Physiological factors that regulate the use of endogenous fat and carbohydrate fuels during endurance exercise

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Exercise causes a dramatic increase in energy requirements because of the metabolic needs of working muscles. Exercise-dependent factors regulate fuel use. Absolute exercise intensity determines the exercise-induced increase in energy demands, whereas exercise intensity relative to an individual’s maximal aerobic capacity (VO₂ max) determines the proportional contribution of different fuel sources (i.e. plasma glucose, plasma fatty acids, muscle glycogen and intramuscular triacylglycerols). Endurance training increases aerobic capacity in muscle and the oxidation of fat during exercise. In addition, exercise-independent factors, such as diet composition, sex, age, and body composition also influence substrate use during exercise. The present review discusses the regulation of substrate use during exercise in human subjects, with a focus on the role of exercise-independent factors.

Energy requirement: Fat metabolism: Carbohydrate metabolism: Endurance exercise

Introduction

During exercise there is a dramatic increase in energy requirements because of the metabolic needs of working muscles. During resting conditions, skeletal muscle consumes approximately 30 % of the body’s total energy requirements, and most (about 80 %) of the muscles’ energy needs at rest are derived from fat, the remainder being derived from carbohydrates (Andres et al. 1956; Dagenais et al. 1976). Exercise can induce up to a 10-fold increase in energy requirements and, therefore, fat and carbohydrate oxidation increase (Klein et al. 1994; Mendenhall et al. 1994) (Fig. 1). The relative contribution of carbohydrate and fat as fuel for working muscle is largely dependent on exercise intensity and duration. Fat is the predominant source of fuel during prolonged low- and moderate-intensity exercise (up to about 65 % of maximal O₂ consumption; VO₂ max) (Jones et al. 1980; Romijn et al. 1993; van Loon et al. 2001); as exercise intensity increases there is a progressive decline in fat oxidation and the relative oxidation of carbohydrate increases (Jones et al. 1980; Coggan, 1991; Romijn et al. 1993; van Loon et al. 2001). Carbohydrate is also the predominant source of energy at the onset of exercise and during short-term exercise, such as sprints (for example, Trump et al. 1996; McKenna et al. 1997; Howlett et al. 1999a). Endurance training increases muscle oxidative capacity and fat oxidation (Horowitz & Klein, 2000a). In addition to these factors that are directly exercise-dependent (for example, exercise intensity, duration, training), exercise-independent factors such as an individual’s sex (for example, Mittendorfer et al. 2002), age (for example, Sial et al. 1996), body composition (for example, Horowitz & Klein, 2000b), chronic diet composition (for example, Schrauwen et al. 2000), and exogenous or dietary fuel availability during exercise (for example, Horowitz et al. 1997; Odland et al. 1998) influence substrate use during exercise. Muscle fibre type might also be an important determinant of substrate metabolism during exercise because mitochondrial density is greater in slow-twitch than fast-twitch fibres (Ingjer, 1979a; Prince et al. 1981). However, Helge et al. (1999) were unable to find any correlation between RER and fibre type in young adult men during moderate-intensity exercise. It is possible that the variability in fibre type composition in these men was not sufficient to demonstrate a relationship between substrate use and fibre type. However, no studies are known

Abbreviations: IMTG, intramuscular triacylglycerols; NEFA, non-esterified fatty acids; PCr, phosphocreatinine; Ra, rate of appearance; Rd, rate of disappearance; TG, triacylglycerols; VO₂ max, maximal O₂ consumption.

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that compared fibre type composition and substrate oxidation in a larger and more diverse group of subjects.

In the present paper, the regulation of substrate use during exercise in human subjects is reviewed, particularly the role of exercise-independent factors.

**Exercise intensity and duration**

Immediately after the onset of exercise (0–30 s) energy is derived predominantly from anaerobic metabolism (Grassi et al. 1996). The switch from aerobic to anaerobic energy metabolism at the onset of exercise is due to some yet unknown mechanism because O2 availability (Bangsbo et al. 2000b; Schrauwen et al. 2000; van Hall et al. 2002) and is mediated by increased β-adrenergic stimulation (Hall et al. 1987; Arner et al. 1990). In addition, exercise stimulates lipolysis of intramuscular TG (IMTG) (Carlson et al. 1971; Essen et al. 1977; Hurley et al. 1986), which releases fatty acids that are directly oxidized by local mitochondria. Table 1 summarizes the body energy stores for a lean individual.

