Effect of Training Status on Regional Disposal of Circulating Free Fatty Acids in the Liver and Skeletal Muscle During Physiological Hyperinsulinemia

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OBJECTIVE — Fat metabolism is increasingly implicated in the pathogenesis of type 2 diabetes. Endurance training has been shown to prevent hepatic steatosis and to alter skeletal muscle fat metabolism, and regional free fatty acid (FFA) uptake adaptations were suggested as a mechanism. Thus, we tested whether endurance training modifies the uptake of plasma FFAs occurring in the liver and in skeletal muscle during anabolic, i.e., hyperinsulinemic, conditions.

RESEARCH DESIGN AND METHODS — Trained and untrained healthy male subjects underwent positron emission tomography scanning of the liver and thigh regions, with the FFA analog 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid, during euglycemic hyperinsulinemia. Tracer influx rate constants in skeletal muscle (MK_i) and liver (LK_i) were multiplied by plasma FFA levels to obtain FFA uptake for skeletal muscle (MFU) and liver (LFU), respectively.

RESULTS — Athletes showed increased Vo_{2max} ($P < 0.0001$), insulin-mediated glucose disposal (*M* value, 61 ± 4 vs. 46 ± 3 μmol · min^{-1} · kg^{-1}, $P = 0.01$), and plasma lactate levels during the clamp and lower percentage of body fat mass ($P = 0.002$). MK_i was 25% higher in athletes than in sedentary men ($P = 0.03$). In all subjects, MK_i and MFU were positively correlated with the *M* value ($r = 0.56$, $P = 0.02$, and $r = 0.51$, $P = 0.03$, respectively) and with plasma lactate levels ($r = 0.63$, $P = 0.006$, and $r = 0.63$, $P = 0.005$, respectively). LK_i was significantly reduced by 20% in the athletes ($P = 0.04$). By multiple regression, LFU was inversely correlated with the two fitness categories ($P = 0.008$), and it was lower in athletes. Linear fitting of liver data showed time consistency, indicating no release of FFAs as a mechanism for the reduced liver retention in athletes.

CONCLUSIONS — We conclude that endurance training promotes insulin-mediated glucose and FFA disposal in skeletal muscle, while lowering hepatic FFA uptake. Such changes may result in a divergent pattern of fat accumulation in the two organs.

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Impaired tissue uptake of plasma free fatty acids (FFAs) is an increasingly recognized finding in patients with reduced glucose tolerance (1,2). Changes in lipid metabolism in these subjects also include raised circulating FFA concentrations (3,4) and fat accumulation in nonadipose tissues, such as liver (5) and skeletal muscle (6), both of which have been strongly implicated in the genesis and/or progression of insulin resistance (3–5).

Tissue FFA uptake is driven by substrate delivery and, independently, is stimulated by insulin in skeletal muscle (1,7,8). The intracellular fate of circulating FFAs entering hepatocytes or myocytes is dependent on nutritional status. In general, lipid stores are consumed during fasting and repleted in the postprandial state. Insulin regulates fat metabolism by suppressing lipid oxidation and stimulating FFA incorporation into triglycerides (9). As a consequence, during insulin stimulation FFA release from skeletal muscle becomes negligible (9) and lipid accumulation becomes tightly dependent on FFA uptake. Recent findings (1) confirm the role of insulin in promoting muscle FFA uptake and show that this response is abolished in muscle cells of patients with type 2 diabetes and restored by exposing these cells to thiazolidinediones.

Endurance training is generally considered to be protective against the development of metabolic disorders. Current knowledge places the enhancement of insulin sensitivity among the main prevention targets of nonalcoholic fatty liver disease (10), and aerobic training has been shown to prevent hepatic steatosis in rats fed a high-fat diet (11). Different from the liver, the apparent paradox of an increased triglyceride and fatty acid binding protein content was described in skeletal muscle of physically trained humans (6,12). Enhanced muscle FFA uptake was suggested as a likely mediator of the accelerated lipid deposition in these subjects. To test the hypothesis that differential uptake of FFA in liver and skeletal muscle (i.e., reduced in liver and enhanced in muscle) might underlie the divergent pattern of fat accumulation observed in athletes, we measured the uptake of serum FFA occurring in these organs under hyperinsulinemic conditions in trained and untrained healthy lean subjects using positron emission to-

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Abbreviations: FFA, free fatty acid; LFU, liver FFA uptake; MFU, skeletal muscle FFA uptake; PET, positron emission tomography; ROI, region of interest.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

mography (PET) and the FFA analog, 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA).

