High intramuscular triglyceride turnover rates and the link to insulin sensitivity: Influence of obesity, type 2 diabetes and physical activity

J.S. Barrett[1], K.L. Whytock[2], J.A. Strauss[1], A.J.M. Wagenmakers[1], S.O. Shepherd[1]

[1]Liverpool John Moores University, [2]Translational Research Institute, AdventHealth, Orlando, FL 32804, USA.

Address for correspondence:
Dr Sam Shepherd
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Tom Reilly Building
Byrom Street
Liverpool
L3 3AF

Abstract

Large intramuscular triglyceride (IMTG) stores in sedentary, obese individuals have been linked to insulin resistance, yet well-trained athletes exhibit high IMTG levels whilst maintaining insulin sensitivity. Contrary to previous assumptions, it is now known that IMTG content *per se* does not result in insulin resistance. Rather, insulin resistance is caused, at least in part, by the presence of high concentrations of harmful lipid metabolites, such as diacylglycerols and ceramides in muscle. Several mechanistic differences between obese sedentary individuals and their highly trained counterparts have been identified, that determine the differential capacity for IMTG synthesis and breakdown in these populations. In this review, we first describe the most up-to-date mechanisms by which a low IMTG turnover rate (both breakdown and synthesis) leads to the accumulation of lipid metabolites and results in skeletal muscle insulin resistance. We then explore current and potential exercise and nutritional strategies which target IMTG turnover in sedentary obese individuals, to improve insulin sensitivity. Overall, improving IMTG turnover should be an important component of successful interventions which aim to prevent the development of insulin resistance in the ever-expanding sedentary, overweight and obese populations.

Novelty Bullet points

- A description of the most up-to-date mechanisms regulating turnover of the IMTG pool.
- An exploration of current and potential exercise/nutritional strategies to target and enhance IMTG turnover in obese individuals
- Overall, highlights the importance of improving IMTG turnover to prevent the development of insulin resistance

Key words

Intramuscular triglyceride, perilipins, insulin resistance, exercise, skeletal muscle, type 2 diabetes

Introduction

Physical inactivity combined with chronic over-consumption of an energy-dense diet causes expansion of adipose tissue depots around the body leading to obesity. The buffering capacity of adipose tissue can become impaired in obesity resulting in spill-over of circulating fatty acids (FA) and triglycerides into non-adipose tissues, such as the liver and skeletal muscle, leading to ectopic lipid deposition (Frayn, 2002). The delivery of excess lipid to skeletal muscle leads to accumulation of intramuscular triglyceride (IMTG) (Bachmann *et al.*, 2001; Chow *et al.*, 2014), which is characteristic of the obese and T2D states. Thus, high IMTG levels in sedentary obese individuals and T2D patients are associated with insulin resistance (Pan *et al.*, 1997; Kelley and Goodpaster, 2001; van Loon *et al.,* 2004). However, it is now known that a high IMTG content *per se* does not result in insulin resistance. Rather, insulin resistance is believed to be caused, at least in part, by the presence of harmful lipid metabolites, such as diacylglycerols (DAGs) and ceramides (Goodpaster et al., 1997; Pan et al., 1997; Forouhi et al., 1999). Indeed, the accumulation of DAGs and ceramides has been shown to disrupt cell function, and specifically the capacity for insulin-stimulated glucose uptake into skeletal muscle via direct interference with the insulin signalling cascade (Yu et al., 2002; Summers and Nelson, 2005; Chaurasia and Summers, 2015). However, defects in insulin signalling are not always observed in insulin resistant individuals (Meyer et al., 2002; Hojlund et al., 2003; Ramos et al., 2021), and the precise mechanism by which lipid metabolites induce insulin resistance is far from certain. Consequently, the link between lipid metabolite accumulation and insulin resistance appears complex, and may be rooted in other factors such as lipid metabolite composition and subcellular localisation (extensively reviewed recently by (Bergman and Goodpaster, 2020). The association between IMTG accumulation and insulin resistance has also been disputed due to endurance trained athletes having a comparable or even higher IMTG content than obese individuals and T2D patients, whilst remaining highly insulin sensitive (Goodpaster et al., 2001; van Loon et al., 2003a). This phenomenon is now well known as the “athlete’s paradox” (Goodpaster *et al.,* 2001). The question of how endurance trained athletes exhibit similar IMTG content compared to obese individuals but are able to combine this with high levels of insulin sensitivity has been the subject of intense research in the last 20 years.

Although the mechanistic link between IMTG accumulation and insulin resistance is not yet fully established, the fundamental difference between endurance athletes when compared to obese individuals with or without T2D is their greater ability to utilise IMTG as a source of fuel during exercise (Schrauwen *et al.*, 2002; van Loon, 2004). Regular breakdown (lipolysis) of IMTG and oxidation of FA during exercise, coupled with elevated rates of FA uptake and IMTG (re)synthesis following exercise, creates a dynamic IMTG pool with a high turnover rate (van Loon, 2004; Moro, Bajpeyi and Smith, 2008). An attractive hypothesis is that regular IMTG turnover maintains insulin sensitivity by regulating the concentration and spatial distribution of lipid metabolites thereby ameliorating their impact on insulin signalling and cell function. However, exercise training appears to have little impact on insulin signalling (Christ-Roberts et al., 2004; Frosig et al., 2007) and does not always alter the concentration of lipid metabolites in muscle (Meyer et al., 2002; Hojlund et al., 2003). Rather, a greater ability to utilise IMTG in trained individuals leads to the hypothesis that the capacity to appropriately adjust FA storage and efficiently breakdown and oxidise FA in line with metabolic demand and FA availability is fundamental to improve insulin sensitivity. Because of this, focus has shifted to identifying the mechanisms that enable a high turnover rate of the IMTG pool in trained individuals in order to be able to create the optimal intervention in obese individuals and people with T2D and subsequently improve insulin sensitivity.

With this in mind, this review will first evaluate the differences in the storage and utilisation of IMTG between trained and more sedentary populations (i.e., obese and elderly individuals and those with T2D) to demonstrate that the dynamic nature of the IMTG pool in trained individuals is a crucial characteristic to the preservation of insulin sensitivity. Based on this information, we will then explore potential strategies to maximise IMTG turnover which could be implemented as interventions to improve insulin sensitivity in obese individuals and T2D patients.

Differences in IMTG storage between trained and sedentary individuals

Although IMTG content itself has no mechanistic link to insulin resistance, in this context it is important to consider the fibre-specific distribution, subcellular location, and morphology of IMTG-containing lipid droplets (LD). In lean, healthy individuals there is a hierarchical distribution between the different fibre types with the majority of

IMTG being stored in type I fibres, followed by type IIa then type IIx fibres (Daemen, van Polanen and Hesselink, 2018; Whytock e*t al.,* 2020). Within skeletal muscle, LDs are located either between the myofibrils (intermyofibrillar [IMF] LDs) or just beneath the surface membrane (subsarcolemmal [SS] LDs) (Nielsen *et al.*, 2017). Using transmission electron microscopy, it was recently shown that type I fibres of healthy males have small LD located in both the intermyofibrillar region and the subsarcolemmal region (Nielsen *et al.,* 2017), whereas type II fibres contain a similar number of LD in the intermyofibrillar region and the subsarcolemmal region but those in the subsarcolemmal region are ~20% larger in diameter (Nielsen *et al.*, 2017). Consequently, the size of subsarcolemmal LD in particular was associated with poorer insulin sensitivity, rather than LD number, at least in healthy untrained males (Nielsen *et al.*, 2017). Daemen *et al,* (2018) extended these observations when comparing differences between trained individuals and patients with T2D. To this end, the elevated IMTG content in trained individuals was explained by a greater number of LD in the intermyofibrillar region of type I fibres, whereas individuals with T2D had a greater number of larger LD in the subsarcolemmal region of type II fibres (Daemen, van Polanen and Hesselink, 2018). It is important to note here that to date the majority of research investigating differences in LD location and morphology has been conducted in males, or without distinction between sex. Thus, differences in LD location and morphology over the lifespan and between sexes should be explored in future studies. Older adults have been shown to have larger LD, fewer mitochondria, and a lower proportion of LD in contact with mitochondria (Crane et al., 2010), likely contributing to age-related decline in mitochondrial function and lipid metabolism.

