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Citation for published version (APA):

van Loon, L. J., Thomason-Hughes, M., Constantin-Teodosiu, D., Koopman, R., Greenhaff, P. L., Hardie, D. G., Keizer, H. A., Saris, W. H., & Wagenmakers, A. J. M. (2005). Inhibition of adipose tissue lipolysis increases intramuscular lipid and glycogen use in vivo in humans. *American Journal of Physiology : Endocrinology and Metabolism*, 289(3), E482-493. <https://doi.org/10.1152/ajpendo.00092.2005>

Document status and date:

Published: 01/01/2005

DOI:

[10.1152/ajpendo.00092.2005](https://doi.org/10.1152/ajpendo.00092.2005)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

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Inhibition of adipose tissue lipolysis increases intramuscular lipid and glycogen use in vivo in humans

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Departments of ¹Movement Sciences and ²Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands; ³School of Life Sciences, University of Dundee, Dundee, Scotland; ⁴School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham; and ⁵School of Sport and Exercise Sciences, University of Birmingham, Birmingham, United Kingdom

Submitted 2 March 2005; accepted in final form 5 May 2005

Van Loon, Luc J. C., Michaela Thomason-Hughes, Dumitru Constantin-Teodosiu, René Koopman, Paul L. Greenhaff, D. Grahame Hardie, Hans A. Keizer, Wim H. M. Saris, and Anton J. M. Wagenmakers. Inhibition of adipose tissue lipolysis increases intramuscular lipid and glycogen use in vivo in humans. *Am J Physiol Endocrinol Metab* 289: E482–E493, 2005. First published May 10, 2005; doi:10.1152/ajpendo.00092.2005.—This study investigates the consequences of inhibition of adipose tissue lipolysis on skeletal muscle substrate use. Ten subjects were studied at rest and during exercise and subsequent recovery under normal, fasting conditions (control trial, CON) and following administration of a nicotinic acid analog (low plasma free fatty acid trial, LFA). Continuous [U-¹³C]palmitate and [6,6-²H₂]glucose infusions were applied to quantify plasma free fatty acid (FFA) and glucose oxidation rates and to estimate intramuscular triacylglycerol (IMTG) and glycogen use. Muscle biopsies were collected to measure 1) fiber type-specific IMTG content; 2) allosteric regulators of hormone-sensitive lipase (HSL), glycogen phosphorylase, and pyruvate dehydrogenase; and 3) the phosphorylation status of HSL at Ser⁵⁶³ and Ser⁵⁶⁵. Administration of a nicotinic acid analog (acipimox) substantially reduced plasma FFA rate of appearance and subsequent plasma FFA concentrations ($P < 0.0001$). At rest, this substantially reduced plasma FFA oxidation rates, which was compensated by an increase in the estimated IMTG use ($P < 0.05$). During exercise, the progressive increase in FFA rate of appearance, uptake, and oxidation was prevented in the LFA trial and matched by greater IMTG and glycogen use. Differential phosphorylation of HSL or relief of its allosteric inhibition by long-chain fatty acyl-CoA could not explain the increase in muscle TG use, but there was evidence to support the contention that regulation may reside at the level of the glucose-fatty acid cycle. This study confirms the hypothesis that plasma FFA availability regulates both intramuscular lipid and glycogen use in vivo in humans.

muscle metabolism; intramyocellular triacylglycerol; fat oxidation; insulin resistance

ELEVATED PLASMA FREE FATTY ACID (FFA) concentrations are associated with obesity and the development of insulin resistance (38, 44). Combined with the fact that, in obese and/or type 2 diabetes patients, skeletal muscle shows a reduced capacity to oxidize FFA (20), this likely explains the increased intramyocellular triacylglycerol (IMTG) storage in these subjects (15, 27, 53). In agreement, various studies have reported a strong relationship between elevated plasma FFA levels, IMTG accretion, and insulin resistance (22, 32, 34). Insights

from various lipid infusion studies suggest that elevated plasma FFA delivery and/or impaired fatty acid (FA) oxidation, result in intramyocellular accumulation of triacylglycerol (TG) and FA metabolites (such as fatty acyl-CoA, diacylglycerol, and ceramides), which are likely to induce defects in the insulin-signaling cascade, causing skeletal muscle insulin resistance (1, 3, 16, 42, 65).