RESEARCH DESIGN AND METHODS

— Nine male endurance athletes (e.g., triathletes, runners) and nine healthy sedentary men volunteered for the study. Endurance athletes competed at the national or international level and had engaged in regular endurance exercise for >10 years. Subjects included in the sedentary group did not exercise regularly. All study subjects were healthy, as judged by history, physical examination, and routine laboratory tests. None of the subjects were taking any medication, and all of them were instructed to suspend any physical activity and to consume a low-fat isocaloric diet, including at least 200 g of carbohydrate for 2 days before the study. Written, informed consent was obtained from all subjects after the study protocol had been accepted by the Joint Commission of Ethics of Turku University and Turku University Central Hospital.

PET scanning

[^{18}F]FTHA was synthesized by previously described methods (8,13), leading to a radiochemical purity of >98%. Subjects were studied after a 10- to 12-h overnight fast. They were positioned in a 15-slice ECAT 931/08–12 tomograph (Siemens/CTI, Knoxville, TN) with a measured in-plane resolution of 6.7 mm and an axial resolution of 6.5 mm. Transmission scans were obtained, to correct subsequent emission scans for the attenuation of γ-photons. Two catheters were inserted, one into a left antecubital vein for the injection of [^{18}F]FTHA and for the infusion of insulin and glucose, the other in a heated (70°C) contralateral radial vein for collection of arterialized venous blood. A primed-continuous infusion of insulin (48 mU · m^{-2} · min^{-1}) was started, and euglycemia was maintained using a 20% glucose infusion, adjusted according to frequent plasma glucose measurements (14). After 115 min had elapsed from the beginning of insulin infusion, [^{18}F]FTHA (178 ± 7 MBq) was injected, and a dynamic 32-min scan of the lower thoracic and liver regions (12 × 15, 4 × 30, 2 × 120, 1 × 180, and 4 × 300 s) was followed by a 15-min scan of the femoral region (6 × 150 s). Serum insulin, FFA, and plasma lactate concentrations were determined every 30 min, as detailed elsewhere (8,13). Blood samples for the measurement of arterialized plasma radioactivity and labeled metabolites were taken throughout the PET study.

Data analysis

All data were corrected for deadtime, decay, and photon attenuation and reconstructed in a 128 × 128 matrix. Final in-plane resolution in reconstructed and Hann-filtered (0.3 cycles/pixel) images was 9.5 mm FWHM (full width at half maximum). Serum metabolite analysis was performed as previously described (8).

Regions of interest (ROIs) were placed on two to four different planes within the right lobe of the liver. One to two small ROIs were placed in the left cardiac chamber to obtain blood time-activity curves, and care was taken to avoid spillover from the myocardial wall. For the femoral regions, ROIs were placed on the anterior, medial, and posterior muscle compartments over four adjacent planes. The nonmetabolized fraction of [^{18}F]FTHA was used to correct the input function (8). Blood and muscle or liver time-activity curves were analyzed graphically (15), i.e., they were fitted to a linear equation in which the slope of the fitted line equals the influx rate constant of [^{18}F]FTHA in the liver (LK_i) and in skeletal muscle (MK_i). FFA uptake indexes in the liver (LFU) and in skeletal muscle (MFU) were estimated by multiplying respective K_i values by mean serum FFA concentrations during PET scanning. For the liver, this represents a minimal estimate, given that the FFA concentration in the portal vein might be slightly higher than that in peripheral blood. To evaluate whether group differences in hepatic tracer retention might depend on tracer outflow from tissue, LK_i was fitted over subsequent time intervals, and LFU was calculated accordingly. In this analysis, a progressive decrease in the estimated parameter would indicate tracer loss (16). For skeletal muscle, previous data rule out this mechanism during insulin infusion in healthy subjects (17).

