Lipotoxicity is believed to be a contributor to metabolic inflexibility. Lipotoxicity is defined as cellular dysfunction resulting from the excessive and ectopic accumulation of lipids in the cell.

One of the best strategies to improve metabolic flexibility is via exercise training. People participating in habitual exercise training are typically of good metabolic health, with high metabolic flexibility. Understanding how adaptations to exercise training lead to improved management of lipids and prevention of lipotoxicity may allow the identification of novel therapeutic targets for the treatment of insulin resistance.

Ectopic fat accumulation

In humans, by far, the largest fat storage site is within white adipocytes. A white adipocyte, from now on referred to as adipocyte, is composed of a large lipid droplet, occupying approximately 90% of the cellular space [4, 5]. The remaining cellular space is occupied by a nucleus and other organelles, including mitochondria. Adipose tissue not only stores fatty acids as triacylglycerol, but also possesses endocrine functions, with the ability to produce and release cytokines (adipokines) including leptin, adiponectin, resistin, acylation stimulating protein, tumor necrosis factor-α, plasminogen activator inhibitor-1 and interleukin-6 [6]. In general, adipokines are proposed to play two main roles, one being to direct whole-body metabolism and the other in inflammatory processes that may occur in the obese state when adipose tissue function is impaired.

Lipids are important in many biological processes such as facilitating hormone synthesis and synthesis of phospholipids that make up cellular membranes. Adipose tissue stores triacylglycerol to fuel metabolic processes upon demand. The latter is the primary reason for lipid storage in adipocytes, that is to provide energy to other tissues when needed. Following lipolysis nonesterified fatty acids (NEFA) are released from adipocytes into circulation and taken up by other tissues including skeletal muscle, liver, heart, kidney and pancreas. In these tissues, lipids are primarily utilized for energy production; however, there is also the capacity for lipid storage at these ectopic sites, probably for the short-term buffering of excessive fat coming into the system. In the healthy state, this so-called ectopic fat accumulation is, under most conditions, rather low, except for the skeletal muscle where the storage of lipids within the muscle cells serves as a readily available substrate source for the mitochondria upon muscle contraction/physical activity. Particularly, long-term endurance exercise activities are characterized by a reliance on the use of intramuscular triglyceride stores for energy production.

Obesity is the result of over nutrition that leads to a net-positive energy balance. When there is a positive energy balance fat storage increases within adipocytes resulting in either hypertrophy, hyperplasia or both [7]. Although the human body has a large capacity for the storage of fat in adipose tissue, ectopic lipid storage is especially prevalent in overweight and obese people, and is believed to be the result of the limited expandability and proliferation of adipocytes [8]. Hence, fatty acids spill over from the adipose tissue into the circulation to be taken up by peripheral tissues. Especially in these ectopic fat stores, fat storage may lead to lipotoxicity. Indeed, hepatic, cardiac and muscular steatosis are linked to diseases such fatty liver disease, steatohepatitis, cirrhosis, heart disease, heart failure, IR and T2D [9–11].

The athletes' paradox

Skeletal muscle from highly trained athletes is characterized with high levels of ectopic fat

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storage in muscle – intramuscular lipid (IMCL) droplets – at a comparable level to individuals with T2D [12]. Moderate-to-high intensity exercise gives rise to a high energy demand at the exercising muscle, and the IMCL may benefit the exercising tissue by supplying free fatty acids at the site of the energy demand [13, 14]. The combined presence of high levels of IMCL along with a high insulin sensitivity is referred to as the 'athletes' paradox'. According to the lipotoxicity theory, IMCLs are associated with metabolic complications. However, athletes presenting with increased IMCL do not possess these same metabolic issues and in fact have improved insulin sensitivity when compared to individuals with insulin resistance and similar IMCL levels, and lean, sedentary people with lower levels of IMCL [12, 15].

