

Symposium 4: New methodologies and insights in the regulation of fat metabolism during exercise

Intramyocellular triacylglycerol as a substrate source during exercise

Luc J. C. van Loon

Nutrition Research Institute Maastricht (NUTRIM), Departments of Movement Sciences and Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

The role of intramyocellular triacylglycerol (IMTG) as a substrate source during exercise has recently regained much attention as a result of the proposed functional relationship between IMTG accumulation and the development of insulin resistance. It has been speculated that elevated NEFA delivery and/or impaired fatty acid (FA) oxidation result in intramyocellular accumulation of triacylglycerol and FA metabolites, which are likely to induce defects in the insulin signalling cascade, causing insulin resistance. The progressive accumulation of IMTG in sedentary patients and patients who are obese and/or have type 2 diabetes should therefore form a major therapeutic target, and efforts should be made to develop interventions that prevent excess IMTG accretion by stimulating their rate of oxidation. Although regular exercise is likely to represent such an effective means, there is much controversy about the actual contribution of the IMTG pool as a substrate source during exercise. The apparent discrepancy in the published literature might be explained by differences in the applied research protocol and the selected subject population, but most of all by the techniques that have been employed to estimate IMTG use during exercise. Data obtained in trained-endurance athletes indicate that athletes can substantially reduce their IMTG pool following a single exercise session. With the growing awareness that skeletal muscle has a tremendous potential to oxidise IMTG during prolonged moderate-intensity exercise, more research is warranted to develop combined exercise, nutritional and/or pharmacological interventions that can stimulate IMTG oxidation in sedentary patients and patients who are obese and/or have type 2 diabetes.

Muscle metabolism: Intramuscular triacylglycerols: Intramyocellular lipid: Magnetic resonance spectroscopy: Insulin resistance

Fat and carbohydrate are the principal substrates that fuel aerobic ATP synthesis in skeletal muscle. Endogenous carbohydrate, mainly stored as muscle and liver glycogen, represents <5% of the total energy storage in an average man. The vast majority of the energy reserves is stored as fat, mainly deposited as triacylglycerol (TG) in subcutaneous and deep visceral adipose tissue. Smaller quantities of TG are present in circulating lipoprotein particles and in lipid droplets inside the muscle fibres (intramyocellular triacylglycerol; IMTG). These intramyocellular lipid droplets are usually located adjacent to the muscle mitochondria (Hoppeler *et al.* 1985), which suggests that they

function as a readily-available pool of fatty acids (FA) for oxidative purposes. Their role in skeletal muscle metabolism has recently regained much interest as a result of the proposed functional relationship between IMTG accumulation and the development of insulin resistance (Shulman, 2000). Initially, the Randle (glucose–FA) cycle was used to explain the mechanism behind FA-induced skeletal muscle insulin resistance. However, more recent findings from various lipid infusion studies have led to the proposal of an alternative mechanism (Shulman, 2000). It has been suggested that elevated plasma NEFA delivery and/or impaired FA oxidation result in the intramyocellular

Abbreviations: EMTG, extramyocellular triacylglycerol; FA, fatty acids; IMTG, intramyocellular triacylglycerol; MRS, magnetic resonance spectroscopy; TG, triacylglycerol.

Corresponding author: Dr Luc J. C. van Loon, fax +31 43 3670976, email L.vanLoon@HB.Unimaas.nl

accumulation of TG and FA metabolites (such as fatty acyl-CoA, diacylglycerol and ceramides), which have been shown to induce defects in the insulin signalling cascade causing insulin resistance (Shulman, 2000; Boden & Shulman, 2002; Hulver & Dohm, 2004). Insulin resistance can subsequently lead to the hyperglycaemic and hyperinsulinaemic state that is associated with type 2 diabetes, and is accompanied by major disturbances in skeletal-muscle substrate metabolism. These disturbances include increased, rather than decreased, muscle glucose oxidation under basal resting and/or exercise conditions and decreased glucose oxidation under insulin-stimulated circumstances, producing a state of metabolic inflexibility (Kelley & Mandarino, 2000). The concomitant impaired capacity to augment fat oxidation during fasting conditions may form one of the key mechanisms that further stimulate IMTG storage in insulin-resistant muscle. This apparent progressive accumulation of IMTG in sedentary patients and patients who are obese and/or have type 2 diabetes should therefore form a major therapeutic target, and efforts should be made to develop effective exercise, nutritional and/or pharmacological interventions that prevent excess IMTG accretion by reducing IMTG storage and/or stimulating IMTG oxidation. However, progress has been complicated by the fact that information on IMTG metabolism is relatively scarce and/or even controversial.