Maximal aerobic power and body fat content

Maximal aerobic power ($V_{O_{2max}}$) was determined by direct analysis of respiratory gases during a continuous incremental maximal cycle ergometer test (Ergoline, Mijnhardt, the Netherlands). Body fat content was estimated from four skinfolds.

Statistical analysis

One-way ANOVA was used for group comparisons. Student's paired *t* test was used for intraindividual comparisons, i.e., fast versus clamp metabolic data. Regression analyses were carried out according to standard techniques. Multivariate analysis was performed where appropriate to examine the impact of simultaneous changes in experimental parameters on regional fatty acid uptake across the study groups. All results are expressed as means ± SE. Level of significance was set at $P \leq 0.05$.

RESULTS

— Athletes had a lower percentage of fat mass (10 ± 1 vs. 17 ± 1%, $P = 0.001$) and higher $V_{O_{2max}}$ (67 ± 2 vs. 48 ± 3 ml · min^{-1} · kg^{-1}, $P < 0.0001$) than untrained subjects; the two groups were otherwise well matched for age (28 ± 1 vs. 27 ± 1 years, NS), BMI (21.6 ± 0.5 vs. 22.7 ± 0.6 kg/m^2, NS), and circulating substrate-hormone levels (Table 1). Insulin-mediated, whole-body glucose disposal rate was ~25% higher in the athletes (Table 1).

MFU

The influx rate constant of FFA in skeletal muscle, MK_i, was significantly higher (by ~25%) in athletes than in sedentary men (Fig. 1); the correspondent ~25% increase in MFU (0.128 ± 0.014 vs. 0.168 ± 0.024) fell just short of statistical significance ($P = 0.18$), due to slightly higher serum FFA levels in sedentary individuals (Fig. 1). Normalizing for this factor [(MFU/individual FFA) × (group average FFA)] led to a significant difference ($P = 0.03$). In the whole study population, MK_i and MFU were positively correlated with plasma lactate levels and whole-body insulin-mediated glucose disposal ($r = 0.63$, $P < 0.01$); the latter relationship was mostly accounted for by the trained group ($r = 0.65$, $P = 0.03$), and MK_i tended to be positively correlated with $V_{O_{2max}}$ ($r = 0.45$, $P = 0.07$).

LFU

Rapid tracer accumulation was observed in the liver, with a progressive increase of the tissue-to-blood radioactivity ratio. Linear fitting was good, with mean *r* values of 1.0 ± 0.0 in both groups (NS). Progressive fitting of liver data demon-

Table 1 —*Metabolic data during fasting and hyperinsulinemia (clamp)*

	Trained	Untrained	*P* (between groups)
Fasting			
Glucose (mmol/l)	5.44 ± 0.14	5.26 ± 0.18	NS
Insulin (pmol/l)	31 ± 3	37 ± 4	NS
FFA (mmol/l)	0.336 ± 0.048	0.406 ± 0.055	NS
Lactate (mmol/l)	1.29 ± 0.13	1.06 ± 0.08	NS
Clamp			
Glucose (mmol/l)	5.35 ± 0.19	5.29 ± 0.11	NS
Insulin (pmol/l)	331 ± 23*	365 ± 17*	NS
FFA (mmol/l)	0.064 ± 0.004*	0.073 ± 0.004*	NS
FFA suppression (%)	78 ± 2	77 ± 4	NS
Lactate (mmol/l)	1.65 ± 0.10*	1.34 ± 0.07*	0.014
M (μmol · min^{-1} · kg^{-1})	61 ± 4	42 ± 4	0.0024

Data are means ± SE. *$P < 0.05$ or less versus fasting (Student's paired t test).

strated time consistency in both groups, thus excluding the occurrence of tracer release from tissue throughout the scanning period (Fig. 2).

LK_i was decreased by ~20% in athletes compared with sedentary men (Fig. 1). LFU was also decreased by 20%, but, similar to MFU, the differences achieved significance after being adjusted for circulating FFA levels ($P = 0.04$).

Multiple regression models were fitted to isolate the independent effect of endurance training on LFU. In these models, LFU was introduced as the dependent variable, and its relationships with the two categories of fitness (the independent variable) were examined while accounting for the influence of biochemical and anthropometric characteristics. An independent inverse association was found between the two training levels and LFU ($P = 0.008$) when adjusting for concurrent plasma lactate and insulin levels.