In recent years, efforts have been made to unpack the contradictory health outcomes associated with IMCL. In these attempts, the leading hypothesis has been that IMCL *per se* is not lipotoxic, but that IMCL is a marker for the accumulation of other bioactive fatty acid intermediates within ectopic fat stores, such as within skeletal muscle. Thus, several studies have examined diacylglyceride (DAG) and ceramide levels, intermediates of fatty acid metabolism, in subjects with varying metabolic health statuses, including endurance-trained athletes [15–24]. The published data from these studies, however, are not conclusive. While one study reports significantly lower intramuscular saturated DAG levels in athletes compared to a lean sedentary group [15] another reported increased levels of DAG, including saturated DAGs, in their athlete cohort [16]. Intramuscular DAGs have also been reported to be lower in obese subjects compared to lean controls [20, 24]. Inconsistencies are also found for the level of ceramides in skeletal muscle, albeit to a lesser extent. Thus, intramuscular ceramides were reported to be higher in several obese cohorts compared to a lean, sedentary group [16, 21–24] and an athlete group [20]. Bergman *et al.* [17], however, found no difference in muscle ceramides in obese subjects compared to athletes. The link between intramuscular ceramides and insulin resistance may be more robust as elevated intramuscular ceramide levels have been reported in patients with T2D [17] and people at risk of developing T2D [19, 22], whereas no such reports with direct data on DAG levels in T2D patients have been made. Notably, the saturated C:18 ceramide species is repeatedly found to be elevated in the muscle from IR and T2D patients [17, 19, 22]. Thus, the athletes' paradox may involve a role for specific fatty acid intermediates; however, the concept is not completely unravelled and is more complex than initially thought. The initial and pioneering studies either used histological approaches using lipid soluble dyes that stain any type of neutral lipids [12, 16, 25–27] or took advantage of noninvasive ^{1}H-NMR spectroscopy to quantify the CH_2 peaks in the ^{1}H spectra, predominantly reflecting fatty acid acyl chains [28–30]. None of these methods measures the so-called bioactive lipids like DAG or ceramides. To measure bioactive lipids, more advanced mass spectrometry on lipids extracted from the host tissue is warranted. However, upon extracting the lipids important information on the cellular location of these bioactive lipids is lost. Another complicating issue, when it comes to DAG analysis, is the subcellular origin of the respective optical isomers of DAG. Incomplete hydrolysis of TAG in lipid droplets (LD) will result in elevated DAG levels. Importantly, though, ATGL-mediated hydrolysis of TAGs in LDs results in sn-2,3 DAG or sn-1,3 DAG [31], whereas the DAG subtype with recognized insulin desensitizing properties is sn-1,2 DAG [32, 33], originating from phospholipid synthesis. For complete comprehension of the complex relationship between lipid moieties and insulin resistance, novel technology like *in situ* application of imaging mass spectrometry can be very helpful [34]. The role of fatty acid intermediates in insulin resistance has been reviewed extensively elsewhere [35].

As an alternative explanation for the athletes' paradox, it has been proposed that the turnover of lipid stores inside the skeletal muscle is a key determinant of lipotoxicity. As such, the nature of lipid storage and breakdown-related enzymes, mitochondrial characteristics and LD characteristics has been the focus of much investigation in order to elucidate the mechanism of lipotoxicity. It has been reported that there is no difference in the intramuscular expression of TAG/DAG biosynthesis-related proteins glycerol-3-phosphate acyltransferase (GPAT), phosphatidate phosphatase (PAP-1) and DAG acyltransferase (DGAT) between healthy, lean and insulin resistant, obese subjects [24]. There is also no difference in ceramide/sphingosine synthesis proteins stearoyl-CoA desaturase-1 (SCD-1), serine palmitoyltransferase (SPT), ceramide synthase, sphingomyelinase and ceramidase.

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Protein phosphatase 2A (PP2A) is upregulated in skeletal muscle of obese subjects concomitant with elevated ceramide levels. PP2A is a direct target of ceramide [36], and its activation by ceramide results in a downregulation of the insulin signalling pathway [37]. While the hydrolysis of TAGs by adipose triglyceride lipase (ATGL) appears to be normal in skeletal muscle of obese individuals, DAG hydrolysis is reduced, possibly due to a reduction in hormone-sensitive lipase (HSL) [20], providing a mechanistic explanation as to how myocellular DAG levels can be elevated. In line, it was also found that HSL content in muscle is reduced in T2D subjects [20]. The rate-limiting step of fatty acid oxidation is at the level of mitochondrial entry, a process facilitated by the enzyme carnitine palmitoyl transferase (CPT). Interestingly, skeletal muscle CPT activity is reduced in obese individuals compared to lean individuals [38]. Thus, lipid breakdown activity in muscle is reduced in obesity and T2D, and there is also reduced lipid input into the mitochondria; two potential mechanistic explanations for the apparent metabolic inflexibility.

In more recent years, research has focused on the way lipids are stored at ectopic sites, such as skeletal muscle. Thus, fatty acids are stored in small lipid droplets that are surrounded by a package of lipid droplet coating proteins. Lipid droplet characteristics, such as size, proximity to mitochondria and expression of proteins involved in the regulation of LD lipolysis, may therefore play a role in governing the efficiency of LD turnover in ectopic lipid sites. The perilipin (PLIN) protein family is one of the most abundant protein families located on LDs. PLIN2, PLIN3 and PLIN5 are muscle expressed PLINs and the most studied in terms of their role in promoting LD hydrolysis in skeletal muscle. PLIN2 and PLIN3 are considered important for the formation of LDs [39, 40] and PLIN3 for budding off of nascent LDs from the endoplasmic reticulum. PLIN5, on the other hand, regulates lipolysis and also associates LDs to the mitochondria [41, 42]. In human muscle, PLIN5 has been shown to be highly associated with ATGL (94 ± 10%) [43]. Work from our laboratory shows that PLIN5 protein is more abundant in muscle from trained individuals compared to lean sedentary, obese and T2D patients [44] suggesting an important role in diminishing lipotoxic effects of muscle lipids. Athletes also present with a higher number of LDs. LD number and PLIN5-associated LDs correlate positively with VO_{2max}, a marker for muscle oxidative capacity [44]. Figure 1 is a series of immunofluorescent images from our laboratory showing the differences in LD dynamics between athletes and patients with T2D.