Physical exercise is likely to stimulate IMTG use and could, therefore, represent an effective means of preventing and/or reducing excess IMTG accretion. However, there is considerable discrepancy in the published literature on the proposed role of IMTG as a substrate source during exercise (Watt *et al.* 2002). This discrepancy may be attributed to the various differences in the research protocols (type, duration and intensity of exercise, dietary status) and the selected subject populations (gender, age, body composition, training status) used, as well as the different techniques that have been employed to estimate IMTG oxidation during exercise. The present brief report provides an overview of the contribution of IMTG as a substrate source during exercise in human subjects. It will mainly focus on the strengths and weaknesses of the different techniques that have been used to estimate IMTG oxidation, as these factors may have contributed to the apparent controversy about the role of IMTG as a substrate source. The overview will conclude with speculation on the efficacy of exercise and/or exercise training as a tool to stimulate IMTG oxidation and prevent and/or reduce excess TG accretion in human skeletal muscle.

Isotopic tracer studies

A common approach to the determination of substrate source utilisation during exercise is to combine indirect calorimetry with the use of stable- and/or radioactive-isotope tracers. In numerous studies continuous intravenous infusions of labelled FA have been applied to determine plasma NEFA oxidation rates. By quantifying the total fat oxidation rate, using indirect calorimetry, the use of other fat sources can be estimated. Although these other fat sources have often been suggested to represent IMTG, it is generally assumed that both muscle and lipoprotein-derived

TG contribute to the oxidation rate of these other fat sources (Oscai *et al.* 1990). In most exercise studies the rate of disappearance of the labelled FA tracer from the circulation has been assumed to represent its rate of oxidation in the muscle. However, at rest, and to a lesser extent during exercise, the rate of disappearance exceeds the rate of oxidation (van Loon *et al.* 2001). Thus, earlier reports on plasma NEFA oxidation rates based on the rate of disappearance of the FA tracer have underestimated the use of muscle and lipoprotein-derived TG during exercise by approximately 10–15%. More recent studies have applied ^{13}C - or ^{14}C -labelled FA tracers, which can be used to quantify tracer oxidation rates directly from the rate of appearance of $^{13}\text{CO}_2$ ($^{14}\text{CO}_2$) in the expired breath. The main assumption when calculating the rate of oxidation of ^{13}C (or ^{14}C)-labelled FA tracers from the excretion of $^{13}\text{CO}_2$ ($^{14}\text{CO}_2$) is that the rate of appearance of $^{13}\text{CO}_2$ in the expired breath accurately reflects the rate of oxidation of the applied tracer. However, this assumption is not always accurate as corrections need to be made for the amount of labelled CO_2 that is produced but not excreted, as a result of the retention of labelled CO_2 in the bicarbonate pools, as well as for label temporarily fixed in other metabolites by way of isotopic exchange reactions within the tricarboxylic acid cycle (Schrauwen *et al.* 1998). An acetate correction factor has been devised to provide a practical means of correction for such C-label retention (Sidossis *et al.* 1995; Schrauwen *et al.* 1998; van Loon *et al.* 2003a). Failure to apply an acetate correction factor leads to a gross underestimation of tracer oxidation rates at rest and, in most cases, a substantial underestimation during exercise. As acetate label recovery reaches an apparent plateau value during exercise, values obtained from previously published studies are often used to correct for label retention in studies performed in other laboratories and/or under different conditions. However, it has been shown that acetate label recovery during exercise depends on the exercise intensity and pre-exercise infusion time, as well as on the individual subjects used in the studies (van Loon *et al.* 2003a). Thus, it is advisable not to use published acetate recovery factors.