Collectively, the regional differences observed between the two study groups led to a 40% increase in the MFU-to-LFU ratio (per unit of tissue) in trained compared with untrained subjects (Fig. 1).

CONCLUSIONS — The present study verified the hypothesis that endurance training is independently associated with an increased uptake of FFAs by skeletal muscle and lower uptake by the liver during hyperinsulinemia. As a result, the MFU-to-LFU ratio was significantly shifted in favor of skeletal muscle in our athlete group. FFA uptake was quantified by PET and [^{18}F]FTHA in the present study. Notably, circulating FFAs comprise a spectrum of different molecules; FTHA is a 17-carbon FFA analog, and the available comparison with 11C-palmitate supports its use as representative of long-chain fatty acids (18). [^{18}F]FTHA enters mitochondria in proportion to the oxidative rate of FFA (13), but after formation of two acetyl-CoA moieties it cannot be oxidized, due to its sulfur atom, and remains trapped. The amount of tracer entering triglycerides is also mostly retained, considering the short experimental time versus the delay required for the release of newly formed triglycerides. Thus, the FFA uptake observed reflects the sum of both processes because the current technique does not allow their dissection. As compared with the leg balance technique, PET allows one to noninvasively and specifically target regional metabolism, and when limited to the present case, the lack of quantification of each intracellular metabolic pathway and rapid tracer breakdown are relative disadvantages. In the whole organism, with a total muscle mass of ~40% of body weight (29 ± 1 kg in our subjects) and an average liver volume of 1.5 l, total MFU and LFU could be estimated from our results to ~30% (muscle) and 25% (liver)

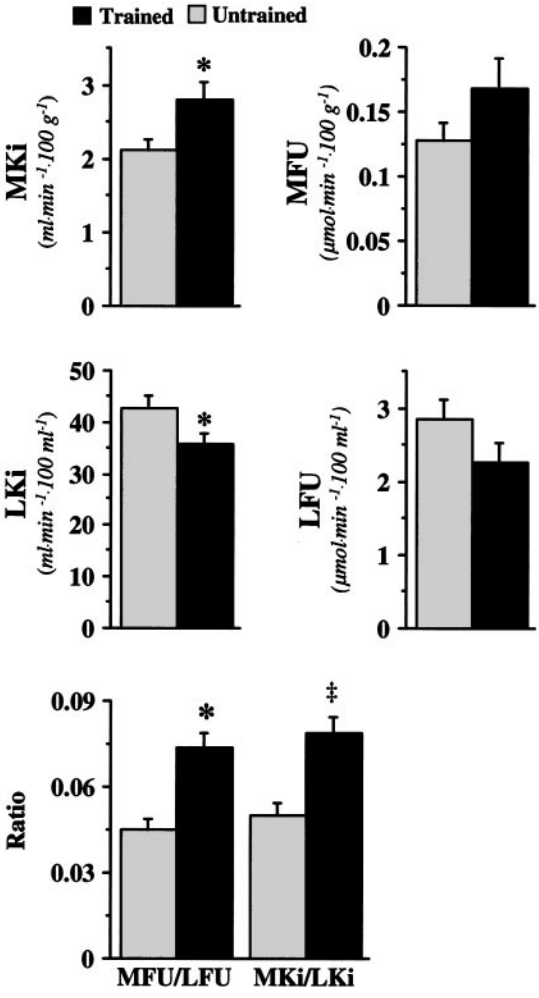

**Figure 1—*Skeletal muscle* (top) *and liver* (middle) *FFA influx rate constants and uptake and their ratios* (bottom) *(per unit tissue) in untrained and trained subjects.* *P ≤ *0.04 and* ‡P = *0.001 versus untrained subjects.*