Interestingly, 8 weeks of a high-calorie, high-fat diet induced insulin resistance in sedentary individuals and resulted in an increase in LD size rather than any changes in LD number (Covington *et al.,* 2017). More recently, we reported that a short-term (7-day) high-calorie, high-fat diet increased LD size and number in type I fibres in both the central and peripheral regions (Whytock *et al.*, 2020). In type IIa fibres LD size increased in both the SS and IMF region but only LD number increased in the SS region, whereas in type IIx fibres only SS-located LD increased in size with no changes in LD number observed (Whytock *et al.*, 2020). This suggests that changes in LD number and size may occur in a hierarchical manner based on both fibre type and subcellular region, at least in response to a high-calorie, high-fat diet. In contrast, a

combined weight loss and exercise training intervention in previously overweight or obese individuals resulted in a decrease in LD size concomitant with improved insulin sensitivity, even in the absence of a reduction in IMTG content (He, Goodpaster and Kelley, 2004). A large number of small LD located in the IMF region, as observed in healthy lean and trained individuals, creates a larger surface area to volume ratio, which is thought to be beneficial for the binding of proteins and lipolytic enzymes to the LD in order to liberate and release FA from the IMTG stored within. Moreover, LD are located in close proximity to mitochondria within skeletal muscle in healthy, trained individuals (Hoppeler *et al.*, 1999; Shaw, Jones and Wagenmakers, 2008), and exercise training in healthy or obese individuals increases the proportion of LD that are in contact with mitochondria (Tarnopolsky *et al.,* 2007; Shepherd *et al.,* 2017b). Together with a large number of small LDs, this adaptation likely creates an efficient means by which to channel FA liberated from IMTG within LD to the mitochondria for subsequent oxidation (Fig. 1). It is important to note here though that increased LD association with mitochondria does not necessarily mean the LD are utilised for oxidation, and this LD-mitochondria interaction may also support triacylglycerol synthesis and LD growth (Benador et al., 2018; Benador et al., 2019).

IMTG turnover in trained vs sedentary individuals.

Cross-sectional comparisons between trained and untrained individuals confirm that endurance-trained individuals have a greater capacity to use IMTG as a substrate during exercise (Klein, Coyle and Wolfe, 1994; Coggan et al., 2000). During moderate-intensity exercise in healthy individuals, IMTG-derived fatty acids contribute ~50% to total fat oxidation, with the remaining ~50% attributable to plasma FA (van Loon *et al.,* 2001). Serial muscle biopsies combined with microscopy-based analyses enable net changes in IMTG content to be determined and using this approach it is now known that IMTG utilisation preferentially occurs in type I fibres from IMTG-containing LD (van Loon et al., 2003a; Shepherd et al., 2013) that are located in the IMF region (Koh *et al.,* 2017; Jevons *et al*., 2020). Moreover, in healthy individuals IMTG utilisation and FA oxidation during exercise is closely related to pre-exercise IMTG content (Shepherd et al., 2013) whereby those with greatest IMTG stores have the greatest IMTG utilisation. Therefore, the high rate of IMTG utilisation observed in healthy, trained individuals must be matched by a large capacity for esterification and storage

of FA as IMTG following exercise. This has been illustrated in a recent study, which demonstrated that myotubes from athletic subjects have higher lipid turnover and lipid oxidation compared to those from sedentary individuals (Lund *et al.,* 2018) Specifically, myotubes from athletes exhibit higher rates of lipolysis and re-esterification of FA into the triacylglycerol (TAG) pool, indicating greater turnover of TAG stores. Importantly, higher complete oxidation and incomplete β-oxidation of FA in myotubes from the athletic population was also observed, suggesting they are able to more effectively rely on FA as a fuel source (Lund *et al.,* 2018). A greater accumulation of FA in myotubes derived from sedentary compared to athletic individuals led the authors to question whether the capacity for IMTG synthesis is downregulated in these individuals, and/or the capacity for lipid metabolite generation is upregulated (Lund *et al.,* 2018). The latter, of course, would consequently reduce insulin sensitivity.

Measuring the fractional synthesis rates (FSR) of IMTG in healthy individuals provides *in vivo* information on the rate of turnover of the IMTG pool. In this regard, IMTG FSR at rest in healthy individuals was first reported to be as high as ~3.4%/h, suggesting that in this cohort complete turnover of the IMTG pool would occur in ~29 h (Sacchetti et al., 2004). Although Bergman *et al.* (2018) have since reported a lower resting IMTG FSR in trained individuals (~1.56%/h), this was still more than 2-fold higher when compared to sedentary, lean individuals (~0.61%/h) (Bergman *et al.,* 2018). Obese individuals have a lower IMTG FSR (~0.42 %/h) than the rates reported for lean, sedentary individuals, and the resting IMTG FSR for obese individuals with pre-diabetes is even lower (~0.21 %/h) (Perreault et al., 2010). With these data, it is no surprise that Bergman *et al.* (2018) reported a positive correlation between IMTG FSR and insulin sensitivity at rest, along with a negative correlation between IMTG synthesis rates and the concentration of key lipid metabolites associated with insulin resistance. There is evidently a strong link between an individual's ability to breakdown and resynthesise IMTG and their level of insulin sensitivity.

As well as measuring IMTG FSR at rest, studies examining IMTG FSR during exercise alongside net changes in IMTG concentration provide further insight into the dynamics of the IMTG pool in trained and sedentary obese and T2D individuals. During 1 hour of moderate-intensity exercise, IMTG FSR is elevated compared to rest and while a

net reduction in IMTG content is observed in trained individuals, IMTG content remains unchanged in obese individuals and those with T2D (Bergman *et al.,* 2018). The latter finding is in line with previous studies measuring IMTG content in biopsies pre- and post-exercise which concluded that there is no net utilisation of IMTG in obese individuals and those with T2D (Kelley and Simoneau, 1994; Blaak and Wagenmakers, 2002). IMTG FSR is elevated during exercise in obesity and T2D (Bergman *et al.,* 2018), which could be due to the high circulating FFA concentrations often observed in these individuals (Axelsen et al., 1999) supplying fatty acids for the synthesis of IMTG. In obese individuals and T2D patients though there is no net change in IMTG content during exercise, but for this to be true IMTG utilisation during exercise must be matched to IMTG FSR. Therefore, by combining pre- and post-exercise measures of IMTG content with estimates of IMTG FSR during exercise, it appears possible that obese and T2D individuals may utilise their IMTG stores, but this occurs in the absence of a net reduction in IMTG content (Bergman *et al.,* 2018), potentially due to replenishment of the IMTG stores from high circulating FFA concentrations (Axelsen et al., 1999). It has also been reported that in individuals with normal glucose tolerance, IMTG FSR during exercise may be reduced, especially compared to individuals with prediabetes (Perreault *et al.,* 2010), obese individuals, and T2D patients (Bergman *et al.,* 2018) who all exhibit only very small changes in IMTG FSR during exercise. Thus, while there is not yet a consensus on how IMTG FSR is altered during exercise in trained, glucose tolerant individuals, these cross-sectional comparisons do highlight an inability to adjust IMTG FSR relative to metabolic demand in obese individuals with pre-diabetes and T2D. Importantly, a net reduction in IMTG content during exercise in endurance trained individuals will theoretically enable a greater capacity for uptake of plasma FFA and storage as IMTG in the post-exercise period. Without a net reduction in IMTG content during exercise in obese individuals, this may limit the capacity for FA's entering skeletal muscle following exercise to be stored as IMTG, and rather these FA's may instead be directed towards the generation of lipid metabolites.