The exact contribution of IMTG to total fat oxidation during exercise is not known because it is difficult to directly measure IMTG oxidation (Wendling et al. 1996; Watt et al. 2002a). Several studies, in which muscle biopsies were taken before and after exercise, found that IMTG concentration declines by 25–40 % after 1–2 h of moderate-intensity cycle ergometer exercise, which could account for 60–75 % of the total amount of fat oxidized (Carlson et al. 1971; Froberg & Mossfeldt, 1971; Essen et al. 1977; Savasi et al. 1996). In contrast, other investigators found that IMTG concentration decreases minimally or not at all after prolonged exercise and therefore does not contribute significantly to total energy production (Kiens et al. 1993; Wendling et al. 1996; Starling et al. 1997; Kiens & Richter, 1998; Bergman et al. 1999). When exercise is performed for more than 4 h, IMTG generally decreased from resting conditions (Froberg & Mossfeldt, 1971; Costill et al. 1973). The reason for the discrepancies between studies is not clear but may be related to difficulties in measuring IMTG concentration in muscle biopsies. Due to the high energy density of fat, only about 2–3 mmol TG/kg dry muscle are required to provide fuel during 2 h of moderate-intensity exercise. This amount of TG represents about 10–15 % of total muscle TG stores, whereas the variability of measuring IMTG concentration is about 25 % (Wendling et al. 1996; Watt et al. 2002a). The poor reproducibility of measuring IMTG concentration makes detection of small changes in IMTG content during exercise nearly impossible. The differences between these studies might, however, also be due to differences in exercise protocols and/or study subject characteristics.

Estimates of IMTG use can also be calculated by using a combination of isotopic tracer and indirect calorimetry tech-

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**Table 1. Body energy stores**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fuel</th>
<th>Energy (MJ)</th>
<th>Time (h)</th>
<th>Distance (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>Triacylglycerol</td>
<td>585-76</td>
<td>168</td>
<td>2000</td>
</tr>
<tr>
<td>Muscle</td>
<td>Triacylglycerol</td>
<td>13-45</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>8-37</td>
<td>2.5</td>
<td>32</td>
</tr>
<tr>
<td>Liver</td>
<td>Triacylglycerol</td>
<td>2-09</td>
<td>0.6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>1-26</td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>Blood</td>
<td>Triacylglycerol, NEFA, glucose</td>
<td>0-42</td>
<td>0-1</td>
<td>1-5</td>
</tr>
</tbody>
</table>

NEFA, non-esterified fatty acids.

* Values are estimates based on a lean individual.
This approach assumes that the difference between whole-body fat oxidation, determined by indirect calorimetry, and plasma fatty acid oxidation, determined by isotope tracers, is due to oxidation of non-plasma fatty acids, presumably derived from IMTG. Data from studies that used this approach suggest that IMTG provides > 50% of the total fat oxidized during prolonged moderate-intensity exercise (Martin et al. 1993; Dyck & Bonen, 1998).

Recently, Guo et al. (2000) used a dual-tracer, pulse-chase technique to directly measure IMTG oxidation during 90 min of moderate-intensity cycling exercise in twelve healthy non-obese men and women and found that: (1) the contribution of IMTG to total fat oxidation was about 50%; (2) the sum of plasma non-esterified fatty acids (NEFA) and IMTG oxidation rates was not different from the total fatty acid oxidation rate measured by using indirect calorimetry; (3) IMTG oxidation rates calculated by using the dual-tracer approach were not different from IMTG calculated as the difference between total fatty acid oxidation (by indirect calorimetry) and plasma fatty acid oxidation. These data suggest that, if there is no other significant source of lipid fuel, this approach can provide a direct measure of IMTG use during exercise that might provide a potentially robust direct measure of IMTG use.

The regulation of IMTG use during exercise has not been carefully studied. However, IMTG use is probably linked to catecholamine release in response to β2-receptor stimulation during exercise. Endurance exercise increases the activity of TG lipase in rat (Langfort et al. 2000) and human muscle (Kjaer et al. 2000); this response is absent in adrenalectomized subjects but can be restored via infusion of adrenalin (Kjaer et al. 2000). These findings are supported by a study that investigated the role of β-receptor stimulation on IMTG use during about 1 h exercise to exhaustion (Cleroux et al. 1989); IMTG use was completely blocked by pharmacological blockage of β3,β3-receptors whereas β1-blockage alone had no effect on IMTG use. However, the exercise-induced activation of TG lipase in muscle might also be controlled by other factors, probably intrinsic in muscle. Langfort et al. (2000) found that electrical stimulation of rat soleus muscle increases TG lipase activity for several minutes but returns to baseline after 60 min of stimulation, even in muscles from animals in which sympathoadrenal organs were removed. Furthermore, addition of propanolol to the incubation medium did not impair the contraction-induced activation of TG lipase (Langfort et al. 2000).