Table 1 summarizes the data on IMCL, LD characteristics and lipid processing markers. In general, differences in health statuses (sedentary, trained, obese and T2D) present with different intramuscular lipid signatures concomitant with differences in regulators of lipid storage and utilization; overall in more healthy conditions, lipid droplet dynamics (storage and release of fatty acids from the droplets) and the protein machinery needed for this tend to be better matched towards oxidative needs. However, deciphering how lipid droplet dynamics is involved in disease development and progression remains a major challenge.

A role for oxidative capacity in explaining the athletes' paradox

A striking difference between athletes and obese and insulin-resistant subjects, who are characterized by similar levels of IMCL, is the oxidative capacity of the muscle. Thus, we and others have shown that T2D patients are characterized by a reduced mitochondrial function [45–48]. One of the hypotheses to explain the athletes' paradox therefore is that not the level of intramuscular lipids *per se*, but their turnover is important. Obviously, one of the determinants of a high lipid droplet turnover is having a high oxidative capacity. Thus, a high oxidative capacity can be hypothesized to prevent lipid-induced lipotoxicity. To test this hypothesis, acute lipid and hyperinsulin infusion studies, a model that mimics the circulating factors of obesity, IR and T2D and induces IR [49, 50], have been used to elucidate the fate of lipids and metabolic effects. Data from our laboratory show that acute lipid infusion combined with hyperinsulinemia leads to IR in young endurance trained and lean sedentary subjects [51] even within 3 h. In the trained group, however, the IR was only ~29% and was accounted for by a reduction in insulin-stimulated glucose oxidation, whereas in the untrained subjects, both insulin-stimulated glucose oxidation and nonoxidative glucose disposal (glycogen storage) were impeded, leading to an overall 70% decrease in insulin sensitivity. We have also reported impaired oxidative and nonoxidative glucose disposal in subjects with T2D in association with increased circulating NEFA levels [45]. Another study reported similar outcomes when studying an obese, lean sedentary and trained

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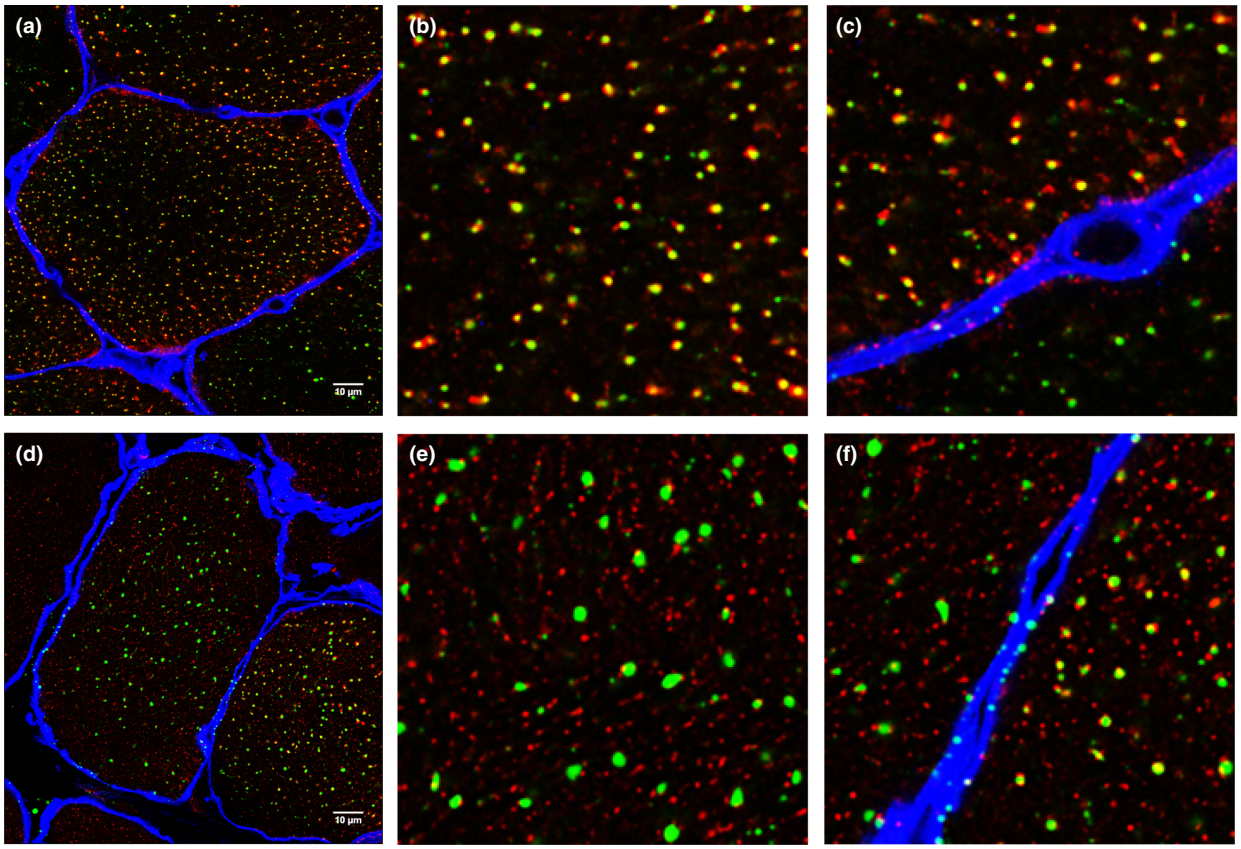