Molecular mechanisms regulating IMTG turnover rate

While the aim of this review is not to provide an in-depth account of the molecular mechanisms that regulate FA uptake and esterification, IMTG storage and breakdown,

it is pertinent that an up-to-date overview of these regulatory mechanisms is provided. For the former, the reader is directed to two excellent reviews (Badin, Langin and Moro, 2013; Lundsgaard, Fritzen and Kiens, 2018).

IMTG Synthesis

Exogenous FA, derived from either adipose tissue or from the diet, are transported in the circulation and taken up into skeletal muscle to be stored as IMTG and/or oxidised as a fuel source. FA uptake into skeletal muscle is regulated primarily by FAT/CD36, although it is likely that this process is mediated by a series of transporter proteins reviewed in detail in (Schwenk *et al.*, 2010; Glatz and Luiken, 2018). Once in skeletal muscle, FA are converted to fatty acyl-CoA and directed to IMTG synthesis. Briefly, FA-CoA undergoes acylation catalysed by key enzymes glycerol-3-phosphate acyltransferase (GPAT), monoacylglycerol acyltransferase (MGAT) and diacylglycerol acyltransferase (DGAT) (Teodoro et al., 2017), ultimately leading to the generation of TAG (or IMTG). The synthesised IMTG are then stored within LD and are a readily available fuel for healthy individuals.

Although the key enzymes that control IMTG synthesis have been identified, little is known about how they regulate this process in skeletal muscle. Following moderate-intensity exercise and a subsequent elevation of lipid availability (induced by an overnight lipid infusion), there is an increase in the protein expression of DGAT1 and GPAT1 (Schenk and Horowitz, 2007) and increased GPAT1 activity (Newsom *et al.*, 2011). Furthermore, overexpression of DGAT1 in rodents results in an increase in TAG content and a decrease in DAG (Liu *et al*., 2007). However, GPAT1 and DGAT1 do not differ in expression between obese and lean individuals (Thrush *et al.,* 2009; Li *et al.,* 2011), and no differences are observed in DGAT1 mRNA expression between endurance trained, obese individuals and those with type 2 diabetes (Bergman *et al.*, 2018), nor in DGAT protein content (Amati *et al.,* 2011). Overall, there is currently no evidence to suggest that the expression or activity of DGAT or GPAT is impaired in obese and T2D individuals. As a result, it could be speculated that the machinery for IMTG synthesis is sufficient in all individuals, and it is the (as yet unknown) activation mechanism which is impaired in obesity and T2D.

IMTG lipolysis

The reduced IMTG utilisation reported in obese individuals and those with T2D could be, at least partly, attributed to impaired rates of lipolysis. Indeed, when compared to lean individuals, obese individuals show impaired ß$_2$- adrenergic-mediated stimulation of lipolysis in skeletal muscle (Blaak *et al.,* 2004). In skeletal muscle, the majority (~98%) of total TAG hydrolase activity (at least at rest) is regulated by adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL). In this regard, it is important to note HSL protein content (Jocken *et al.*, 2007) and HSL phosphorylation (at Ser^{563}, Ser^{555} and Ser^{659}) (Jocken *et al.*, 2008) are both lower in obese compared to lean individuals. However, it was reported that individuals with T2D actually had greater ATGL protein expression compared to lean and obese individuals (Badin *et al.*, 2011). Because ATGL may have a higher affinity to TAG (Haemmerle *et al.*, 2006), and HSL a higher affinity to DAG (Fredrikson *et al.,* 1981; Haemmerle *et al.*, 2002), it has been suggested that in obesity and T2D the imbalance between ATGL and HSL protein content favours DAG accumulation, and this contributes to the disruption of insulin signalling. Indeed, overexpression of ATGL in myotubes from lean, healthy, insulin-sensitive individuals induced DAG and ceramide accumulation, which was associated with reduced insulin-stimulated glycogen synthesis and reduced activation of IRS-1 and Akt (Badin *et al.*, 2011). Although, this imbalance was reported by Jocken *et al.,* (2008) with greater ATGL content and lower HSL content in obese individuals with T2D compared to lean (Jocken *et a*l., 2008), it was not evident in non-obese T2D, questioning its role in the development of insulin resistance. Moreover, it is now known that DAG that is derived from ATGL-mediated lipolysis is unable to activate the atypical PKC isoforms known to disrupt the insulin signalling cascade (Eichmann *et al.*, 2012). Additionally, Bergman *et al.,* (2018) has more recently shown that elevated IMTG content in obese individuals is not due to an imbalance between HSL and ATGL content, but more likely due to the specific species of ceramides present in obese individuals, and the subcellular location in which they are stored (Bergman *et al*., 2018).

LD proteins and their regulation of IMTG turnover

Surrounding a core of TAG and cholesterol esters, LDs have a phospholipid monolayer that is now known to be coated with numerous proteins which likely determines the functional role of each LD. The perilipin proteins (PLIN) are the group

of LD proteins most extensively investigated (Morales, Bucarey and Espinosa, 2017), with PLIN2, 3, 4 and 5 all being expressed in human skeletal muscle. Research conducted over the last decade has started to uncover a potential role for the PLIN proteins in both IMTG storage and lipolysis.

The role of PLIN in IMTG storage - It is evident from *in vitro* studies that the knockout of PLIN2 or PLIN5 in skeletal muscle compromises TAG storage (Bosma *et al.*, 2012; Gallardo-Montejano *et al.,* 2016). It makes sense then, that the overexpression of these PLIN isoforms results in quite the opposite, promoting TAG storage (Xu *et al.*, 2005; Bosma *et al.*, 2012; Gallardo-Montejano *et al.*, 2016). Similarly, the suppression of PLIN3 reduced LD maturation and TAG incorporation into IMTG stores in HeLa cells (Bulankina *et al.,* 2009), whereas in skeletal muscle myotubes augmenting PLIN3 gene expression increases IMTG content (Kleinert *et al.,* 2016). PLIN4 is purported to be the most abundant PLIN in skeletal muscle (Deshmukh *et al.*, 2015), yet its knockout in mice has no effect on skeletal muscle IMTG concentrations (Chen *et al*., 2013), questioning the importance of this protein in IMTG storage.

Human biopsy studies demonstrate that PLIN2 protein expression is greater in trained versus sedentary individuals (Amati *et al.*, 2011; Shaw *et al.,* 2012; Shepherd *et al.,* 2013), females versus males (Shaw *et al.,* 2009; Peters *et al.,* 2012), and type 1 versus type 2 fibres (Shaw *et al.*, 2009), suggesting that PLIN2 is closely related to IMTG content in healthy individuals. The same observations also extend to PLIN3 (Peters *et al.*, 2012; Shepherd *et al.*, 2017b) and PLIN5 (Shepherd *et al*., 2013; Shepherd *et al.*, 2017a; Shepherd *et al.*, 2017b; Daemen, van Polanen and Hesselink, 2018). Furthermore, when exercise training augments IMTG content, increases in PLIN2 (Shaw *et al.*, 2012; Shepherd *et al.,* 2013), PLIN3 (Shepherd *et al.,* 2017b) and PLIN5 (Peters *et al.,* 2012; Shepherd *et al.*, 2013) are also observed. Much less research has been conducted on PLIN4, although we recently showed that PLIN4 protein expression is greater in trained versus untrained individuals (Shepherd *et al.,* 2017b). Despite this, endurance training fails to augment PLIN4 mRNA or protein expression in healthy individuals (Peters *et al.,* 2012; Pourteymour *et al.*, 2015). Together, these data suggest that the expression of PLIN2, PLIN3 and PLIN5 is closely related to IMTG content, at least in healthy individuals or following a period of exercise training. This may be an important adaptation in order to support greater IMTG storage, especially

in the face of elevated FA availability and turnover. Indeed, in response to an acute lipid infusion (Shepherd et al., 2017a) or 48 h of fasting (Gemmink *et al.*, 2016) (both conditions increase FA availability) in trained individuals, there appears to be a redistribution of the pre-existing PLIN2, PLIN3 and PLIN5 protein pool (which could be from either LD-bound or non-LD-bound) to the expanded LD pool. Importantly, this redistribution was not apparent in sedentary individuals (Shepherd et al., 2017a) and the capacity to redistribute PLIN5 to maintain coverage of the expanded LD pool was associated with a greater maintenance of insulin sensitivity (Gemmink *et al.*, 2016). More recently, we showed that in elite triathletes, post-exercise increases in IMTG content occurred *prior* to a redistribution of the PLIN (2,3,5) protein pool (Jevons *et al.*, 2020). Taken together, this suggests that the PLIN proteins do not play a direct role in IMTG synthesis but coating of LD with PLINs may be an important adaptation which supports IMTG storage.