Fatty acids derived from plasma TG (Ie. VLDL-TG) can also be taken up by muscle and oxidized as fuel. Few studies have evaluated the contribution of plasma TG to total energy production, but the available data suggest it is unlikely that VLDL-TG is an important source of fuel during exercise. During resting conditions, plasma TG may account for 5–10% of total fat oxidation (Ryan & Schwartz, 1965; Wolfe et al. 1985). There is also indirect evidence that only a very small fraction of total energy production is derived from plasma TG during exercise (Mackie et al. 1980; Kiens & Lithell, 1989; Turcotte et al. 1992). For example, Guo et al. (2000) and Kiens & Lithell (1989) found that both total plasma TG and VLDL-TG uptake by skeletal muscle is negligible during exercise. In addition, the activation of muscle lipoprotein lipase, the enzyme that hydrolyses plasma TG, occurs about 8–20 h after an exercise bout is completed (Seip et al. 1995; Greiwe et al. 2000; Pilegaard et al. 2002). Therefore, it is probable that muscle uptake of VLDL-TG after exercise is important for replenishing IMTG stores consumed during exercise.

At rest, the oxidation of plasma NEFA (derived from lipolysis of adipose tissue TG) can entirely account for total fat oxidation (Sidossis et al. 1995). Studies in which stable-isotope tracer techniques were applied found that during low- and moderate-intensity exercise, plasma NEFA and IMTG contribute equally to total fat oxidation (Sidossis et al. 1997, 1998; van Loon et al. 2001); during high-intensity exercise, total fat oxidation decreases, in part, because of a decrease in plasma fatty acid availability (Romijn et al. 1993, 1995) and a decrease in the use of IMTG (van Loon et al. 2001). Raising plasma fatty acid concentrations to 1–2 mmol/l by intravenously infusing a lipid emulsion and heparin during the exercise bout increases fat oxidation by about 30% (Romijn et al. 1995) but does not completely restore it to the rate observed during moderate-intensity exercise (Romijn et al. 1993). Thus, high-intensity exercise probably decreases the capacity of skeletal muscle to oxidize fatty acids. The suppression of fat oxidation during high-intensity exercise may be related to increased glycogen metabolism in muscle. The high rate of muscle glycogenolysis during high-intensity exercise increases the amount of acetyl-CoA derived from glycogen, which presumably increases malonyl-CoA concentrations in muscle (Elayan & Winder, 1991; Sadiuk et al. 1993). Malonyl-CoA inhibits carnitine O-palmitoyltransferase-I, the enzyme responsible for long-chain fatty acid entry into mitochondria (McGarry et al. 1977, 1983; Robinson & Zammit, 1982; Rasmussen & Wolfe, 1999). Therefore, high rates of glycogenolysis during high-intensity exercise can reduce fat oxidation by impairing long-chain fatty acid transport into the mitochondria by inhibiting carnitine O-palmitoyltransferase-I (Sidossis et al. 1997).

Glucose delivered by the bloodstream (derived from hepatic glycogenolysis and gluconeogenesis) or released locally by breakdown of intramuscular glycogen can be used by skeletal muscle as fuel. At rest and during low-intensity exercise, blood glucose is the predominant source of carbohydrate for oxidation; the oxidation of blood glucose increases with increasing exercise intensity and reaches values of approximately three times resting values during high-intensity exercise (van Loon et al. 2001). The contribution of muscle glycogen to total energy expenditure is negligible at rest, but increases with increasing exercise intensity and accounts for the majority (> 75%) of total carbohydrate oxidation during high-intensity exercise (van Loon et al. 2001). The increase in plasma glucose use during exercise is mediated by increased translocation of glucose transporter-4 vesicles to the plasma membrane, an increase in the insulin sensitivity of glucose transporter-4-mediated glucose transport, and an increase in glucose transporter-4 protein (Holloszy et al. 1998). Hepatic glucose production increases, primarily by increased glucagon secretion during mild- and moderate-intensity exercise (Wasserman et al. 1989; Kjaer et al. 1993; Wasserman, 1995; Coker et al. 2001), to maintain plasma glucose con-
centration when muscle glucose uptake is increased by exercise (Coggan et al. 1992a; Holloszy et al. 1998; van Loon et al. 2001; Helge et al. 2003). However, other factors such as a rise in catecholamine concentrations might also contribute to glucose production, particularly at higher exercise intensities (Coggan, 1991; Sigal et al. 1994, 1996, 2000; Wasserman, 1995; Coggan et al. 1997; Coker et al. 2001). The increase in muscle glycogen use is caused by several factors. Muscle contraction increases muscle cytosolic Ca concentration, which activates phosphorylase causing glycogen breakdown (Holloszy et al. 1998). However, the increase in cytosolic Ca is short-lived and is only responsible for glycogen breakdown during the onset of exercise. Glycogen breakdown is also mediated continuously during exercise by β-adrenergic stimulation and a cAMP-activated mechanism of phosphorylase activation (Holloszy et al. 1998), which can eventually lead to complete muscle glycogen depletion.