Fig. 1 *Cross-sectional immunofluorescent image of a human m. vastus lateralis section of a type I muscle fibre from a trained athlete (a, b. c) and a patient with T2D (d, e, f). Lipid droplets are stained in green, the lipid droplet coat protein PLIN5 is stained in red, and the basement membrane protein laminin is stained in blue. These images exemplify a few of the characteristic differences related to lipotoxicity and fat storage between insulin-sensitive trained individuals and insulin-resistant sedentary individuals. In the trained state, the fat is stored in more lipid droplets that are smaller (panel a). A large proportion of lipid droplets are associated with PLIN5 (yellow, panel b) and not membrane associated (panel c). Individuals with T2D present with fewer but larger lipid droplets (panel d). More cytosolic PLIN5 is observed with less association with lipid droplets (panel e). A greater number of lipid droplets are found within the vicinity of the cellular membrane (panel f).*

group [52]. All three subject groups developed IR upon lipid infusion, but the obese group had a greater degree of IR compared to the other two groups. While glucose oxidation did not change, nonoxidative glucose disposal was reduced in the sedentary and obese group but increased in the athletes. Possibly reflective of good metabolic flexibility, the lipid infusion did not increase IMCL levels in the athletes, whereas in the sedentary subjects, IMCL levels were increased [51]. Although it is tempting to speculate that this higher fat oxidative capacity prevents the accumulation of lipid intermediates, levels of specific lipid species have unfortunately not been reported in most of these studies. One study did report on specific IMCL species and found that lipid infusion specifically increased intramuscular DAGs in lean, sedentary but not trained athletes [53].

As another model to elevate plasma NEFA levels, we employed a 60-h fasting protocol to induce insulin resistance [54]. Upon fasting, IMCL levels were also increased with a specific increase in the number and size of PLIN5-associated LDs. PLIN5-positive LD size negatively correlated with fasting-induced reduction in ADP-driven state-3 respiration and maximal uncoupled respiration [54]. Taken together, this suggests that PLIN5 protects the muscle from fasting-induced reduction in

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Table 1 *Human studies investigating intramuscular lipid storage and intramuscular lipid storage regulators and markers of mitochondrial function. Studies were included on the basis that they investigated intramuscular lipid storage and/or intramuscular lipid storage regulators. ↑ indicates increased expression; ↓ indicates decreased expression; → indicates no change*