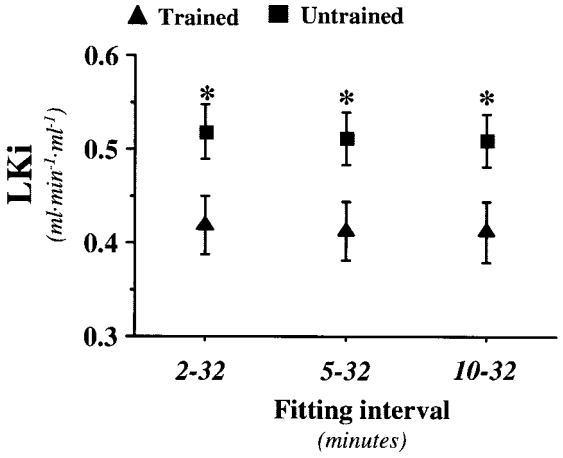

Figure 2—*Model fitting during progressive scanning time frames. A time-dependent decline in* LK_i *would be expected in case of tracer release. Instead, the data showed time consistency of liver FFA influx rate constant* (LK_i) *in untrained (squares) and trained (triangles) subjects.* *$P < 0.05$.

of total serum FFA turnover, as previously measured in healthy individuals during insulin infusion studies (19). Notably, similar amounts of serum FFA were handled in the two organs despite a 15-fold difference in mass. Previous studies showing that postabsorptive lipid oxidation and skeletal muscle oxidative capacity are higher in trained compared with untrained subjects (6) rule against defective FFA breakdown as a mechanism for skeletal muscle triglyceride accumulation in athletes, and the evidence that muscle triglyceride content was unaffected during exercise led to the hypothesis that concurrent synthesis overrides hydrolysis in male subjects (20). The present study was conducted in the insulinized state, in which FFA oxidation is almost fully suppressed and triglyceride synthesis is enhanced and tightly dependent on FFA uptake. In this situation, we found a 25% increase in MFU and a 20% decrease in LFU in trained versus sedentary individuals. Such changes had been anticipated by previous evidence (12,21); to the best of our knowledge, the present study demonstrates them for the first time in humans. It should be noted that uptake values and group differences shown in the present work would most likely be amplified in a physiological postprandial situation, in which suppression of lipolysis is balanced by the introduction of lipids with the meal, and circulating FFA levels are higher than those during an insulin clamp. In this situation, an enhanced MK_i, as observed in our athletes, would support the notion of a causal link between higher MFU and increased FFA transporter/triglyceride content in endurance-trained humans (6,12,21). Further along this line of reasoning, the correlation observed between MFU or MK_i and known indicators of fitness, namely insulin-mediated glucose disposal and $V_{O_{2max}}$, may explain the observed link between muscle FFA transporters/triglyceride content and the degree of endurance training (6,21).

The current finding of a downregulation of LFU in athletes is compatible with the concept that higher insulin sensitivity and/or fitness may prevent excessive hepatic fat accumulation (10,11), particularly since the present experiments were conducted in a metabolic state in which hepatic triglyceride synthesis is stimulated and FFA catabolism is inhibited. Though the influence of visceral FFA release on the calculation of LFU could not be assessed, due to the lack of portal measurements, a higher suppression of visceral lipolysis in trained subjects, due to increased insulin sensitivity and decreased abdominal fat mass (22), would amplify the group differences reported here.

Different from findings in type 2 diabetic patients with defective insulin-regulated lipolysis and from (intralipid) studies revealing substrate competition at high serum FFA concentrations (4), our data showed no evidence of reciprocal inhibition between glucose and FFA uptake in skeletal muscle under normally suppressed serum FFA levels. On the contrary, skeletal muscle FFA and whole-body glucose uptake (mainly reflecting muscle uptake) were proportionally increased in all subjects, and they were augmented to the same extent, i.e., by ~25%, in the athletes compared with the sedentary individuals. The current data underscore the composite nature of the insulin-sensitivity concept, of which the sole assessment of insulin-mediated glucose uptake represents one part only. Different substrates need to be available to support muscle performance at different intensity levels, as exercise capacity is optimized by predominant use of FFAs at low-moderate intensities and of glucose (from muscle glycogen and from blood) during high-intensity work (23). In keeping with this notion, the simultaneous increase in the uptake of both substrates, FFAs and glucose, in trained subjects might be not surprising when considering that hyperinsulinemia normally signals a situation in which provision of fuel is not rate limiting. In the whole body, glucose, but not lipid oxidation, was found to be enhanced in trained compared with sedentary subjects during euglycemic hyperinsulinemia (24). In the liver, glucose storage was shown to be increased, and net FFA uptake decreased, by prior exercise during hyperglycemia and mild hyperinsulinemia in dogs (25), which is in accord with the subsequent need for a prompt enhancement in fasting hepatic glucose production in athletes (26). In skeletal muscle, training was associated with increased protein content of GLUT4, insulin receptor, glycogen synthase, and glycogen synthase activity, enhancing glucose uptake during exercise and during insulin stimulation (27,28). Higher triglyceride stores were observed in skeletal muscle of trained men, together with elevated oxidative metabolism at rest (6) and triglyceride consumption during a bout of exercise (24); both glycogen and triglyceride repletion occurred, though with different time courses, in the postexercise period (29). Partially at variance with these findings, no influence of exercise or endurance training on muscle triglyceride stores could be found in men after a strict and prolonged dietary regimen (20). In our study, patients were instructed to consume a low-fat, high-carbohydrate isocaloric diet, reflecting the condition of the former rather the latter studies, and we cannot exclude that closer monitoring would have resulted in different findings. The cross-sectional nature of the present study does not allow us to dissect the outcome of endurance training from the effect of genetic or non–exercise-related environmental influences. Supporting the interaction between FFA and glucose metabolism, longitudinal studies (30) showed that enhanced fasting rates of fat oxidation were