**Exercise training**

The effect of endurance training on substrate metabolism has been previously reviewed (Holloszy et al. 1998; Rasmussen & Wolfe, 1999; Horowitz & Klein, 2000a). Endurance exercise training increases the oxidation of fatty acids and spares muscle glycogen and blood glucose use during submaximal exercise (Holloszy & Coyle, 1984; Horowitz & Klein, 2000a). Several adaptations within skeletal muscle itself facilitate the increased oxidation of fatty acids: increased density of the mitochondria in the skeletal muscles, which increases the capacity for fat oxidation (Holloszy, 1967; Ingjer, 1979b); a proliferation of capillaries within skeletal muscle, which enhances plasma fatty acid delivery to muscle (Ingjer, 1979b; Saltin & Gollnick, 1983); an increase in carnitine transferase, which facilitates fatty acid transport across the mitochondria membrane (Mole et al. 1971); an increase in fatty acid-binding proteins, which regulate myocyte fatty acid transport (Turcotte et al. 1991, 1999); and changes in muscle fibre type that favour fat oxidation (Andersen & Henriksson, 1977; Henriksson, 1992; Mujika & Padilla, 2001).

The training-induced increase in total fat oxidation is not due to an increase in the mobilization and oxidation of adipose tissue TG. Both cross-sectional studies of trained and untrained subjects and longitudinal training studies have found that training either does not change or actually decreases the rate of appearance (Ra) of fatty acids into plasma during exercise performed at the same absolute intensity (Jansson & Kaijser, 1987; Martin et al. 1993; Klein et al. 1994; Horowitz & Klein, 2000a). Plasma catecholamine concentrations, which stimulate lipolysis of adipose tissue TG during exercise, are lower during exercise performed at the same absolute intensity before and after training (Wolfel et al. 1990; Phillips et al. 1996b; Friedlander et al. 1998b) despite a training-induced increase in the capacity to secrete adrenaline (Kjaer & Galbo, 1988). In addition, training does not alter lipolytic sensitivity to adrenaline. Although maximally stimulated lipolytic activity (at adrenaline concentrations between 10^{-6} and 10^{-4} mol/l) is greater in adipocytes obtained from endurance-trained subjects than in those from untrained subjects (Despres et al. 1984; Crampes et al. 1986, 1989; Riviere et al. 1989), lipolytic activity is the same or slightly lower in adipocytes from endurance-trained subjects at physiological adrenaline concentrations (between 10^{-10} and 10^{-8} mol/l; Crampes et al. 1986, 1989). Moreover, whole-body and regional adipose tissue lipolytic sensitivity to adrenaline infusion in vivo is the same before and after training (Stallknecht et al. 1995; Horowitz et al. 1999). During exercise performed at the same relative intensity, whole-body lipolytic rates are greater in endurance-trained than in untrained individuals (Klein et al. 1996), but the mechanism responsible for the increased lipolytic response is not known.

Most available data indicate that the training-induced increase in fat oxidation during exercise is due primarily to an increase in the oxidation of non-plasma fatty acids. The exact source of these non-plasma fatty acids is not entirely clear. It is possible that increased oxidation of VLDL-TG contributes to the training-induced increase in total fat oxidation during exercise. Herd et al. (2001) found greater leg VLDL-TG uptake in response to endurance training, which was probably mediated by exercise-induced activation of muscle lipoprotein lipase. However, other investigators have found plasma triacylglycerols are not an important fuel during exercise even after short-term training (Kiens & Lithell, 1989; Guo et al. 2000). It is probable that fatty acids derived from IMTG are an important contributor to the increase in fat oxidation in trained subjects. Data from studies that measured IMTG content in muscle biopsies, however, yield conflicting results. Some (Hurley et al. 1986; Phillips et al. 1996c) but not all (Kiens et al. 1993; Dyck & Bonen, 1998; Bergman et al. 1999) studies showed a greater depletion of IMTG during exercise performed after, rather than before, training. The technical difficulty in measuring IMTG concentration in muscle biopsies (Wendling et al. 1996; Watt et al. 2002a) may have contributed to the differences between studies. It is also unclear how endurance training might increase IMTG lipolysis during exercise because the catecholamine response during exercise is decreased (Galbo et al. 1977; Winder et al. 1979) and skeletal muscle β-adrenergic receptor density remains the same (Klein et al. 1996). Therefore, if endurance training increases reliance on IMTG it must affect muscle sympathetic nervous system activation or other, as yet unknown, factors that regulate IMTG lipolysis.