The role of PLIN in IMTG breakdown - Research has also focused on the potential role of PLIN2, PLIN3 and PLIN5 in supporting IMTG breakdown and utilisation during exercise. A role for the PLIN proteins in TAG breakdown stems from evidence showing that PLIN2, PLIN3 and PLIN5 can interact with the key lipolytic enzymes ATGL and HSL (Anthonsen *et al.*, 1998; Granneman *et al.,* 2011; Macpherson *et al.*, 2013). Moreover, both PLIN2 and PLIN5 are thought to suppress lipolysis at rest by preventing the interaction between ATGL and CGI-58, whereas this inhibition is relieved permitting ATGL to interact with CGI-58 upon lipolytic stimulation (Wang *et al.,* 2011; Macpherson *et al.*, 2013). PLIN3 knockout in myotubes results in a reduction of lipid oxidation (Covington *et al.*, 2014). However, in response to an endurance exercise bout PLIN3 expression is positively correlated to whole-muscle homogenate palmitate oxidation rates as well as whole-body cumulative fat oxidation (Covington *et al.*, 2014). Recently, AMPK phosphorylation of PLIN3 was shown to bring about conformational changes to PLIN3 that expose the C-terminus and promote LD dispersion to facilitate lipolysis (Zhu *et al.*, 2019). This new data potentially underpins the relationship between PLIN3 and lipolysis.

In human studies, we initially showed that LD labelled with PLIN2 or PLIN5 are preferentially broken down in lean, sedentary individuals during 1 h of moderate-intensity exercise (Shepherd *et al.,* 2012; Shepherd *et al.,* 2013). However, when

assessed following six weeks of endurance training or sprint interval training, only LD labelled with PLIN5 were preferentially targeted for breakdown in an equivalent bout of exercise (Shepherd *et al.,* 2013). More recently, we have shown that during more prolonged (4 h) of moderate-intensity exercise in elite triathletes, this preferential use of PLIN labelled LD is not apparent. The use of LD not labelled with PLIN proteins could be attributed to a very high rate of IMTG turnover compared to sedentary individuals, especially during such prolonged exercise. Nevertheless, it does appear that PLIN5 plays a key functional role regulating IMTG breakdown, since 1 h of moderate-intensity exercise led to a redistribution of HSL specifically to LD labelled with PLIN5 (Whytock *et al.*, 2018).

FA as signalling molecules

It is now beginning to be understood that FA play a crucial role in skeletal muscle adaptation to exercise, by acting as ligands for peroxisome proliferator-activated receptor (PPAR) α and δ to support transcription of genes involved in lipid metabolism (Banner *et al.,* 1993). An extensive review of this topic is beyond the scope of the current paper (readers are directed to (Funai and Semenkovich, 2011) but it is pertinent to briefly consider the importance of FA as signalling molecules in the context of adaptation. In humans, suppression of lipolysis with nicotinic acid prior to exercise resulted in reduced mRNA expression of Peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha (PGC-1α), PPARα and PPARδ, demonstrating a role for FA availability in exercise-induced gene expression (Watt *et al.,* 2004). In contrast, treatment with a PPARδ agonist resulted in a dose-dependent increase in skeletal muscle FA oxidation in mice, as well as increased expression of mRNA encoded for proteins involved in FA catabolism, such as *β*-oxidation enzymes, FA transport proteins and uncoupling proteins (Tanaka *et al.*, 2003).

The source of the FA that act as ligands and activate PPARα and PPARδ is not yet confirmed but could be linked to the activation of intramuscular lipases acting upon IMTG. In support, ATGL-mediated hydrolysis of triacylglycerol promotes activation of PGC-1α and PPARα signalling in order to upregulate mitochondrial biogenesis. Moreover, ATGL-mediated lipolysis activates SIRT-1, the protein responsible for the deacetylation, and therefore activation, of PGC-1α (Khan *et al.,* 2015). Until recently, the mechanism by which ATGL-mediated lipolysis promotes mitochondrial biogenesis

was unknown. However, Najt *et al.* (2020) recently reported that monounsaturated FA are able to activate SIRT-1, thereby enhancing PGC-1α/PPARα signalling. Importantly, these monounsaturated FA were derived from intracellular LDs (Najt *et al.*, 2020), permitting speculation that FA liberated from IMTG stored in LD may play a role in promoting mitochondrial biogenesis, although evidence for this is not yet available. Najt *et al.* (2020) also identified a role for PLIN5 as a FA binding protein, which is able to bind LD-derived monounsaturated FA and transport them to the nucleus, at least in response to cAMP/PKA-mediated lipolytic stimulation in hepatocytes. This observation is consistent with that of Gallardo-Monejano *et al.* (2016), who reported that fasting-induced lipolysis stimulates PKA-mediated phosphorylation of PLIN5 followed by its translocation to the nucleus (Gallardo-Montejano *et al.,* 2016). Here, PLIN5 interacts with SIRT-1 and PGC-1a to increase transcription of proteins involved in FA catabolism. The translocation of PLIN5 was also shown for the first time to influence the transcriptional regulation of mitochondrial respiration and mitochondrial biogenesis (Gallardo-Montejano *et al.,* 2016). Taken together, it appears that intracellular LDs are more than a source of FA for oxidation but may play a key role in the regulation of mitochondrial biogenesis and the FA catabolism programme. Theoretically then, enhancing the utilisation and turnover of the IMTG pool could be one strategy to stimulate mitochondrial biogenesis and increase the capacity for fat oxidation. We will now explore potential strategies that could be used to induce these adaptations and subsequently enhance insulin sensitivity and discuss whether these have application in clinical populations.

Strategies to improve IMTG turnover

Exercise training

It is well known that endurance exercise training is a powerful stimulus to augment oxidative capacity and IMTG utilisation during exercise (Baldwin et al., 1972; Kiens et al., 1993; Phillips et al., 1996; Bergman et al., 1999; van Loon, 2004). Following endurance training there is an increase in the number of IMTG-containing LDs that are in direct contact with mitochondria (Tarnopolsky et al., 2007; Devries et al., 2013; Shepherd et al., 2017a), which together with expansion of the mitochondrial network enhances the total capacity for FA ß-oxidation (Granata, Jamnick and Bishop, 2018). In sedentary lean and obese individuals endurance training also augments the

expression of proteins that regulate IMTG breakdown and LD dynamics, including ATGL (Alsted *et al.*, 2009), PLIN2 (Shaw *et al.,* 2012; Shepherd *et al*., 2013), PLIN3 (Shepherd *et al.*, 2017a) and PLIN5 (Shepherd *et al.,* 2017a). Notably, the increased expression of the PLIN proteins occurs primarily in type I fibres, which may explain why following a period of endurance training the increase in IMTG utilisation is also predominantly in type I fibres (Van Proeyen *et al.,* 2011a; Van Proeyen *et al.,* 2011b; Shepherd *et al.,* 2013). Importantly, the augmented use of IMTG following endurance training is related to an improvement in insulin sensitivity (Van Proeyen *et al.,* 2011a; Van Proeyen *et al.,* 2011b; Shepherd *et al.,* 2013).