Insulin resistance can subsequently lead to the development of the hyperglycemic and hyperinsulinemic state that is associated with type 2 diabetes and accompanied by major disturbances in skeletal muscle substrate metabolism (17, 37). These disturbances produce a state of metabolic inflexibility that stimulates IMTG storage at the expense of its oxidation (20). Recent data suggest that the structural imbalance between FFA uptake and IMTG storage and its oxidation, and not the actual size or distribution of the IMTG pool, is responsible for the development of skeletal muscle insulin resistance (10, 48, 53). It could be speculated that greater metabolic flexibility combined with an elevated IMTG turnover rate would prevent excessive accumulation of intramyocellular FA metabolites as well as reduce the degree of lipid peroxidation (42) due to a decreased resident time of the intramyocellular lipids. Therefore, efforts should be made to develop interventions that stimulate IMTG oxidation, which could be of great importance in the prevention and/or treatment of skeletal muscle insulin resistance (52). However, the latter is complicated, as information on the regulation of IMTG metabolism is lacking (17).

Physical exercise stimulates IMTG use (23, 52, 54) and, as such, represents an effective means to prevent and/or reduce excess IMTG accretion. Recently, we (54) observed a progressive decline in IMTG oxidation rate during prolonged moderate-intensity exercise in healthy trained men. The latter was inversely correlated with a concomitant increase in plasma FFA concentration (54), supporting the contention that elevated plasma FFA levels suppress IMTG oxidation during exercise (40, 41, 54, 56). Consequently, we hypothesized that plasma FFA concentrations regulate the balance between IMTG storage and oxidation. With hormone-sensitive lipase (HSL) (24) as the site at which IMTG mobilization and/or oxidation is likely regulated, we speculated that HSL activity is not only regulated by muscle contraction (25) and adrenalin

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(21, 26, 59), but may also be modified by changes in plasma FFA availability. Indirect evidence for this is provided by the in vitro observation that HSL is inhibited both by oleic acid and oleoyl-CoA in adipose tissue (18). The presence of such a feedback inhibition mechanism in muscle would serve to protect against excessive intracellular accumulation of FA (metabolites) during increased plasma FFA provision. If this hypothesis is correct, reducing plasma FFA provision might reduce intramyocellular FA (metabolite) concentrations, disinhibit HSL, and increase IMTG mobilization and oxidation at rest and/or during prolonged exercise.

In the present study, contemporary stable isotope methodology and fluorescence microscopy were applied to quantify substrate source utilization at rest and during prolonged moderate-intensity exercise under normal fasting conditions (control trial, CON) and following specific inhibition of adipose tissue lipolysis, thereby reducing plasma FFA availability (low FFA trial, LFA). In addition, we aimed to elucidate the mechanisms responsible for the anticipated shift in skeletal muscle substrate use. We speculated that an acute reduction in plasma FFA availability modulates skeletal muscle substrate use by way of the Randle cycle, by activating AMP-activated protein kinase (AMPK), by increasing acetyl-CoA carboxylase (ACC) phosphorylation, and/or by stimulating muscle HSL by site-specific phosphorylation (at Ser⁵⁶³ or Ser⁵⁶⁵). This study provides novel insight into the role of plasma FFA availability as a main factor in modulating IMTG utilization and introduces adipose tissue lipolytic inhibition as a practical intervention to increase both intramuscular lipid and glycogen use at rest and during exercise.

METHODS

Subjects. Ten fit, active male subjects [age 23 ± 1 yr, height 1.82 ± 0.03 m, body wt 74 ± 3 kg, fat-free mass 64 ± 3 kg, maximal power output (W_{\max}) 388 ± 14 W, and maximal oxygen uptake capacity

($\dot{V}O_{2\max}$) 62 ± 3 ml·kg body wt⁻¹·min⁻¹] were selected to participate in this study. Subjects were informed about the nature and risks of the experimental procedures before their written informed consent was obtained. This study was approved by the local Medical Ethics Committee.

Pretesting. W_{\max} and $\dot{V}O_{2\max}$ were measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) during an incremental exhaustive exercise test. Body composition was assessed using the hydrostatic weighing method in the morning after an overnight fast. Body fat percentage was calculated using Siri's equation (46).

Diet and activity before testing. All subjects maintained normal dietary and physical activity patterns throughout the experimental period. In addition, they filled out a food intake diary for 2 days before the first exercise trial to keep their dietary intake as identical as possible before the other trials. The evening before each trial, subjects received the same standardized meal (41.2 kJ/kg body wt, containing 72, 11, and 17% energy carbohydrate, fat, and protein, respectively).

Experimental trials. Each subject performed three trials separated by at least a 1-wk interval. Each trial consisted of 90 min of resting measurements, followed by 120 min of cycling exercise (50% W_{\max}) and 120 min of recovery. In the two main trials, a [U-¹³C]palmitate and a [6,6-²H₂]glucose tracer were infused continuously at rest and during exercise with breath, blood, and muscle samples collected at regular intervals. In the LFA trial, plasma FFA availability was reduced by oral administration of a peripheral lipolytic inhibitor (2× 250 mg acipimox). In the CON trial, a placebo was provided. A third trial was performed to determine the acetate recovery factor to correct [U-¹³C]palmitate oxidation rates accurately for carbon label retention in the bicarbonate pool(s) and by way of isotopic exchange reactions in the TCA cycle (45).