Subjects	Lipid species	LD characteristics	Lipid processing units	Reference
Lean, sedentary (LS) Endurance trained (ET)	↑IMTG (ET) →Saturated IMTG ↓C16:0, C16:1; ↑C18:0, C18:2 ↓Saturated DAG (ET) ↓C16:0, C16:1, C18:0; ↑C18:1, C18:2 →Phospholipids ↓C16:0, C18:2; ↑C18:0, C18:1		↑SCD-1 (ET)	Bergman B. C. *et al.* [15]
Lean, sedentary (LS) Endurance trained (ET)	→IMCL	↑PLIN2 (ET) ↑PLIN3 (ET) ↑PLIN5 (ET)		Shepherd S. O. *et al.* [59]
Lean, sedentary (LS) Endurance trained (ET) Obese (OB)	↑IMCL (ET & OB) ↑DAG (ET) ↑Saturated DAG (ET) ↑Mono-unsaturated DAG (ET) ↑Poly-unsaturated DAG (OBS) ↑C14:0/18:0, C16:0/18:0, C16:0/18:1, C16:1/18:0, C18:0/18:1, DI-C18:0; ↓DI-C16:1 (ET) ↑C16:1/18:1, DI-C14:0, DI-C16:1; ↓C16:0/18:0, DI-C18:0 (OB) ↑Ceramide, saturated and unsaturated (OB) ↑C18:1, C24:0, C24:1, DHC16; ↓C14:0 (OB) ↓Sphingosine ↑Sphingosine-1-phosphate	↑LD density (ET & OB)	↑mitochondrial density (ET) ↑SDH (ET) ↓SDH (OB) ↑PLIN5 (ET) ↓PLIN5 (OB) ↑SCD1 (ET) ↑ATGL (ET)	Amati, F. *et al.* [16]
Endurance trained (ET) Obese (OB)			↑ATGL (OB) →CGI-58 ↓HSL (OB)	Jocken, J. W. *et al.* (Study 1) [20]
Lean non-smoking (LS) Lean smoking (LSm)	→TAG ↑Saturated TAG (LSm) ↑C16:0, C16:1 (LSm) →DAG ↑Saturated DAG (LSm) ↓C18:1 (LSm)		↑IRS-1 (LSm)	Bergman, B. C. *et al.* [18]

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Table 1 (*Continued*)

Subjects	Lipid species	LD characteristics	Lipid processing units	Reference
Lean, sedentary (LS) Obese (OB)	↑Ceramide (OB) ↑Mono- and polyunsaturated (OB) ↑C20:0, C20:4 (OB) →DAG ↓Mono-unsaturated (OB) ↓C22:6 (OB)		→SCD-1 →SREBP-1c →mtGPAT →DGAT →PAP-1 ↑PP2A (OB) →SPT →ceramide synthase →acid ceramidase →CERK →A-SMase →N-SMase	Thrush, A. B. *et al.* [24]
Lean, sedentary (LS) Obese (OB)	↑Ceramide (OB)			Adams, J. M. *et al.* [21]
Lean, sedentary (LS) Obese (OB)			↑PFK (OB) ↑GAPDH (OB) ↓COX (OB) ↑FABP (OB) ↓CPT (OB)	Simoneau, J. A. *et al.* [38]
Lean, sedentary (LS) Obese/Obese diabetic (OB/OBD)	↑IMCL (OB/OBD) ↑DAG 18:1 (OB/OBD) ↑Saturated ceramide (OB/OBD)		↓DAG hydrolysis (OB/OBD)	Moro, C. *et al.* [22]
Endurance trained (ET) Obese (OB) Diabetic (T2D)	→ceramides ↑C18:0 (T2D) ↑C24:0 (ET)			Bergman, B. C. *et al.* [17]
Healthy control (HC) Non-obese Diabetic (NOD) Obese diabetic (OBD)			↓ATGL (NOD) ↑ATGL (OBD) ↓HSL (OBD)	Jocken, J. W. *et al.* (Study 2) [20]
Endurance trained (ET) Lean, sedentary (LS) Obese (OB) Diabetic (T2D)	↑IMCL (ET & T2D)	↑LD number (ET) →LD size ↑PLIN5 (ET)		Gemmink, A. *et al.*[44]
Lean, sedentary (LS) Lean, sedentary T2D offspring (LOff) Obese (OB) Obese diabetic (OBD)	↑TAG (OB & OBD) ↑DAG (OB & OBD) ↑Ceramide (LOff & OB) ↑↑Ceramide(OBD) ↓Sphingomyelin(OBD)		↑Sphingomyeinase (OBD) ↑Sphinganine (OBD) ↓Ceramidase (LOff)	Straczkowski, M. *et al.* [23]

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mitochondrial function possibly through maintained flux of fatty acid oxidation.

Effects of acute and chronic exercise on lipotoxicity

Having a good understanding of the differences in lipid content characteristics between athletes and type 2 diabetes patients, what can be referred to as two extremes at the spectrum of metabolic health, provides valuable information regarding the underlying molecular mechanisms of lipotoxicity. However, whilst generating such data may be helpful for the identification of therapeutic targets, it is also important to realize that exercise *per se* is one of the best strategies to treat and prevent T2D. Therefore, it is also important to understand the effects of acute exercise bouts and long-term exercise programmes on endogenous lipids and the progression of metabolic disease.