Several studies that have used FA isotope tracers have shown that during moderate-intensity exercise approximately 40–60% of the total fat oxidation is accounted for by plasma-derived NEFA oxidation in endurance-trained male subjects following an overnight fast (Romijn *et al.* 1993; Sidossis *et al.* 1998; Coyle *et al.* 2001; van Loon *et al.* 2001). These findings suggest that other fat sources can contribute substantially to total fat oxidation during exercise. However, the relative contribution to total energy expenditure has been shown to depend on exercise intensity (Romijn *et al.* 1993; van Loon *et al.* 2001), exercise duration (Romijn *et al.* 1993; van Loon *et al.* 2003b), training status (Martin *et al.* 1993; Phillips *et al.* 1996; Schrauwen *et al.* 2002) and diet (Schrauwen *et al.* 2000; Coyle *et al.* 2001), and seems to be substantially lower in patients who are obese and/or have type 2 diabetes (Blaak *et al.* 2000). Consequently, the results of these stable-isotope studies suggest that IMTG and/or lipoprotein-derived TG have the capacity to form an important substrate source during exercise. Even though the oxidation rate of IMTG plus

lipoprotein-derived TG appears to be most pronounced in highly-trained endurance athletes exercising at a moderate-intensity workload (van Loon *et al.* 2001; Fig. 1), recent findings indicate that lean sedentary males can substantially (2–3-fold) increase their capacity to use these TG sources after 3 months of low-intensity endurance training (Schrauwen *et al.* 2002).

In addition to the previously mentioned studies that applied stable-isotope methodology at the whole-body level, other research groups have applied similar methodology to the working muscle or limb. Most of these studies have observed considerable NEFA uptake and/or oxidation in the active limb (van Hall *et al.* 1999). While at rest a large proportion of plasma NEFA uptake has been shown to be re-esterified into the IMTG pool, muscle contraction markedly increases the proportion of NEFA uptake that is routed towards oxidation, at the expense of re-esterification and storage in muscle TG stores (Sacchetti *et al.* 2002). Although leg RQ values tend to be generally higher than whole-body RER values, plasma NEFA oxidation rates do not fully account for total fat oxidation in the leg. For example, recent data by van Hall *et al.* (2002) show considerable FA oxidation in the working leg during 2 h of one-leg knee-extensor exercise at 65% of the maximal leg power output. In line with whole-body tracer measurements, 68 (SE 32) % of the total fat oxidation in the leg is accounted for by plasma NEFA oxidation during the first 30–60 min of exercise, which leaves a considerable contribution to be made by the use of other fat sources. In accordance with recent data (van Loon *et al.* 2003b), which show a progressive increase in plasma NEFA

oxidation rate and a concomitant decrease in the use of other fat sources with the duration of moderate-intensity exercise, data by van Hall *et al.* (2002) for the working limb also suggest that the reliance on muscle-derived TG is reduced with exercise duration.

In summary, the FA tracer methodology described indirectly estimates muscle plus lipoprotein-derived TG oxidation during exercise. Most stable-isotope studies suggest that these TG form an important substrate source during moderate-intensity exercise in both endurance-trained athletes as well as in lean healthy men not involved in any regular exercise training. As re-esterification of plasma NEFA uptake into the IMTG pool is markedly reduced during exercise, these studies also imply that IMTG contents are reduced following prolonged exercise.