Protocol. A schematic outline of the study protocol is provided in Fig. 1. After an overnight fast, subjects arrived at the laboratory at 8:00 AM by car or public transportation. After 30 min of supine rest, a percutaneous muscle biopsy was taken from the vastus lateralis muscle. A Teflon catheter (Baxter, Utrecht, The Netherlands) was inserted into an antecubital vein of one arm for blood sampling, and another catheter was inserted in the contralateral arm for isotope

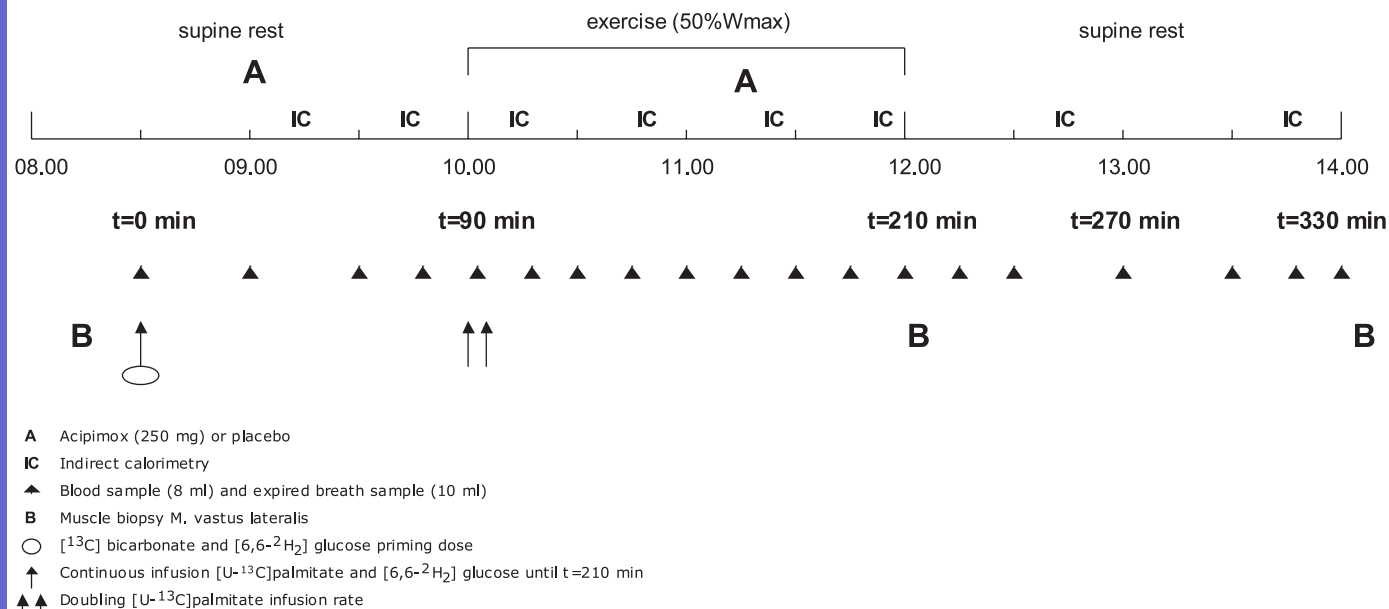


Fig. 1. Schematic outline of the study protocol. After initial blood, breath, and muscle biopsy sample, a continuous infusion with [U-¹³C]palmitate and [6,6-²H₂]glucose was started for 210 min. At rest and during exercise and postexercise recovery, whole body substrate use was studied under normal fasting conditions (control trial, CON) and under reduced plasma free fatty acid (FFA) availability following administration of the nicotinic acid analog acipimox (low-FFA trial, LFA).