Acute exercise is known to transiently increase the capacity for glucose uptake in skeletal muscle, amongst others via the activation of AMPK. Interestingly, acute exercise may also have effects on several intramuscular lipid species. We and others have shown an acute bout of exercise reduces IMCL content in healthy individuals [30, 55–57]. This reduction can be accounted for by a reduction in LD density and size [56]. Specifically, from pre- to postexercise, there is a significant reduction in size for PLIN2-associated LDs and no change in PLIN2-negative LDs. This suggests that there is preferential oxidation of PLIN2-associated lipids with acute exercise. Another study reports no change in PLIN5-associated LD before and after an acute exercise bout [43]. However, IMCL was not reduced with the exercise bout, potentially due to insufficient exercise intensity or duration or a combination of both for the recreationally active cohort that was used. Few studies have investigated the effect of acute exercise on IMCL in insulin resistant or T2D patients. One hour of endurance training per day reduces IMCL content after 3 days in T2D, healthy sedentary and trained individuals [58]. On the other hand, lipid intermediates, ceramide and dihydroceramides, are increased immediately after exercise in patients with T2D, obese participants and endurance-trained athletes. Thus, in this postexercise period that is characterized by elevated glucose uptake, bioactive insulin desensitizing lipids appear elevated. During recovery, however, total ceramides and several long-chain saturated ceramide species are significantly reduced compared to baseline in the T2D cohort [17]. Sphingosine and sphingosine-1-phosphate followed a similar pattern, with an increase immediately postexercise and return to baseline during recovery in all the subjects. Understanding how these acute changes affect metabolic health outcomes is one of the upcoming challenges we are facing.

While acute exercise has the potential to reduce muscle lipid content, chronic exercise training augments muscle lipid storage. However, endurance-trained athletes and obese insulin-resistant volunteers vary in many long-term metabolic adaptations, and examining the effect of chronic exercise training in populations prone to developing diabetes may reveal more interesting information regarding the role of lipotoxicity in the development of insulin resistance. PLIN2, PLIN3 and PLIN5 protein expressions are higher in trained subjects compared to sedentary subjects [59, 60], and PLIN5 expression is also higher in trained compared to obese[16] and trained compared to T2D [44]. Endurance exercise training increases ATGL protein expression and HSL activation, by phosphorylation, concomitant with a reduction in IMCL in both lean [61] and obese subjects [62]. It is hypothesized that also with chronic exercise training the reduction in TAG intermediates, such as DAGs and ceramides, may explain the beneficial effects of exercise training, such as improvement in insulin sensitivity [63, 64]. Indeed, a 16-week aerobic training programme in older, overweight and obese subjects resulted in an improvement in insulin sensitivity concomitant with an increase in IMCL but with significant reductions in muscle DAG and ceramides [25]. Succinate dehydrogenase (SDH), a marker for oxidative capacity, and glycogen content were both increased following the training protocol. This indicates that mitochondrial function and nonoxidative glucose disposal were both improved in this subject cohort. Eight weeks of exercise training in obese individuals also resulted in a reduction in IMCL content [62], more specifically in a reduction in ceramides and a nearly significant reduction in total DAGs [65].

While the role of intramuscular lipid species in the muscle is not completely known, current literature certainly points to elevated ceramide as the more notorious player in metabolic disease. Of note, the C18:0 ceramide species that is elevated in IR and T2D patients [17, 19, 22] is reduced 2 h after an acute exercise bout [17] but also after at least 4 weeks of training in obese subjects [63, 65]. This

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Table 2 *Human studies investigating intramuscular lipid storage and intramuscular lipid storage regulators and markers of mitochondrial function following exercise intervention. Studies were included on the basis that they investigated intramuscular lipid storage and/or intramuscular lipid storage regulators. ↑ indicates increased expression; ↓ indicates decreased expression; → indicates no change*

Subjects	Exercise	Lipid species	LD characteristics	Lipid processing units	Reference
Lean, sedentary	Acute 1-h cycle ergometer 65% of VO_{2max}	↓IMCL	↓PLIN2 ↓LD density ↓ PLIN2-positive LD ↑PLIN2-negative LD		Shepherd, S. O. *et al.* [56]
Recreationally active	Acute 1-h cycle ergometer 60% of VO_{2max}	→IMCL	→PLIN5		Mason, R. R. *et al.* [43]
Endurance trained (ET) Obese (OB) Diabetic (T2D)	Acute 1.5-h cycle ergometer 50% of VO_{2max}	↑Sphingosine-1-phosphate ↓Ceramide, recovery (T2D) ↓C18:0, C22:0, C23:0, C24:0, recovery (T2D) ↓C23:0, recovery (OB) ↑C14:0, C16:0, C18:0, exercise (T2D) ↓C16:0, exercise (ET)			Bergman, B. C. *et al.* [17]
Endurance trained (ET) Lean, sedentary (LS) Diabetic (T2D)	Acute, 7 days 1-h cycle ergometer 60% of VO_{2max}	↓IMCL, 3 days			Ith, M. *et al.* [58]
Lean, sedentary	Chronic, 8 weeks Cycle ergometer 3–5 sessions/week 40- to 90-min sessions Intermittent exercise 2- to 6-min duration at 45%–55% and 60%–85% VO_{2max}	↓IMCL		↑ATGL ↑p-HSL	Alsted, T. J. *et al.* [61]