Biochemical estimate of muscle triacylglycerol content

Not all researchers agree with the contention that IMTG stores are oxidised during exercise, as studies applying muscle TG extraction analyses to muscle samples collected before and after prolonged moderate-intensity exercise in both trained and untrained subjects have provided contradictory findings (Watt *et al.* 2002). For example, it has recently been suggested that IMTG stores do not play an important role during moderate-intensity exercise in trained men as opposed to trained women (Roepstorff *et al.* 2002; Steffensen *et al.* 2002), but rather form an important substrate source during post-exercise recovery (Kiens & Richter, 1998). Watt *et al.* (2002) have suggested that the apparent discrepancy may be the result of the high between-biopsy variability (>23%) obtained when using the TG extraction technique to estimate net changes in IMTG content. The latter can be explained by the presence of extramyocellular lipid and/or by differences in fibre type composition in the muscle samples obtained (Wendling *et al.* 1996). Watt *et al.* (2002) have concluded that most studies applying direct muscle TG extraction analyses to muscle biopsies before and after prolonged exercise report small, but not significant, reductions in muscle TG content. In addition, they have reported a much lower between-biopsy variability (<12%) when TG extraction analyses are performed on muscle samples taken from endurance-trained athletes (Watt *et al.* 2002). This finding may partly explain why most of the studies that report significant net reductions in muscle TG content are those studies that have been performed with endurance-trained athletes. Clearly, the expected problems with the high between-biopsy variability in muscle TG content when using the TG extraction method could be of even more importance in sedentary patients and in patients who are obese and/or have type 2 diabetes. The reason is the large subcutaneous fat layers and the suggested increase in extramyocellular lipid deposits in these subjects, which are likely to result in substantial contamination of muscle biopsy samples. Clearly, additional techniques need to be applied in the investigation of the effects of exercise and/or exercise training on muscle TG use in sedentary patients and in patients who are obese and/or have type 2 diabetes.

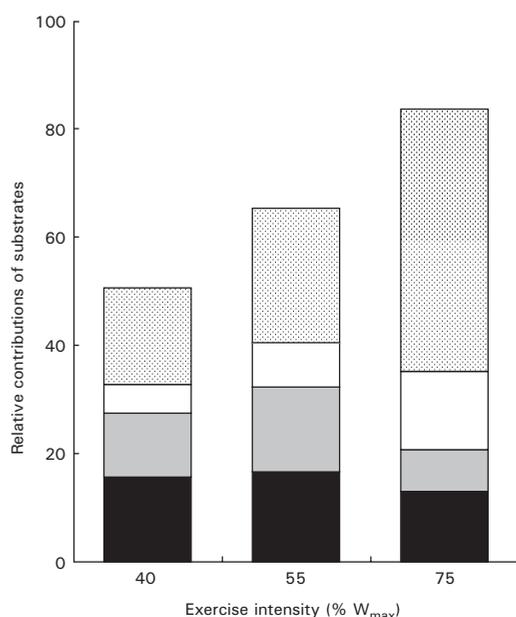


Fig. 1. Energy expenditure as a function of exercise intensity (expressed as a percentage of maximal workload capacity (W_{max})). The relative contribution of plasma glucose (□), muscle glycogen (▒), plasma NEFA (■) and other fat sources (sum of muscle and lipoprotein-derived triacylglycerols; □) to total energy expenditure at different levels of exercise intensity are illustrated. (Adapted from van Loon *et al.* 2001.)

Magnetic resonance spectroscopy

With the introduction of magnetic resonance spectroscopy (MRS), a non-invasive means of quantifying both the intra- and extramyocellular lipid content has become available (Boesch *et al.* 1999; Boesch & Kreis, 2000). The proton MRS technique can be used to measure the resonances from methyl and methylene groups of IMTG. The resonance signal appears as multiple peaks on the proton spectrum of the skeletal muscle of interest. These multiple peaks have been linked to different compartments, and have been assigned to represent either IMTG or EMTG content. In MRS literature these variables are often referred to as intra- and extramyocellular lipid (termed IMCL and EMCL respectively). Although the differentiation between IMTG and EMTG based on a shift in the resonance frequency is not entirely unambiguous, several studies have reported good correlations between IMTG content as determined by MRS and by other techniques (Howald *et al.* 2002; van Loon *et al.* 2003c). Whereas the IMTG signal is independent of the angle between the leg and the static magnetic field, the amplitude of the EMTG signal has been shown to be highly variable. Thus, in contrast to the IMTG signal, the EMTG signal should not be regarded as a representative indicator of the actual EMTG content in a muscle (Boesch *et al.* 1999; Boesch & Kreis, 2000). There is still uncertainty as to what the EMTG signal actually represents. In the author's laboratory, as well as in others (Levin *et al.* 2001), structural analyses of large numbers of muscle cross-sections using various microscopy techniques have failed to provide evidence for the presence of substantial extramyocellular lipid depots and/or adipocytes situated between muscle fibres. Clearly, more research is warranted to investigate the physiological structure(s) responsible for the shift in resonance frequency.