**Diet**

Almost a century ago, Krogh & Lindhard (1920) and Christensen & Hansen (1939) observed that fat oxidation during exercise is increased in response to short-term consumption of a high-fat diet. Similar results have been obtained more recently (Phinney et al. 1983; Helge et al. 1996; Schrauwen et al. 2000) in studies that investigated fat oxidation during exercise after long-term (> 7 d) adaptation to a high-fat diet. The mechanisms responsible for the increase in fat oxidation are not entirely clear. High-fat diets increase the rate of plasma NEFA appearance in plasma and fat oxidation at rest (Mittendorfer & Sidossis, 2001), and increase plasma NEFA availability (Phinney et al. 1983), and decrease muscle (Phinney et al. 1983) and
liver (Hultman & Nilsson, 1971) glycogen stores during exercise. Although it has been proposed that increased plasma fatty acid availability is responsible for the increase in fat oxidation in response to high-fat diets, Schrauben et al. (2000) found that the rate of plasma NEFA oxidation was not different after high-carbohydrate or high-fat diets. Therefore, the high-fat diet-induced increase in fat oxidation in response to high-fat diets is probably due to the use of plasma TG or IMTG. This notion is supported by findings from studies that show that longer-term adaptation to high-fat diets increases the capacity of muscle for plasma TG uptake by increasing lipoprotein lipase activity (Kiens et al. 1987; Helge & Kiens, 1997) and IMTG storage (Kiens et al. 1987; Helge et al. 1998) whereas high-carbohydrate diets increase muscle glycogen stores (Helge et al. 2001). Helge et al. (2001) found that increased uptake and oxidation of plasma NEFA and VLDL-TG during exercise accounted for the increase in leg plasma fatty acid oxidation after 7 weeks of training while consuming a high-fat diet. Use of IMTG (determined as the difference between total fat use and the measured oxidation of plasma NEFA and VLDL-TG uptake) also tended to be higher with a high-fat diet but the increase was not statistically significant, possibly because of inadequate statistical power. In summary, these findings demonstrate that diet composition causes changes in substrate use during exercise.

**Body composition**

Obese individuals have increased TG stores in both adipose tissue and skeletal muscle (Phillips et al. 1996a). Therefore, endurance exercise may be particularly beneficial for obese individuals because of mobilization from both adipose tissue and skeletal muscle TG depots. The rate of lipolysis, determined by measuring NEFA Ra, during moderate-intensity endurance exercise is similar in lean and obese subjects (Kanaley et al. 1993; Horowitz & Klein, 2000b). However, the effect of obesity on fat oxidation is less clear because of conflicting results from different studies that found higher rates of fat oxidation in obese women and no difference in fat oxidation between lean and obese women during exercise (Kanaley et al. 1993; Colberg et al. 1995; Ardevol et al. 1998). Differences in aerobic fitness and age could have contributed to the discrepant findings between these studies. By matching lean and obese women on fat-free mass, aerobic capacity, and age, it was recently found that obese women had greater total fat oxidation than lean women but plasma NEFA oxidation was the same in both groups while carbohydrate oxidation rates were lower in obese than lean women (Horowitz & Klein, 2000b). This indicates increased reliance on other fatty acid sources, presumably derived from IMTG and possibly VLDL-TG, and sparing of carbohydrates in obese compared with lean women. Little is known about the effects of obesity on substrate use during exercise in men. Recently, Goodpaster et al. (2002) found a tendency for higher rates of total fat oxidation in obese compared with non-obese men during moderate-intensity exercise; this was due to higher rates of non-plasma, presumably IMTG-derived, fatty acid oxidation. In a study conducted in lean, overweight and obese men, who were matched on age and aerobic fitness, it was found that NEFA Ra and plasma NEFA oxidation during moderate-intensity exercise was highest in the lean and lowest in the obese group, whereas the rate of whole-body fat oxidation was not different between groups (B Mittendorfer and S Klein, unpublished results). This indicates that lean and obese men oxidize the same amount of fat during exercise but obese men rely more on IMTG and less on plasma NEFA than lean men. Endurance exercise training has similar effects in lean and obese subjects, and results in an increase in total fat oxidation during exercise, due to an increase in non-plasma fatty acid oxidation in obese men (van Aggel-Leijssen et al. 2002) and women (van Aggel-Leijssen et al. 2001). In these studies, exercise was performed at the same absolute intensity before and after training, and NEFA Ra was not affected by training. The composite of these data indicates that obesity is associated with increased use of non-plasma fatty acids, presumably derived from IMTG, during exercise in both men and women. In addition, the effect of training on substrate metabolism is similar in lean and obese subjects.