the strongest predictor of exercise-related improvement in insulin sensitivity. Interestingly, in animal studies (25), prior exercise augmented the capability of the liver to extract glucose under fasting and insulin-stimulated conditions.

Our findings are relevant to the therapeutic or preventive potential of exercise in patients with, or in those at risk of, type 2 diabetes, in whom numerous metabolic defects involve both glucose and lipid metabolism. In fact, drugs acting to reduce lipid content in the liver of type 2 diabetic subjects improved their glycemic control (5); those drugs were also shown to stimulate MFU in cell cultures (1), though the benefits of such an effect on whole-body metabolic homeostasis could not be explored due to the in vitro study design. In our data, the enhanced removal of circulating FFAs by skeletal muscle in the athletes may be one mechanism by which FFA uptake by other organs, e.g., the liver, was reduced, thus preventing excessive hepatic fat accumulation. The signal(s) for such organ cross-talk remain, however, putative.

We conclude that endurance training concertedly promoted insulin-mediated glucose and FFA disposal in skeletal muscle, while lowering hepatic FFA uptake. Consequently, serum FFAs were diverted from the liver to skeletal muscle. Such changes may result into a divergent pattern of fat accumulation in the two organs.

References

1. Wilmsen HM, Ciaraldi TP, Carter L, Reehman N, Mudaliar SR, Henry RR: Thiazolidinediones upregulate impaired fatty acid uptake in skeletal muscle of type 2 diabetic subjects. *Am J Physiol Endocrinol Metab* 285:E354–E362, 2003
2. Mensink M, Blaak EE, van Baak MA, Wagenmakers AJ, Saris WH: Plasma free fatty acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. *Diabetes* 50:2548–2554, 2001
3. Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard BV: Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status: relation to plasma triglyceride and apolipoprotein B concentrations: Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Arterioscler Thromb Vasc Biol* 17:64–71, 1997
4. Randle PJ: Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 14:263–283, 1998
5. Bajaj M, Suraamornkul S, Pratipanawatr T, Hardies LJ, Pratipanawatr W, Glass L, Cersosimo E, Miyazaki Y, DeFronzo RA: Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. *Diabetes* 52:1364–1370, 2003
6. Goodpaster BH, He J, Watkins S, Kelley DE: Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86:5755–5761, 2001
7. Kelley DE, Mokan M, Simoneau JA, Mandarino LJ: Interaction between glucose and free fatty acid metabolism in human skeletal muscle. *J Clin Invest* 92:91–98, 1993
8. Maki MT, Haaparanta M, Nuutila P, Oikonen V, Luotolahti M, Eskola O, Knuuti JM: Free fatty acid uptake in the myocardium and skeletal muscle using fluorine-18-fluoro-6-thia-heptadecanoic acid. *J Nucl Med* 39:1320–1327, 1998
9. Kelley DE, Goodpaster B, Wing RR, Simoneau JA: Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol* 277:E1130–E1141, 1999
10. Haque M, Sanyal AJ: The metabolic abnormalities associated with non-alcoholic fatty liver disease. *Best Pract Res Clin Gastroenterol* 16:709–731, 2002
11. Gorski J, Oscai LB, Palmer WK: Hepatic lipid metabolism in exercise and training. *Med Sci Sports Exerc* 22:213–221, 1990
12. Kiens B, Kristiansen S, Jensen P, Richter EA, Turcotte LP: Membrane associated fatty acid binding protein (FABPpm) in human skeletal muscle is increased by endurance training. *Biochem Biophys Res Commun* 231:463–465, 1997