Other forms of exercise training can also increase the capacity for IMTG utilisation during exercise. For example, 6 weeks of sprint interval training in sedentary individuals enhances IMTG utilisation during a single bout of moderate-intensity exercise, to a similar degree as endurance training (Shepherd *et al.,* 2013; Scribbans *et al.*, 2014) at least in lean, sedentary individuals. Underpinning this, several different forms of high intensity (or sprint) interval training have been shown to enhance skeletal muscle oxidative capacity (Little *et al.*, 2010; MacInnis and Gibala, 2017; Astorino and Schubert, 2018), and we have also reported an increased expression of PLIN2 and PLIN5 following sprint interval training (Shepherd *et al.,* 2013). Interestingly, in lean, sedentary individuals 6 weeks of whole-body resistance training also increases IMTG utilisation during a single bout of moderate-intensity exercise (Shepherd *et al.*, 2014), although the net changes in IMTG content during exercise following training are typically less than when compared to endurance or sprint interval training (Shepherd *et al.,* 2013). Given that resistance training can enhance mitochondrial content and oxidative capacity (Tang, Hartman and Phillips, 2006; Balakrishnan *et al.,* 2010; Pesta *et al.,* 2011) as well as resting IMTG content (Shepherd *et al.*, 2014), this finding is perhaps not unexpected. Moreover, because both high intensity interval training and resistance training improve insulin sensitivity in sedentary (Ishii et al., 1998) and obese individuals (Croymans et al., 2013; Ryan et al., 2020), it is tempting to speculate that an enhanced capacity for IMTG utilisation during exercise could, at least in part, contribute to this effect. Although this is yet to be investigated directly, high intensity interval training in obese individuals augments several adaptations that would support greater IMTG turnover, including increased mitochondrial content (Gibala *et al*., 2006; Burgomaster *et al.,* 2008; Larsen *et al.,* 2015; Chrois *et al.*, 2020) and mitochondrial

interaction with LDs, increased HSL and CD36 content (Talanian *et al.,* 2010) and greater protein expression of PLIN2, PLIN3, and PLIN5 (Shepherd et al., 2013; Shepherd *et al.*, 2017b).

As discussed above, exercise stimulates IMTG utilisation in lean, healthy individuals (Shepherd *et al.,* 2013; Scribbans *et al.*, 2014), and to a lesser extent in those with obesity and T2D (Shepherd *et al.,* 2017a; Bergman *et al*., 2018). However, a poor capacity to simultaneously reduce the rate of IMTG synthesis during exercise in individuals with obesity and T2D results in a minimal to zero decrease in IMTG content post-exercise (van Loon *et al.,* 2004). The result of this is a limited capacity for FA's entering skeletal muscle following exercise to be stored as IMTG and interfere with insulin signalling. Thus, in sedentary and obese individuals, additional strategies to exercise alone may be required to augment IMTG utilisation (or turnover) in order to create a net decrease in IMTG content post-exercise. In this context, the question then arises as to whether the insulin sensitising-effect of regular exercise training can be enhanced, by manipulating the conditions under which exercise is undertaken in order to maximise IMTG utilisation and obtain a post-exercise decrease in IMTG content (Fig. 2).

Anti-lipolytic drug therapy

During prolonged exercise there is a progressive decline in IMTG oxidation rate which is inversely related to the concomitant increase in plasma free fatty acid (FFA) concentrations (Romijn et al., 1993; Romijn et al., 1995; van Loon et al., 2003a). Thus, it is purported that elevated plasma FFA concentrations may suppress IMTG utilisation during exercise. Pharmacological inhibition of adipose tissue lipolysis, via the anti-lipolytic agent Acipimox, before and during exercise abolishes the progressive rise in plasma FFA during exercise and results in enhanced IMTG oxidation in lean, healthy individuals (van Loon et al., 2005a). Individuals with obesity and T2D exhibit elevated circulating plasma FFA and triglyceride concentrations, which is linked to the development of insulin resistance (Boden, 2003), and therefore could also be part of the mechanism by which exercise-induced IMTG utilisation is suppressed in these individuals. In support, inhibition of adipose tissue lipolysis before and during exercise in obese T2D patients increases IMTG oxidation at rest, during 60 minutes of moderate-intensity exercise, and for several hours' post-exercise (van Loon et al.,

2005b). Moreover, this was accompanied by a superior rate of glycogen oxidation, and greater post-exercise insulin sensitivity (van Loon et al., 2005b).

Several studies show that short-term Acipimox treatment (250 mg, two-to-three times per day) for up to 2 weeks can reduce fasting plasma free fatty acids and increase insulin sensitivity and glucose control in obese and T2D individuals (Bajaj *et al.,* 2005; Daniele *et al.,* 2014; Phielix *et al.,* 2014; van de Weijer *et al.*, 2015). Furthermore, 8 weeks of Acipimox treatment lowers plasma free fatty acids, cholesterol and triglyceride concentrations in obese individuals and T2D patients (Crepaldi *et al*., 1988; Stuyt, Kleinjans and Stalenhoef, 1998). Lower plasma free fatty acid levels reduce the availability of FA for uptake into skeletal muscle, potentially minimising the accumulation of lipid in this tissue. Despite these positive changes to blood lipids and insulin sensitivity, longer term Acipimox treatment results in a rebound rise in fasting plasma FFA (Fulcher *et al*., 1992; Vaag and Beck-Nielsen, 1992; Saloranta *et al.,* 1993) and both hepatic and skeletal muscle insulin sensitivity is unchanged (Makimura *et al*., 2016). Therefore, while chronic treatment with Acipimox does not seem feasible, combining exercise with anti-lipolytic therapy may represent an effective strategy to augment insulin sensitivity in individuals with obesity and T2D.

Fasted exercise

As high plasma FFA concentrations suppress IMTG utilisation during exercise, a simple strategy to reduce FFA availability would be to feed carbohydrate, since carbohydrate ingestion increases plasma insulin levels and subsequently suppresses circulating FFA through insulin-mediated inhibition of HSL in adipose tissue (Watt *et al., 2004)*. However, insulin also suppresses HSL activity in skeletal muscle (Enoksson *et al.*, 1998), and therefore carbohydrate ingestion would theoretically lead to a decrease in IMTG utilisation. The overnight fasted state is characterised by low plasma insulin concentrations, and therefore skeletal muscle lipolytic enzyme activity remains functional (Horowitz et al., 1997; Arkinstall et al., 2001). Indeed, in healthy individuals, two hours of moderate-intensity cycling in the overnight-fasted compared to carbohydrate-fed state led to greater IMTG utilisation in type 1 fibres (De Bock *et al.,* 2005). Similar results were obtained in overweight/obese males, although in this population IMTG utilisation was more pronounced in both type 1 and type 2 fibres following fasted compared to postprandial exercise (Edinburgh *et al.,* 2020). It should