**Sex effects**

Women usually have more body fat (Clarys et al. 1999; Mittendorfer et al. 2003) and are less fit (Davis et al. 2002) than men. These differences complicate the investigation of the effect of gender on substrate use during exercise, because both body composition and fitness, as discussed earlier (p. 101), independently influence the rate of lipolysis and fat oxidation during endurance exercise. Several studies found that the rate of fat oxidation was higher in both untrained and trained women than men (Costill et al. 1979; Powers et al. 1980; Froberg & Pedersen, 1984; Blatchford et al. 1985; Keim et al. 1996; Friedlander et al. 1998b; Burguera et al. 2000; Carter et al. 2001; Roepstorff et al. 2002; Steffensen et al. 2002) whereas others found that women use more fat and less carbohydrate than men (Froberg & Pedersen, 1984; Blatchford et al. 1985; Tarnopolsky et al. 1990; Horton et al. 1998; Carter et al. 2001). Furthermore, menstrual cycle phase can affect substrate kinetics during exercise. Fat oxidation is higher, carbohydrate oxidation is lower, and glucose Ra and rate of disappearance (Rd) are lower during high-intensity exercise performed during the luteal than the follicular phase of the menstrual cycle (Zderic et al. 2001). In contrast, fatty acid kinetics is the same during the follicular and luteal phases of the menstrual cycle at rest (Heiling & Jensen, 1992; Corsmit et al. 1994; Zderic et al. 2001) and during low- and moderate-intensity exercise (Zderic et al. 2001; Horton et al. 2002).

The independent effect of gender on lipid metabolism was recently examined during moderate-intensity endurance exercise, by studying young adult men and women who were matched on adiposity (percentage body fat: 24 (se 2) and 25 (se 1) % in men and women, respectively), aerobic fitness (49 (se 2) and 47 (se 1) ml/kg fat-free mass per min in men and women, respectively) and age (33 (se 3) and 29 (se 4) in men and women, respectively) (Mittendorfer et al. 2002). It was found that NEFA Ra and Rd during exercise were greater in women than in men, but the rate of total fatty acid oxidation was similar in both
groups. Compared with men, women oxidized more plasma NEFA, derived primarily from adipose tissue TG, and less non-plasma NEFA, presumably derived primarily from IMTG and possibly VLDL-TG. The greater reliance on plasma NEFA as a fuel in women than men was probably a result of greater NEFA availability in women than in men. In contrast, studies performed at the August Krogh Institute in Copenhagen (Roepstorff et al. 2002; Steffensen et al. 2002) found that IMTG use during exercise, determined by evaluating fat content in muscle biopsies, was greater in women than men, who were matched on aerobic fitness but not body composition. Limitations in using IMTG content as a measure of IMTG oxidation during exercise and differences in matching men and women on body composition may be responsible for the discrepancy between studies.