The MRS technique offers excellent reproducibility, and seems to represent an accurate quantitative assessment of IMTG content. Subsequently, various research groups have applied MRS to the quantification of IMTG content in different populations, which has greatly improved the understanding of the proposed relationship between obesity, elevated IMTG contents and the development of insulin resistance (Shulman, 2000). All studies applying MRS to the assessment of muscle lipid content before and after prolonged endurance exercise have reported substantial (approximately 20–40%) decreases in IMTG concentration in healthy trained and untrained male and trained female subjects (Boesch *et al.* 1997, 1999; Krssak *et al.* 2000; Rico-Sanz *et al.* 2000; Brechtel *et al.* 2001; Decombaz *et al.* 2001; Larson-Meyer *et al.* 2002; Johnson *et al.* 2003; Schrauwen-Hinderling *et al.* 2003; van Loon *et al.* 2003c). In most of these studies IMTG content has been determined in lower leg muscle (tibialis and/or soleus muscle), which is in contrast to the majority of studies using biochemical estimates of muscle TG utilisation that have generally used muscle samples from the vastus lateralis. However, more recent MRS studies have now also reported similar relative decreases in IMTG content in the vastus lateralis muscle following prolonged moderate-intensity exercise (Johnson *et al.* 2003; Schrauwen-Hinderling *et al.* 2003; van Loon *et al.* 2003c). Although the relative

changes in IMTG content seem to be of similar magnitude, these studies show that there are large differences in IMTG content between various muscle groups within similar subject populations. The absolute IMTG concentrations observed in the vastus lateralis (Johnson *et al.* 2003; van Loon *et al.* 2003c) are well above IMTG contents reported for tibialis and/or soleus muscle (Rico-Sanz *et al.* 1998, 2000; Decombaz *et al.* 2001; Hwang *et al.* 2001), which can only partly be explained by differences in muscle fibre type composition (Hwang *et al.* 2001). This finding indicates that local net changes in muscle TG content cannot generally be translated towards whole-body IMTG use.

As most exercise studies have focused on IMTG depletion, far less data are available on post-exercise IMTG repletion and the role of dietary fat intake in post-exercise IMTG storage (Decombaz *et al.* 2000, 2001; Larson-Meyer *et al.* 2002; van Loon *et al.* 2003c). The latter is in contrast to the numerous studies on muscle glycogen use and the efficacy of dietary interventions to accelerate muscle glycogen synthesis, which have led to the recommendation that endurance athletes should use high-carbohydrate low-fat diets. Interestingly, recent MRS data have shown that the use of low-fat (<10–15% energy from fat) as well as normal-fat (approximately 35–40% energy from fat) diets can substantially impair post-exercise IMTG repletion (Decombaz *et al.* 2000, 2001; Larson-Meyer *et al.* 2002; van Loon *et al.* 2003c) compared with the use of relatively-high-fat diets. Clearly, the use of MRS to non-invasively determine changes in IMTG content over time during prospective nutritional, exercise and/or pharmacological interventions will be of great value in future research.

In summary, in accordance with most stable-isotope data, studies using MRS as a means of quantifying both intra- and extramyocellular lipid content all seem to support the contention that substantial net decreases in IMTG content (approximately 20–40%) occur following prolonged endurance exercise in both trained as well as healthy fit but untrained subjects. It is also interesting that these MRS studies have shown marked net reductions in IMTG content in both trained male and female athletes, following both running as well as cycling exercise, in an overnight fasted as well as in a fed state.