554 be noted though that when the exercise duration is extended to 3 h, there appears to
555 be no effect of carbohydrate feeding before (Fell et al., 2021) and/or during
556 (Stellingwerff et al., 2007; Fell et al., 2021) exercise on IMTG utilisation. In this case,
557 it is possible that the duration of exercise overrides the inhibitory effect of carbohydrate
558 feeding on IMTG utilisation. Nevertheless, it is worth noting that limiting glucose
559 availability by exercising in the fasted state encourages skeletal muscle cells to
560 increase transcriptional activities of factors that upregulate the fatty acid oxidation
561 programme and thereby induce metabolic adaptations for efficient lipid oxidation
562 (Canto et al., 2010). Six weeks of endurance training in lean, healthy individuals in a
563 fasted state was more effective for increasing skeletal muscle oxidative capacity,
564 CD36 and $FATBP_m$ content, and net IMTG breakdown during a single exercise bout
565 compared to undertaking the same training in a fed condition (Van Proeyen *et al*.,
566 2011b). Moreover, 6 weeks of endurance training in a fasted state in
567 overweight/obese individuals augmented skeletal muscle remodelling of
568 phospholipids (Edinburgh *et al.,* 2020). Thus, fasted exercise appears to augment
569 changes in skeletal muscle phospholipids, by reducing saturated FFA, that correlate
570 with improved post-prandial insulinemia.
571
572 Whether these beneficial adaptations to fasted exercise can enhance the insulin-
573 sensitising effect of exercise training for obese individuals and individuals with T2D
574 has been investigated in two studies to date. Edinburgh *et al.,* (2020) reported
575 increased oral glucose sensitivity in overweight/obese individuals completing 6 weeks
576 of training in the fasted state. Additionally, 12-weeks of endurance training in males
577 diagnosed with T2D and randomised to exercising in an overnight fasted state or after
578 breakfast, saw greater improvements in HbA1c when an exercise programme Is
579 completed in the fed state compared to the fasted state. There was no difference in
580 the ability to reduce fat mass, increase fat oxidation or improve HDL concentrations
581 between nutritional strategies (Verboven *et al.*, 2020). While fasted exercise typically
582 enhances IMTG utilisation, it is not yet clear whether this translates into greater
583 benefits to insulin sensitivity and other markers of cardiometabolic health.
584
585 *Training with low muscle glycogen availability*
586

587 The “train-low” paradigm has gained interest over the last decade, and typically
588 consists of performing an initial bout of high intensity exercise (to reduce muscle
589 glycogen concentrations) before a second exercise bout is performed several hours
590 later or the following morning. Importantly, the ingestion of carbohydrate is restricted
591 between sessions to prevent muscle glycogen resynthesis, such that the second
592 exercise bout is commenced with low muscle glycogen concentrations. Under these
593 conditions, rates of whole-body fat oxidation are augmented (Hansen *et al.*, 2005; Yeo
594 *et al.,* 2008b; Morton *et al.,* 2009; Hulston *et al.,* 2010) and while there is no direct
595 evidence for an increased IMTG utilisation when exercising with low muscle glycogen,
596 one would speculate that this does occur. If true, this strategy could prove to be
597 applicable for individuals with metabolic disease. Note though that research in this
598 area to date is limited to highly trained males.
599
600 Importantly, in trained males systematically commencing exercise with reduced
601 muscle glycogen availability augments the activation of signalling proteins (Baar and
602 McGee, 2008) leading to elevated gene expression of mitochondrial proteins (Bartlett,
603 Hawley and Morton, 2015). The signalling pathways stimulated by a reduced muscle
604 glycogen availability have been explained in detail elsewhere (Hawley *et al.*, 2018;
605 Hearris *et al*., 2018; Impey *et al.*, 2018). Briefly, low muscle glycogen availability
606 stimulates greater activity of AMPK and p38MAPK (Wojtaszewski *et al.*, 2003; Chan
607 *et al.*, 2004), which in turn leads to activation and translocation of p53 and PGC-1α to
608 the nucleus and mitochondria (Bartlett *et al.,* 2013; Andrade-Souza *et al.,* 2019). Here,
609 these proteins help regulate the transcription of key mitochondrial proteins and those
610 involved in mitochondrial fusion and fission. Exercising under conditions of reduced
611 muscle glycogen availability also enhances circulating FFA concentrations, which in
612 turn activates the nuclear transcription factor PPARδ (Philp *et al.,* 2013), to upregulate
613 the expression of proteins linked to lipid metabolism, including ß-HAD (Yeo *et al.*,
614 2008b; Hulston *et al.,* 2010), HSL (Arkinstall *et al.*, 2004), and the FA transport
615 proteins, FATBP and CD36 (Arkinstall *et al.*, 2004; De Bock *et al.,* 2008; Lane *et al.*,
616 2015). With reduced CHO oxidation, there is a concomitant increase in total lipid
617 oxidation (Hearris *et al*., 2019) and step wise increases in AMPK activation showing
618 that “train-low” provides a potent stimulus for promoting endurance adaptation.
619

Studies which incorporate training sessions that are commenced with low muscle glycogen availability over several weeks report increased activity and content of the mitochondrial proteins citrate synthase, β-HAD, and SDH (Hansen et al., 2005; Yeo et al., 2008a; Morton et al., 2009), alongside elevations in whole-body fat oxidation (Yeo et al., 2008a), and result in a greater contribution of IMTG to total energy expenditure during moderate-intensity exercise in well-trained cyclists (Hulston *et al.,* 2010). Increasing oxidative enzyme capacity supports greater fat oxidation, which in athletic populations, is a key consideration for improving substrate utilisation and promoting glycogen sparing, thereby enhancing performance. From a clinical perspective, manipulating carbohydrate availability around exercise is also of relevance, since the adaptations outlined above would likely contribute to an improved IMTG turnover and underpin an increase in insulin sensitivity. Of course, using a prior exercise session to create a stimulus of low muscle glycogen availability (i.e., the 'traditional' train-low model) is challenging in sedentary, overweight/obese individuals, who likely will not perform exercise of sufficient intensity and/or duration to reduce glycogen below the 'threshold' required to stimulate the signalling responses mentioned above. Therefore, whilst effective, the traditional train-low model may only be adopted in overweight/obese individuals with high motivation and who have access to a specialist support network.

Low carbohydrate, high fat (LCHF) diet

Using diet may be a more appropriate strategy to generate a state of low muscle glycogen availability in sedentary overweight/obese individuals with or without T2D. To target fat oxidation, macronutrient intake can be manipulated by either a non-ketogenic low-carbohydrate high-fat diet (where fat supplies 60-65% fat), or by a ketogenic low-carbohydrate high-fat diet (where fat supplies 75-80% of daily intake) (Burke *et al.,* 2020). The purpose of a ketogenic diet is to induce fasting-like effects and leads to the production of ketone bodies, which can provide an additional substrate for oxidative energy production. Moreover, a ketogenic diet therefore leads to increases in whole-body fat oxidation rates, and a subsequent reduction in whole-body carbohydrate oxidation, which can be explained entirely by a decrease in muscle glycogen utilization (Starling *et al.,* 1997; Burke *et al.*, 2000; Helge *et al.,* 2001). Unlike

652 the above methods of glycogen manipulation, a ketogenic diet is typically a chronic
653 strategy to maximise fat as a source of fuel (Burke, 2021).
654
655 The specific adaptations to a ketogenic diet, and the timeline of these adaptive
656 responses, are controversial (Lindseth, 2017; Burke *et al.*, 2020), but in terms of
657 exercise capacity, they seem to be related to increased delivery, uptake and oxidation
658 of free fatty acids in skeletal muscle (see detailed review by (Burke, 2021). Adaptations
659 to a ketogenic diet in trained athletes can occur as quickly as within 5 days but are
660 often accompanied by feelings of fatigue due to exposure to this extreme change in
661 diet (Burke *et al.,* 2017). Over a longer period (12 weeks) though, it was recently
662 reported that a LCHF ketogenic diet combined with exercise increased mitochondrial
663 respiratory control ratio, ATP production and muscle triglyceride content (Miller *et al.*,
664 2020). Whilst there is still little data on changes in mitochondrial proteins, LD proteins
665 or IMTG content in human skeletal muscle in response to a ketogenic diet, there is a
666 robust increase in whole-body fat oxidation.
667
668 A non-ketogenic LCHF diet also enhances whole-body fat oxidation studies
669 investigating the mechanisms for greater oxidative capacity due to high fat diet
670 typically investigate this short-term using a non-ketogenic LCHF diet. Following 5 days
671 of a high fat diet, increases in whole-body fat oxidation occurred, despite reduced
672 skeletal muscle mitochondrial respiration (Leckey *et al.*, 2018). Importantly, a 12%
673 increase in FAT/CD36 protein was observed, which suggests an increased capacity
674 for FA uptake (Leckey *et al.*, 2018). Therefore, it is likely that increased whole-body
675 fat oxidation following a non-ketogenic LCHF diet is the result of increased transport
676 and delivery of FA to skeletal muscle. To date, no study has detailed the mechanisms
677 underpinning the increase in fat utilisation in human skeletal muscle, yet previous
678 studies has shown that short-term exposure (~5 days) to a high fat diet increases
679 IMTG content (Yeo et al., 2008a), HSL content (Stellingwerff *et al.,* 2006), and the
680 protein abundance of FAT/CD36 (Cameron-Smith *et al.*, 2003) and carnitine palmitoyl
681 transferase (Goedecke *et al.,* 1999). These adaptations demonstrate an adaptive
682 response which could improve the capacity of the exercising muscle to increase the
683 breakdown and resynthesis of IMTG stores in response to long-term ingestion of a
684 LCHF diet. Future studies should then investigate whether an improved turnover of fat