Table 2 (*Continued*)

Subjects	Exercise	Lipid species	LD characteristics	Lipid processing units	Reference
Lean, sedentary (LS) Obese (OB)	Chronic, 12 weeks Cycle ergometer 2–3 sessions/week 30- to 60-min sessions 50%–70% VO_{2max}		↑PLIN5		Peters, S. J. *et al.* [68]
Obese	Chronic, 4 weeks Sprint interval training (SIT), cycle ergometer 3 sessions/week 4–7 sprints/week 30 s 200% W_{max}, 2 min recovery 30 W Moderate intensity continuous training (MICT), cycle ergometer 5 sessions/week 40- to 60-min sessions 65% VO_{2max}	→IMCL ↓Ceramide ↓C18:0 ↑DAG C18:1/18:2, C18:0/18:2	↑PLIN2 ↑PLIN3 ↑PLIN5	↑COX	Shepherd, S. O. *et al.* [63]
Obese	Chronic, 8 weeks Cycle ergometer 5 sessions/week 1-h sessions 65%–70% VO_{2max}	→TAG ↓DAG C16:0 ↓Ceramide ↓C16:0, C16:1, C18:1, C18:2, C20:0		↑CPT	Bruce, C. R. *et al.* [65]
Obese	Chronic, 8 weeks Cycling and running 5 sessions/week Progressive 35%–85% VO_{2max}	↓IMCL	↑PLIN3 ↑PLIN5	↑ATGL ↑p-HSL	Louche, K. *et al.* [62]
Obese	Chronic, 16 weeks Walking and cycling 4–5 sessions/week 45-min sessions 75% max heart rate	↑IMCL ↓DAG ↓Ceramide		↑SDH	Dube, J. J. *et al.* [25]
Type 2 diabetics	Chronic, 6 months Walking, cycling, cross-country skiing 3 sessions/week 40-min sessions 75% VO_{2max}	↑IMCL	↑PLIN2 →LD size	↑COX	Shaw, C. S. *et al.* [67]

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is in line with another study in obese individuals showing that – although IMCL levels were unaltered after 4 weeks of training – insulin sensitivity was improved along with a reduction in intramyocellular ceramides [63]. Interestingly, ceramide levels correlated negatively with PLIN expression [63] suggesting that the expression of PLINs is important for the promotion of neutral lipid storage rather than bioactive lipid intermediates.

In obese subjects, 4 weeks of exercise training also results in increased PLIN2, PLIN3 and PLIN5 expression and increased mitochondrial size and number [63]. Interestingly, mitochondrial size has been positively correlated with insulin sensitivity [66]. As a result of the increase in mitochondrial size, an increase in LDs associated with mitochondria was found, which in turn correlated positively with PLIN levels [63]. The timing and sequence of the respective adaptive responses of mitochondria, LDs and their coating proteins remain to be elucidated. In a study examining the longer term effects of exercise training on muscle lipid metabolism in T2D patients, PLIN2 muscle expression was increased after 8 weeks of endurance training while IMCL elevations were only observed after 24 weeks of training [67]. However, the relationship between muscle PLIN expression and exercise training is not