Histochemistry and fluorescence microscopy

Both the biochemical method and the use of MRS to measure muscle TG content are restricted to the quantification of net muscle TG content in mixed muscle samples and, therefore, do not facilitate direct localisation of the muscle lipid depots or differentiation between muscle fibre types. The latter could be of importance, as muscle fibre type recruitment during prolonged moderate-intensity exercise relies mainly on the use of type I muscle fibres. Recently, conventional oil red O staining of muscle cross-sections has been combined with (immuno)fluorescence microscopy to facilitate such a selective quantification of muscle lipid content in type I and II muscle fibres (Koopman *et al.* 2001; van Loon *et al.* 2003a,b). Fluorescence microscopy does not eliminate all problems

associated with the heterogeneity of repeat muscle biopsy samples. However, an important advantage of this method is that contamination of muscle biopsy samples with extramyocellular fat and/or differences in muscle fibre type composition can be visualised directly and taken into account during the quantification procedures. Using the fluorescence method (Fig. 2) an approximately 3-fold greater lipid content in the type I v. type II muscle fibres has recently been observed. This finding is in agreement with earlier findings from fibre type-specific quantification of lipid content using biochemical TG estimation in dissected muscle fibres (Essen *et al.* 1975), as well as with oil red O staining using conventional light microscopy (Malenfant *et al.* 2001).

As a result of the apparent discrepancy in the existing literature on the capacity of human skeletal muscle to oxidise and reduce net IMTG content, the role of IMTG as a substrate source during moderate-intensity exercise has recently been investigated in eight endurance-trained male cyclists following an overnight fast (van Loon *et al.* 2003b). These conditions were selected because they result in maximal fat oxidation rates (Fig. 1). (Immuno)fluorescence microscopy was applied to oil red O-stained muscle cross-sections prepared from biopsies taken from the vastus lateralis before and immediately after a 2 h exercise trial (approximately 60% $V_{O_{2max}}$) and a >60% net reduction in intramyocellular lipid content in the type I muscle fibres only was observed (Fig. 2). This finding is in accordance with the preferred recruitment of type I muscle fibres during prolonged endurance exercise and shows that IMTG depletion has been underestimated in the past as a result of the inability to differentiate between fibre types. Clearly, future research investigating the role of exercise and/or exercise training as a means of reducing IMTG content should consider fibre type-specific changes.

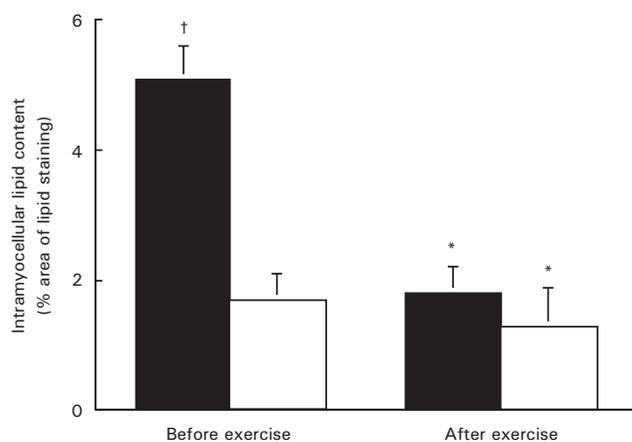


Fig. 2. Mean fibre type-specific (type I, ■; type II, □) intramyocellular lipid content (% area of lipid stained) before and immediately after exercise as determined by quantitative fluorescence microscopy on oil red O-stained muscle cross-sections. Values are means with their standard errors represented by vertical bars. Mean values were significantly lower than pre-exercise values: * $P < 0.05$. Mean values were significantly higher than those for type II muscle fibres: † $P < 0.05$. (Adapted from van Loon *et al.* 2003b.)