685 at a whole-body and muscle level contribute to the improved glycaemic control
686 reported following a LCHF diet (Ahmed et al., 2020).
687
688 Whilst the above addresses the potential impact of chronic changes in diet on IMTG
689 turnover, it should also be noted that macronutrient intake in the hours and days
690 following an exercise bout can impact IMTG stores. For example, traditional sports
691 nutrition guidelines suggest the consumption of a high carbohydrate diet following
692 exercise in order to support glycogen resynthesis (Ivy, 1991). However, when diets
693 high in carbohydrate, and therefore low in fat (<15% energy from fat), are consumed
694 post-exercise, IMTG resynthesis is shown to be substantially impaired (Decombaz et
695 al., 2000; Decombaz et al., 2001; Larson-Meyer, Newcomer and Hunter, 2002). Even
696 when the diet is made up of ~24% energy from fat (which could be considered quite
697 typical of a high carbohydrate diet), IMTG repletion over the subsequent 48 h following
698 exercise is still impaired (van Loon et al., 2003b). To date, work in this area has only
699 used healthy, trained individuals who have high rates of IMTG utilisation during
700 exercise resulting in a net decrease in IMTG content post-exercise. Given that a net
701 decrease in IMTG content following exercise appears to be absent in obese individuals
702 or T2D patients, a high-fat diet in the period following exercise could theoretically
703 contribute to aberrant storage of IMTG and the generation of lipid metabolites. Future
704 research is required to examine this though.
705
706 *Weight loss and Calorie restriction (CR) on IMTG utilisation*
707
708 Weight loss achieved via a restriction in caloric intake (but where the relative
709 macronutrient contribution remains the same) is a powerful strategy to improve insulin
710 sensitivity in overweight and obese individuals with or without T2D (Moore *et al.,*
711 *2000)*. Notably, diet-induced weight loss often results in a decrease in IMTG content
712 (Goodpaster *et al.,* 2000; Dube *et al.,* 2011), and this occurs alongside reductions in
713 the concentrations DAGs and ceramides in muscle (Dube *et al.,* 2011), suggesting a
714 remodelling of the intramuscular lipid pool.
715
716 Six months of CR increased markers of mitochondrial biogenesis and mitochondrial
717 DNA and reduced oxidative stress markers in overweight individuals (Civitarese *et al.,*
718 2007), but key enzymes of the TCA cycle, β-oxidation and electron transport chain

were unchanged. Over 16-weeks, CR has shown to increase citrate synthase activity (Menshikova *et al.*, 2017) and reduce skeletal muscle lipid content (Goodpaster *et al.,* 2000), but does not alter mitochondria volume or enzymes from the beta oxidation pathway (Menshikova *et al.*, 2017). Even when similar weight loss is achieved, a CR intervention alone does not achieve improvements in mitochondrial content or electron transport chain enzyme activity, whereas a CR plus exercise intervention does (Toledo *et al.,* 2008). CR has also been shown to reduce IMTG in skeletal muscle, as well as mRNA of genes involved in lipogenesis and FA transport yet showed no change in mitochondrial content or mRNA genes involved in mitochondrial biogenesis (Sparks *et al.,* 2017).

A combined intervention of exercise and CR in an athletic population increases IMTG content (Nadeau *et al.,* 2006), and skeletal muscle oxidative capacity (Pruchnic *et al.*, 2004; Nadeau *et al.,* 2006). Conversely, weight loss induced by a combination of exercise and CR in obese individuals either decreases (Rabol *et al.,* 2009), or does not change IMTG content, but leads to reductions in LD size and increases mitochondrial content (He, Goodpaster and Kelley, 2004). This suggests that a combined exercise and weight loss intervention remodels the LD pool such that the ability to utilise fat as a fuel source and regularly turnover IMTG would be enhanced, rather than simply reducing the amount of IMTG in skeletal muscle. Considering long-term CR interventions fail to improve oxidative capacity, a combined intervention of CR and exercise to reduce lipid metabolites and improve mitochondrial oxidative capacity, is likely the most powerful strategy to increase IMTG utilisation and improve skeletal muscle lipid turnover in individuals at risk of T2D.

Summary

It is now widely agreed that the absolute level of IMTG is unrelated to insulin resistance, but rather a high rate of turnover of the IMTG pool appears to be fundamental to the preservation of insulin sensitivity. Thus, in the trained state IMTG represents a highly dynamic lipid pool within muscle that can be adjusted relative to metabolic demand. Chronic exercise training creates a stimulus of regular breakdown and resynthesis of the IMTG pool, reducing the potential for accumulation of toxic lipid metabolites and therefore the risk of skeletal muscle insulin resistance. In obese

individuals and people with T2D there is a collective imbalance between the rate of FA uptake into muscle, esterification and storage, IMTG breakdown and oxidation. Despite many years of research, the molecular mechanisms underlying this imbalance are yet to be fully understood. The discovery that the PLIN proteins may play a role regulating LD dynamics has provided additional understanding over the last decade, but efforts to identify the mechanisms responsible for the low turnover of the IMTG pool in obesity and T2D should continue to form the basis of future work. Exercise training studies demonstrate that improved IMTG turnover is a key adaptation contributing to improved insulin sensitivity at both the skeletal muscle and whole-body level. Importantly, there appears to be potential for the use of pharmacological or nutritional strategies to maximise the insulin-sensitising effect of exercise interventions. Future work is now required to test these interventions in the longer term in clinical populations, with consideration for how these interventions can be successfully adopted in the real world.