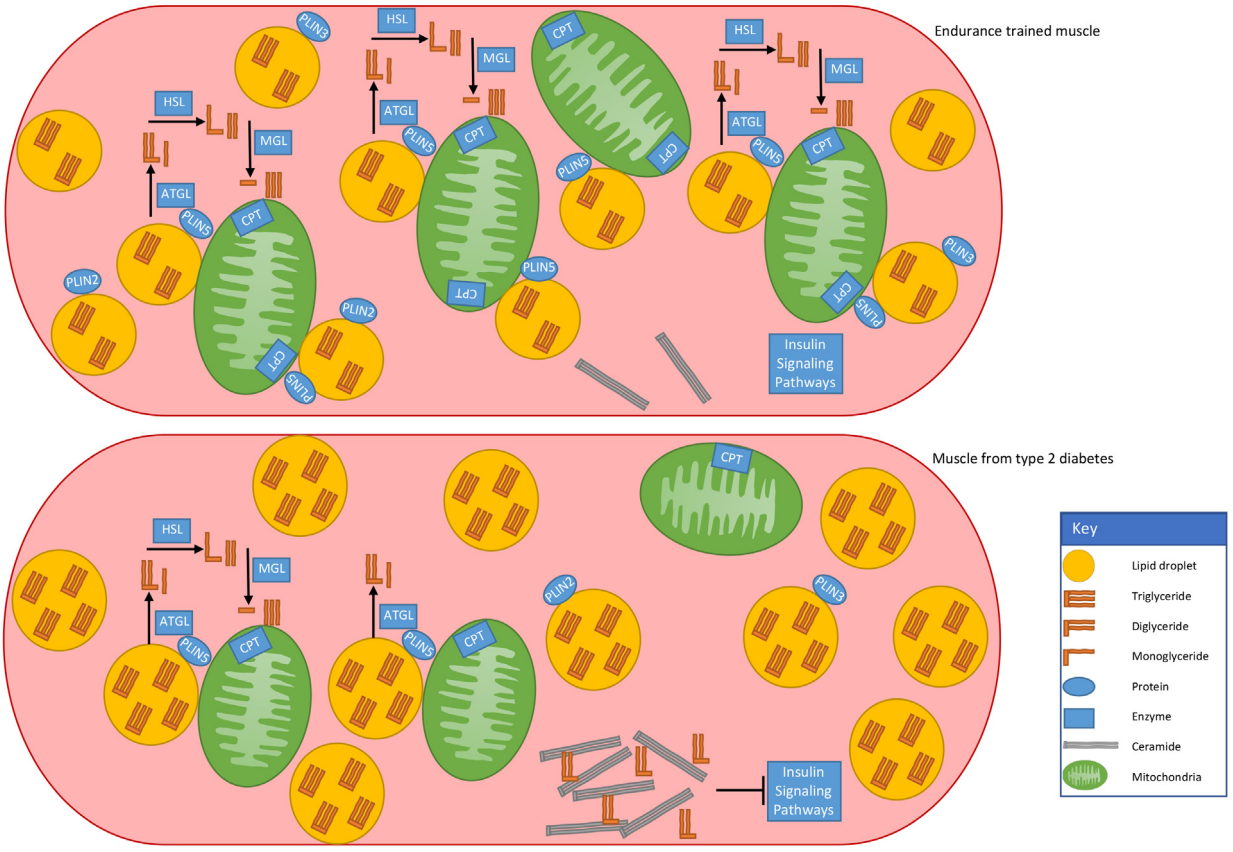

Fig. 2 *Schematic representation of lipid processing within an endurance-trained muscle vs a type 2 diabetic muscle. The endurance-trained muscle is characterized by a greater number of PLIN proteins and PLIN-associated LDs and PLIN-associated mitochondria. Endurance training also increases HSL activity and CPT inside the mitochondria and mitochondrial size and number. Therefore, lipid hydrolysis and oxidation are more efficient in endurance-trained muscle. Ceramides are kept low, and insulin signalling is maintained. In T2D, there is less PLIN and LD-mitochondria association and reduced HSL activity. Lipid hydrolysis is inefficient and lipid intermediates such as ceramides and DAGs accumulate, inhibiting insulin signalling pathways.*

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consistently reported as 8 weeks of training increased PLIN3 and PLIN5 expression, but not PLIN2 in obese subjects [62], although PLIN2 was increased in T2D patients with 8-weeks of training [67], and 12 weeks of exercise training resulted only in increased PLIN5 in obese subjects [68]. Table 2 summarizes the effects of exercise on IMCL, LD characteristics and lipid processing markers.

It is also interesting to note that changes in insulin sensitivity and intramuscular lipids are not always accompanied by weight loss or even body fat loss [63, 65] suggesting that fat promoting turnover of ectopic lipids or redistribution of fat away from ectopic sites towards inert storage in adipocytes is of importance in the pathogenesis of metabolic disease rather than loss of fat mass *per se*. Exercise-induced improvements in mitochondrial function and fat oxidative capacity may be responsible for keeping fatty acid intermediates low in skeletal muscle, suggesting that the turnover of intramuscular triglycerides, including their controlled release from the lipid droplet, transport towards the mitochondria and intramitochondrial combustion is an important determinant of skeletal muscle lipotoxicity. Figure 2 is a schematic representation of how lipid processing may occur in an exercise trained and T2D muscle tissue.

Conclusion

Lipotoxicity is characterized by an increase in ectopic lipid storage, not necessarily of triglycerides, but bioactive insulin desensitizing lipid species such as ceramides. Ceramides play a role in suppressing insulin signalling pathways and are thereby factors that impede insulin sensitivity. Accumulation of muscle lipids due to lipid over supply results in a reduction in lipid hydrolysis efficiency. Therefore, lipotoxicity is the failure of muscle to deal with excess lipid uptake. Acute exercise has immediate beneficial effects such as reducing intramuscular lipids in obese and T2D patients, thereby stimulating lipid hydrolysis and oxidation. However, muscle adaptations to long-term exercise programmes are important in improving health outcomes and reversing insulin resistance. Exercise programmes increase muscle PLIN and lipid hydrolysing enzymes. Furthermore, toxic lipid species such as ceramides are reduced upon physical exercise training, alleviating the inhibition of insulin signalling pathways and thus contributing to improving insulin sensitivity.

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Conflict of interest statement

No conflicts of interest to declare.

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