Intramyocellular triacylglycerol content, insulin resistance and exercise

Various studies have reported a strong positive correlation between IMTG content and insulin resistance. This finding has led to the proposed functional relationship between elevated plasma NEFA levels, IMTG accumulation and the development of insulin resistance (Shulman, 2000). As such, elevated IMTG content is now often regarded as a risk factor for the development of insulin resistance and type 2 diabetes. However, the positive correlation between IMTG content and insulin resistance would have disappeared entirely if endurance-trained athletes had been included in these correlation studies, because endurance-trained athletes are markedly insulin sensitive, despite having an elevated muscle TG content (Goodpaster *et al.* 2001). The apparent increase in IMTG-storage capacity in the athlete may represent one of the many metabolic responses to endurance training, similar to increased muscle glycogen storage and increased mitochondrial density (Hoppeler *et al.* 1985). A recent comparison of oil red O-stained muscle cross-sections obtained from overweight patients with type 2 diabetes, weight-matched normoglycaemic controls and highly-trained endurance athletes has shown a >75% higher IMTG content in the trained athletes compared with the overweight patients with type 2 diabetes and their normoglycaemic controls. About 40% of this difference is attributable to the higher type I muscle fibre content of the trained athletes. As no further differences in the structural characteristics and relative distribution pattern of the IMTG deposits between these groups could be detected, the data imply that elevated IMTG contents alone are unlikely to be directly responsible for inducing skeletal muscle insulin resistance. Thus, in sedentary patients and in patients who are obese and/or have type 2 diabetes the elevated IMTG contents may simply act as an indicator of a structural imbalance between plasma FA uptake and FA oxidation. It seems likely, therefore, that the capacity to oxidise IMTG is one of the factors that modulates the association between IMTG accretion and the development of insulin resistance. Fig. 3 shows a comparison between stable-isotope tracer data for whole-body substrate use during exercise as obtained by Blaak *et al.* (2000) for patients with type 2 diabetes and obese controls with data from Schrauwen *et al.* (2002) for a group of sedentary lean subjects before and after a 3-month training programme and data from van Loon *et al.* (2001) for highly-trained endurance athletes. These data suggest that the capacity to oxidise IMTG plus lipoprotein-derived TG during exercise is greatly reduced in patients who are obese and/or have type 2 diabetes, and that it can be augmented in healthy sedentary lean subjects by participation in an exercise training programme. From these data it could be speculated that the difference between trained athletes and patients who are obese and/or have type 2 diabetes in the relationship between muscle TG content and insulin sensitivity may be explained by the capacity to oxidise the IMTG stores, which would indicate that it is not the actual size of the IMTG stores but rather the mobility of these stores that may be linked with insulin resistance.

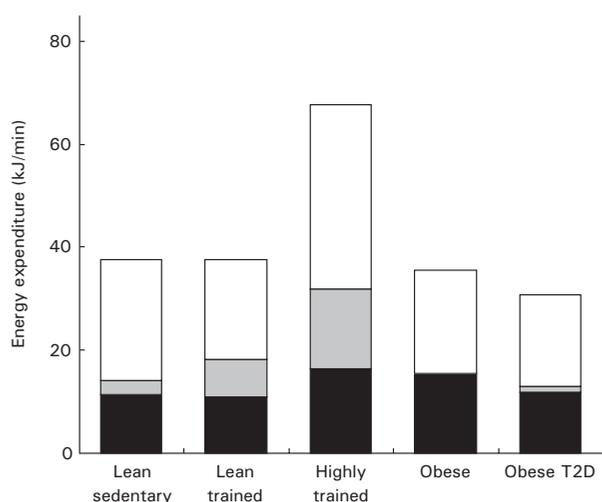


Fig. 3. Substrate utilisation in sedentary lean subjects before and after 3 months of low-intensity endurance training (Schrauwen *et al.* 2002), in highly-trained endurance athletes (van Loon *et al.* 2001) and in obese patients with type 2 diabetes (T2D) and their obese controls (Blaak *et al.* 2000) during moderate intensity exercise (50–60% $\dot{V}_{O_{2max}}$). Total fat and carbohydrate oxidation rates were determined by indirect calorimetry, plasma NEFA oxidation rates were measured by the intravenous [$U-^{13}C$]palmitate infusion, with corrections for ^{13}C label retention using an [$1,2-^{13}C$]acetate correction factor, determined in independent additional trials. (□), Endogenous carbohydrates; (▒), muscle and lipoprotein-derived triacylglycerol; (■), plasma NEFA.

In summary, with the awareness that endurance-trained athletes have the capacity to substantially reduce their IMTG pool by undertaking even a single exercise session, more research is warranted to define ways of preventing and/or reducing IMTG accumulation in sedentary patients and in patients who are obese and/or have type 2 diabetes. These interventions should focus on reducing plasma FA availability as well as stimulating IMTG oxidation to improve the balance between FA uptake and oxidation. It seems clear that an increase in the capacity to oxidise IMTG by way of regular exercise training in combination with nutritional and/or pharmacological assistance could prove to be a very effective approach.

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