Author statements

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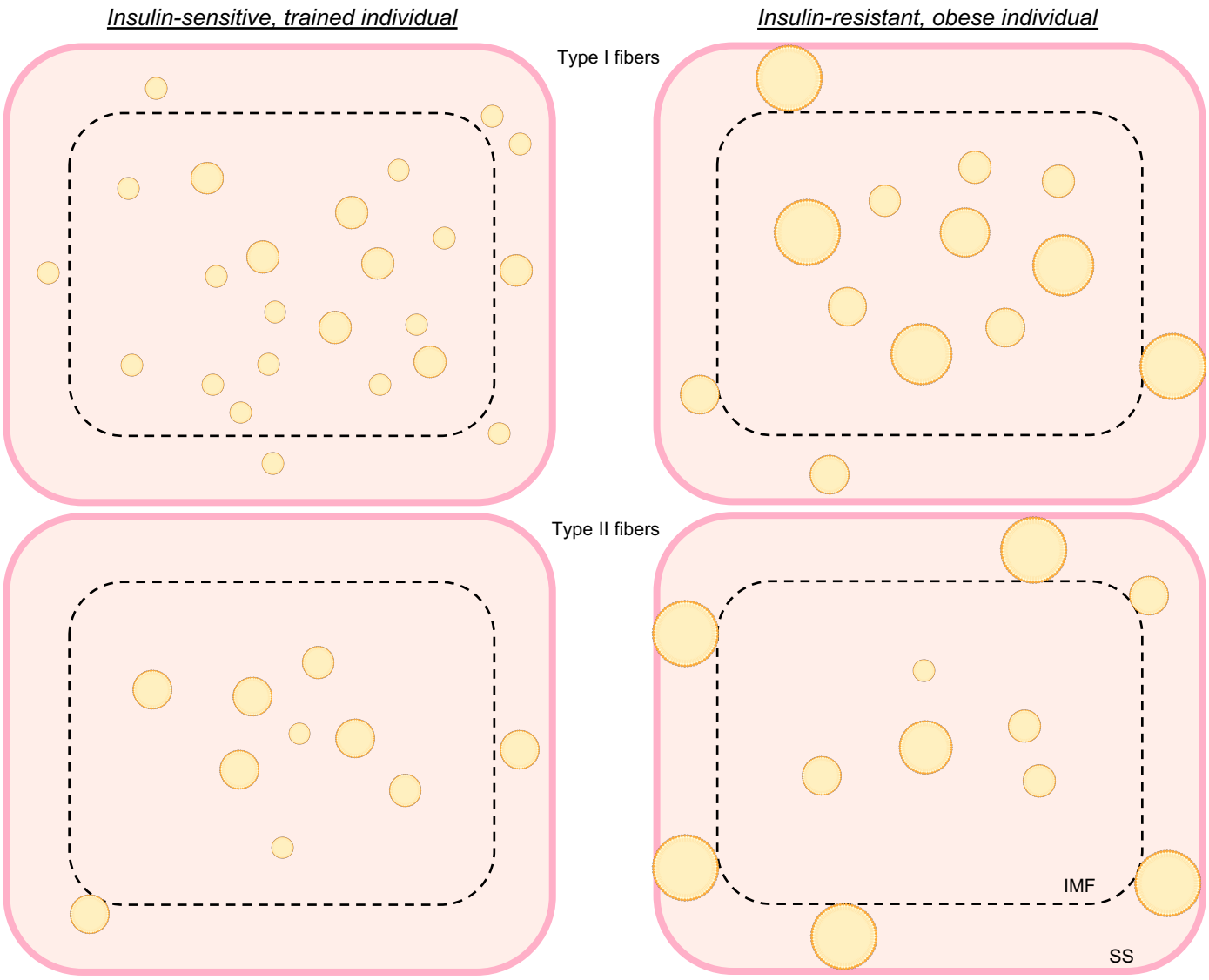
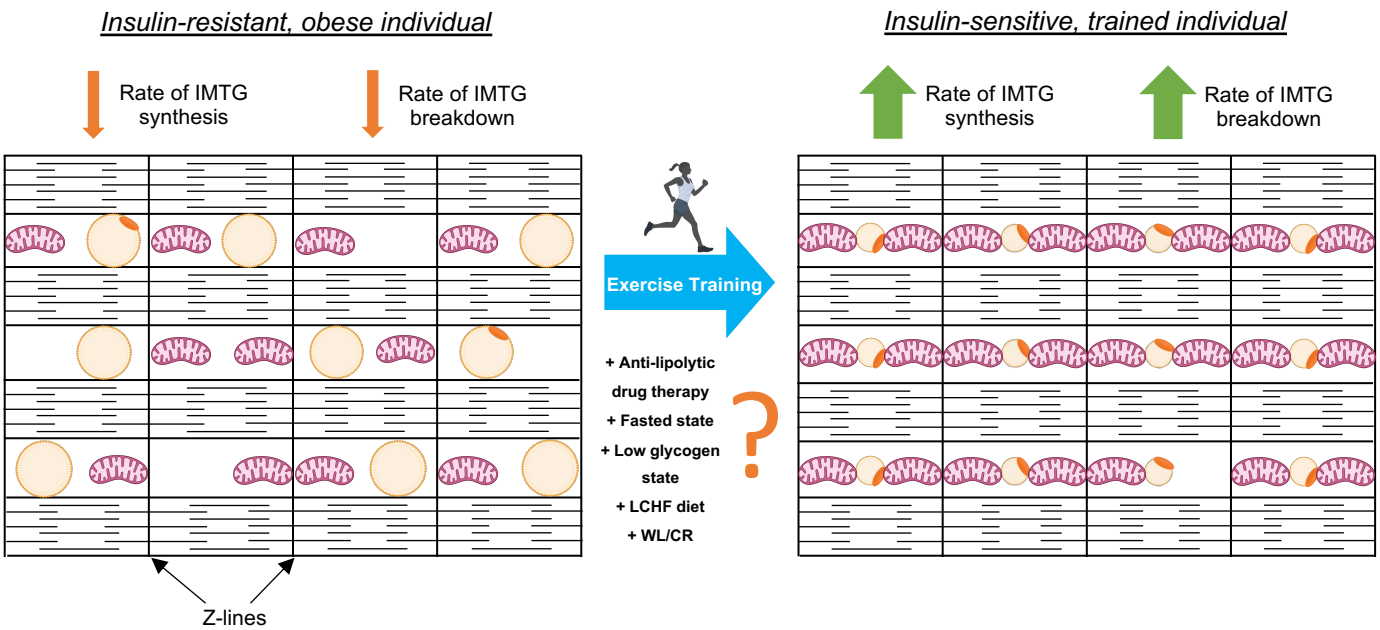
Figure 1. A representation of the subcellular location of LD in skeletal muscle in insulin-resistant, obese individuals and insulin-sensitive, trained individuals.

Insulin-sensitive, trained individuals typically exhibit a large number of small LD which are primarily located in the intermyofibrillar region of type I fibres. Moreover, the number of LD is two-to-three-fold greater in type I compared to type II fibres in trained individuals. In contrast, insulin-resistant, obese individuals tend to exhibit LD that are much large in size compared to insulin-sensitive, trained individuals. Furthermore, in insulin-resistant individuals a higher proportion of LD appear to be present in the subsarcolemmal region compared to trained individuals. This is especially true in type II fibres, where the number and size of SS LD is ~two-fold greater compared to trained individuals (Daemen et al., 2018). Thus, the muscle of trained individuals is characterised by a large number of small LD located in the intermyofibrillar region of type I fibres, whereas the muscle of insulin-resistant obese individuals is characterised by large LDs stored in the subsarcolemmal region of type II fibres.

Figure 2. Schematic overview of the distribution of LD and mitochondria in skeletal muscle in insulin-resistant, obese individuals and insulin-sensitive, trained individuals.

Endurance, resistance, or high intensity interval exercise training promotes a shift towards a more structured network of LD and mitochondria; that is, more LD are labelled with PLIN proteins (PLIN2, 3, 4 or 5, but indicated as one by the small orange ellipse) and are located in close proximity to the mitochondria. This collective adaptation to exercise training enables more efficient breakdown and oxidation of IMTG stored in LD. This is important in order to create a net decrease in IMTG following exercise, which subsequently increases the capacity for IMTG synthesis and storage in the hours following exercise. Thus, following training the overall turnover rate of the IMTG pool is enhanced, which reduces the risk of generating and accumulating toxic lipid metabolites that would otherwise contribute to the development of insulin resistance. In obesity and T2D there is a poor capacity to reduce the rate of IMTG synthesis during exercise and therefore generate a net decrease in IMTG content post-exercise. Additional strategies to exercise alone may therefore be required to create a post-exercise decrease in IMTG content in obese individuals. We propose several co-strategies (anti-lipolytic drug therapy, fasted exercise, training with low muscle glycogen, a LCHF diet or WL/CR) that target an improved rate of IMTG turnover, and therefore maximize the insulin-sensitising effects of exercise training alone in obese individuals. Future work is now required to test these interventions in the longer term in clinical populations, with consideration for how these interventions can be successfully adopted in the real world.


Insulin-sensitive, trained individual
Insulin-resistant, obese individual
Type I fibers
Type II fibers
IMF
SS


Insulin-resistant, obese individual
Rate of IMTG synthesis
Rate of IMTG breakdown
Z-lines
Exercise Training
+ Anti-lipolytic drug therapy
+ Fasted state
+ Low glycogen state
+ LCHF diet
+ WL/CR
?
Insulin-sensitive, trained individual
Rate of IMTG synthesis
Rate of IMTG breakdown