13. Takala TO, Nuutila P, Pulkki K, Oikonen V, Gronroos T, Savunen T, Vahasilta T, Luotolahti M, Kallajoki M, Bergman J, Forsback S, Knuuti J: 14(R,S)-[18F] fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in myocardium and skeletal muscle. *Eur J Nucl Med Mol Imaging* 29:1617–1622, 2002
14. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
15. Patlak CS, Blasberg RG: Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data: generalizations. *J Cereb Blood Flow Metab* 5:584–590, 1985
16. Lammertsma AA, Brooks DJ, Frackowiak RS, Beaney RP, Herold S, Heather JD, Palmer AJ, Jones T: Measurement of glucose utilisation with [18F]2-fluoro-2-deoxy-D-glucose: a comparison of different analytical methods. *J Cereb Blood Flow Metab* 7:161–172, 1987
17. Turpeinen AK, Takala TO, Nuutila P, Axelin T, Luotolahti M, Haaparanta M, Bergman J, Hamalainen H, Iida H, Maki M, Uusitupa MI, Knuuti J: Impaired free fatty acid uptake in skeletal muscle but not in myocardium in patients with impaired glucose tolerance: studies with PET and 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid. *Diabetes* 48:1245–1250, 1999
18. Knuuti J, Takala TO, Nagren K, Sipila H, Turpeinen AK, Uusitupa MI, Nuutila P: Myocardial fatty acid oxidation in patients with impaired glucose tolerance. *Diabetologia* 44:184–187, 2001
19. Bonadonna RC, Groop LC, Zych K, Shank M, DeFronzo RA: Dose-dependent effect of insulin on plasma free fatty acid turnover and oxidation in humans. *Am J Physiol Endocrinol Metab* 259:E736–E750, 1990
20. Steffensen CH, Roepstorff C, Madsen M, Kiens B: Myocellular triacylglycerol breakdown in females but not in males during exercise. *Am J Physiol Endocrinol Metab* 282:E634–E642, 2002
21. Turcotte LP, Swenberger JR, Tucker MZ, Yee AJ: Training-induced elevation in FABP(PM) is associated with increased palmitate use in contracting muscle. *J Appl Physiol* 87:285–293, 1999
22. Janssen I, Katzmarzyk PT, Ross R, Leon AS, Skinner JS, Rao DC, Wilmore JH, Rankinen T, Bouchard C: Fitness alters the associations of BMI and waist circumference with total and abdominal fat. *Obes Res* 12:525–537, 2004
23. Gollnick PD: Metabolism of substrates: energy substrate metabolism during exercise and as modified by training. *Fed Proc* 44:353–357, 1985
24. Matzinger O, Schneiter P, Tappy L: Effects of fatty acids on exercise plus insulin-induced glucose utilization in trained and sedentary subjects. *Am J Physiol Endocrinol Metab* 282:E125–E131, 2002
25. Pencek RR, James F, Lacy DB, Jabbour K, Williams PE, Fueger PT, Wasserman DH: Interaction of insulin and prior exercise in control of hepatic metabolism of a glucose load. *Diabetes* 52:1897–1903, 2003
26. van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA, Saris WH: Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *J Physiol* 553:611–625, 2003
27. Kristiansen S, Gade J, Wojtaszewski JF, Kiens B, Richter EA: Glucose uptake is increased in trained vs. untrained muscle during heavy exercise. *J Appl Physiol* 89:1151–1158, 2000
28. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JF, Dela F: Strength train-ing increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes* 53:294–305, 2004

29. Kiens B, Richter EA: Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am J Physiol* 275: E332–E337, 1998
30. Goodpaster BH, Katsiaras A, Kelley DE: Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 52:2191–2197, 2003