The mechanism(s) responsible for the gender differences in lipolysis of adipose tissue TG observed in our subjects during exercise is not known. Most of the increase in lipolytic activity that occurs during exercise is mediated through catecholamine stimulation of adipose tissue β-adrenergic receptors (Arner et al. 1990; Hellstrom et al. 1996; Mora-Rodriguez et al. 2001). However, plasma catecholamine concentrations during exercise were similar in both male and female subjects. Moreover, it is unlikely that these differences in lipolytic sensitivity to β-adrenergic stimulation were responsible for the gender differences in lipid kinetics. Studies performed in vitro in isolated human adipocytes exposed to physiological concentrations of catecholamines (Leibel & Hirsch, 1987; Crampes et al. 1989; Wahrenberg et al. 1991; Mauriege et al. 1999) and in vivo in human subjects during catecholamine infusion (Jensen et al. 1996; Millet et al. 1998) found that adipose tissue lipolytic sensitivity was similar in men and women. However, activation of α-adrenergic receptors, which inhibits lipolysis, may also be involved in determining the net lipolytic response to exercise (Hellstrom et al. 1996; Stich et al. 1999), so gender differences in α-adrenergic receptor activity might have influenced lipolytic rates during exercise. Using the microdialysis technique, it has been shown that local adipose tissue α-adrenergic receptor blockade during endurance exercise increased regional glycerol release from abdominal subcutaneous adipose tissue in men (Hellstrom et al. 1996) but not women (Hellstrom et al. 1996). These results suggest that α-adrenergic receptor activity inhibits lipolysis during exercise in men but is not involved in the regulation of lipolysis during exercise in women.

Several studies have evaluated the metabolic response to endurance exercise training in men and women (for example, Martin et al. 1993; Phillips et al. 1996b; Friedlander et al. 1998b; Horowitz et al. 2000). In one study, exercise training caused a greater increase in fat oxidation during exercise in women than in men (Friedlander et al. 1998a,b). However, these findings are confounded by the fact that the increase in aerobic fitness was also greater in women (25%) than in men (9%). It has been found that 12 weeks of endurance training in women increased total fat oxidation by about 25% during exercise performed at the same absolute intensity (Horowitz et al. 2000). This response is the same as that reported in men after a similar training-induced increase in fitness (Martin et al. 1993; Phillips et al. 1996b). The difference between plasma NEFA oxidation, assessed by isotope tracer methods, and whole-body fat oxidation, assessed by indirect calorimetry, suggests that the training-induced increase in fat oxidation was due primarily to an increase in the oxidation of non-plasma fatty acids, presumably IMTG. In contrast, Steffensen et al. (2002) found no effect of training status on IMTG use, assessed by measuring IMTG content in muscle biopsies, during moderate-intensity exercise in men or women, when exercise was performed at the same relative intensity in a cross-sectional study of untrained, moderately and highly trained subjects. However, total fat oxidation was higher in highly trained than sedentary and moderately trained subjects. The reason for the discrepancy in the source of oxidized TG between studies is not clear but may be related to differences in study design (longitudinal training v. cross-sectional analysis) and the methods used to assess IMTG use (muscle biopsy v. isotope tracer and indirect calorimetry techniques).

**Aging**

Most studies investigating the effect of ageing on substrate metabolism during exercise have focused on measuring the RER to determine whole-body fat and carbohydrate oxidation rates. During brief (3–8 min) stages of incremental exercise, RER at any given absolute exercise intensity is greater in old compared with young subjects (Robinson, 1938; Durmin & Mikulcic, 1956; Julius et al. 1967; Montoye, 1982). This suggests that old subjects rely more on carbohydrate than fat as a fuel during exercise performed at the same absolute intensity, which was supported by higher blood lactate levels during submaximal exercise in older subjects (Robinson, 1938; Astrand, 1958; Straddell, 1964; Silverman & Mazzeo, 1996). However, given the lower aerobic capacity and fitness (V̇O₂max) in old than young individuals (for example, Hagberg et al. 1988; Kohrt et al. 1991; Schwartz et al. 1991; Coggan et al. 1993; Rooyackers et al. 1996; Sial et al. 1996), a greater dependence on carbohydrate for energy in old individuals would be expected during exercise performed at the same absolute, but higher relative, intensity (Jones et al. 1980; Coggan, 1991; Romijn et al. 1993; van Loon et al. 2001). Studies that evaluated RER during exercise performed at the same relative intensity have reported conflicting results, and RER has been reported to be lower (Hagberg et al. 1988), the same (Tankersley et al. 1991), and higher (Silverman & Mazzeo, 1996) in elderly compared with young subjects. However, these studies did not carefully control for dietary intake or training status (Hagberg et al. 1988; Tankersley et al. 1991), which can affect RER. In a study conducted in sedentary, lean young (26 (SE 2) years) and old (73 (SE 2) years) subjects, matched on gender, BMI, and fat-free mass, it was found that RER was higher in old than in young subjects during 60 min of cycle ergometer exercise performed at either the same absolute or relative intensity (Sial et al. 1996). Therefore, most of the available data indicate that ageing is associated with significant alterations in substrate oxidation during endurance exercise, manifested by a shift from using fat to carbohydrate as a fuel.
Age-related changes in skeletal muscle itself favour the oxidation of carbohydrate over fat and may be responsible for the shift in substrate metabolism observed in elderly subjects. Muscle mitochondrial oxidative enzymes, which are needed for fat oxidation, decrease with increasing age (Meredith et al. 1989). Mitochondrial citrate synthase, succinate dehydrogenase, and β-hydroxy-CoA-dehydrogenase activities are lower in old (age 57–74 years) than in young (age 20–38 years) individuals (Coggan et al. 1992b, 1993; McCully et al. 1993) because of both decreased mitochondrial density (Conley et al. 2000) and enzyme activity relative to muscle mitochondrial volume (Trounce et al. 1989; Rooyackers et al. 1996). In addition, compared with young adults, elderly subjects have a lower rate of phosphocreatine (PCr) resynthesis after exercise, suggesting a decreased rate of O₂ consumption during exercise (McCully et al. 1993; Conley et al. 2000). Furthermore, the inorganic phosphate : PCr value at any given power output, which reflects the balance between ATP hydrolysis (energy use) and ATP resynthesis (energy production), is greater in old than in young men (Coggan et al. 1993). These observations indicate that muscle oxidative capacity is diminished in old individuals, and that glucose may be preferred over fatty acids as a fuel during exercise in old individuals.

Aging is associated with decreased lipolytic sensitivity to β-adrenergic stimulation (Lomqvist et al. 1990; Ford et al. 1995) and lower sympathoadrenal response to exercise (Kohrt et al. 1993), suggesting that diminished lipolysis of adipose tissue TG might contribute to the observed decrease in fat oxidation. However, it has been found that fatty acid Ra in plasma during exercise performed at the same absolute intensity (about 800 ml O₂ consumed/min) was about 35 % higher in old (73 (SE 2) years) than young (26 (SE 2) years) subjects (Sial et al. 1996). Despite greater NEFA release into plasma and plasma NEFA uptake during exercise performed at the same absolute intensity, whole-body fat oxidation was lower in old than in young subjects. Therefore, it is probable that an age-related decrease in the respiratory capacity of skeletal muscle itself was responsible for the lower rate of fat oxidation during exercise in older than younger subjects, because adipose tissue lipolysis and plasma NEFA availability were not rate-limiting.

The decrease in fat oxidation is associated with an increase in carbohydrate oxidation during exercise in elderly subjects. Increased plasma glucose availability is not responsible for the increase in glucose oxidation. It has been found that the glucose Ra into plasma and the Rd from plasma was not different in young and old subjects when exercise was performed at the same absolute intensity. These data suggest that muscle glycogen use was higher in older than younger subjects (Sial et al. 1996).

Endurance training in elderly individuals increases skeletal muscle mitochondrial enzymes and respiratory capacity (Suominen et al. 1977). In addition, the inorganic phosphate : PCr value in muscle at any given power output in trained older men was lower compared with older sedentary men but similar to the value observed in sedentary young men (Coggan et al. 1993). It was found that 16 weeks of supervised endurance training did not affect whole-body lipolytic rate (NEFA Ra or glycerol Ra), but decreased glucose Ra and caused an increase in fat oxidation and a decrease in carbohydrate oxidation during exercise to values observed in untrained young adults. (Sial et al. 1998). Therefore, the adaptation to training in old subjects is similar to that seen in young adult subjects. The shift in substrate oxidation was probably due to changes within skeletal muscle itself, possibly an increase in the fractional oxidation of plasma fatty acids taken up by muscle and/or an increase in the use of intramuscular triacylglycerols.

Summary and perspectives
Substrate use during exercise is influenced by factors that are directly related to the exercise bout itself, such as exercise intensity and duration, and a variety of other factors, that are independent of exercise, such as an individual’s sex, body composition, age and diet. All of these variables have to be considered as possible confounding factors between groups when evaluating substrate metabolism during exercise. Future investigations should concentrate on defining the contribution of IMTG and plasma TG to fuel use during exercise; this will probably involve use and development of novel techniques. A better understanding of the regulation of the use of these lipid sources is particularly important because it is yet unknown how variations in the availability of these lipid sources affect their use as fuel during exercise. Currently available isotope tracer techniques do not allow the distinction of these TG sources to total energy consumption during exercise, and measurement of IMTG use by muscle biopsy techniques is particularly difficult in subjects with increased muscle fat stores. Therefore, the contribution of IMTG and plasma TG to total energy consumption in obese individuals and individuals with insulin resistance, who have increased IMTG stores and plasma TG concentrations, remains largely unknown.

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