infusion. Thereafter, a resting blood sample was taken, and expired breath samples were collected into Vacutainer tubes. Subsequently, subjects were administered a single intravenous dose of $\text{NaH}^{13}\text{CO}_3$ (0.06375 mg/kg) to prime the bicarbonate pool(s), followed by a [6,6- $^2\text{H}_2$]glucose prime (13.5 $\mu\text{mol/kg}$). Thereafter, a continuous infusion of [6,6- $^2\text{H}_2$]glucose (0.3 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and [U- ^{13}C] palmitate (0.01 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) (or [1,2- ^{13}C]acetate in the acetate recovery trial) was started ($t = 0$ min) via a calibrated IVAC pump (IVAC 560, San Diego, CA) and continued for 210 min. At $t = 0$ min and $t = 165$ min, a capsule containing 250 mg of acipimox (Nedios, Byk, Zwabenburg, The Netherlands) or a placebo was orally administered. At $t = 90$ min, subjects started to exercise on a cycle ergometer at a workload of 50% W_{max} for a 120-min period. While subjects were at rest, $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were measured continuously (Oxycon- β , Mijnhart); during exercise $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were measured for 5 min every 15 min before sampling of blood and expired breath. Immediately after cessation of exercise, a second muscle biopsy was taken, after which the subjects rested supine for 120 min during which $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were again measured continuously. After 120 min of postexercise recovery, a third muscle biopsy was taken. Breath and blood samples were collected at $t = 0, 30, 60, 75$, and 90 min (at rest) and at $t = 105, 120, 135, 150, 165, 180, 195$, and 210 min (during exercise). In addition, blood samples were collected at $t = 225, 240, 270, 300, 315$, and 330 min (during postexercise recovery).

Tracer infusion. Infusion rates of [U- ^{13}C]palmitate and [6,6- $^2\text{H}_2$]glucose averaged 9.3 ± 0.2 and 276.1 ± 4.3 $\text{nmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectively. At the onset of exercise [U- ^{13}C]palmitate infusion rates were doubled (18.6 ± 0.4 $\text{nmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). In the acetate recovery trial, a corresponding amount of ^{13}C was infused, resulting in an average [1,2- ^{13}C]acetate infusion rate of 71.2 ± 1.6 and 142.4 ± 3.1 $\text{nmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ at rest and during exercise, respectively. Palmitate, glucose, and acetate tracer concentrations in the infusates averaged 1.06 ± 0.02 , 21.3 ± 0.6 , and 4.64 ± 0.10 mmol/l, respectively.

Blood and breath sample analysis. Blood samples (7 ml) were collected in EDTA-containing tubes and centrifuged at 1,000 g for 10 min at 4°C . Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -80°C . Plasma glucose (Uni Kit III; Roche, Basel, Switzerland), lactate (11), FFA (NEFA-C; Wako Chemicals, Neuss, Germany), free glycerol (148270; Roche Diagnostics, Indianapolis, IN), and triglyceride (GPO-Trinder 337B; Sigma Diagnostics, St Louis, MO) concentrations were analyzed with a COBAS semiautomatic analyzer (Roche).

Expired-breath samples were analyzed for $^{13}\text{C}/^{12}\text{C}$ ratio by gas chromatography-continuous flow isotope ratio mass spectrometry (GC-IRMS; Finnigan MAT 252, Bremen, Germany). For determination of plasma palmitate and FFA kinetics, FFA were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Palmitate concentration was determined on an analytic gas chromatograph with flame ionization detection using heptadecanoic acid as an internal standard and on average comprised $22.9 \pm 1.0\%$ of total FFA. Isotope tracer-to-tracee ratio (TTR) of [U- ^{13}C]palmitate was determined using GC-combustion-IRMS (Finnigan MAT 252). After derivatization, plasma [6,6- $^2\text{H}_2$]glucose enrichment was determined by electron ionization GC-MS (Finnigan INCOS-XL). Glucose (Uni Kit III, Roche) and acetate (Kit 148261, Boehringer) concentrations in the infusates were determined with the COBAS FARA.

Calculations. From respiratory measurements, total fat and carbohydrate oxidation rates were calculated using the nonprotein respiratory quotient (33).

$$\text{fat oxidation rate} = 1.695\dot{V}\text{O}_2 - 1.701\dot{V}\text{CO}_2 \quad (1)$$

$$\text{carbohydrate oxidation rate} = 4.585\dot{V}\text{CO}_2 - 3.226\dot{V}\text{O}_2 \quad (2)$$

with $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ in liters per minute and oxidation rates in grams per minute. Breath and plasma enrichments are expressed as TTR:

$$\text{TTR} = (^{13}\text{C}/^{12}\text{C})_{\text{sa}} - (^{13}\text{C}/^{12}\text{C})_{\text{bk}} \quad (3)$$

in which sa indicates sample and bk indicates background value. Rates of palmitate and glucose appearance (R_a) and disappearance (R_d) were calculated using the single-pool non-steady-state Steele equations adapted for stable isotope methodology, as described elsewhere (63). As such, plasma palmitate and glucose R_d were calculated by correcting the R_a for the time-dependent changes in plasma metabolite concentration.

$$R_a = \frac{F - V[(C_2 + C_1)/2] [(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2} \quad (4)$$

$$R_d = R_a - V \cdot \left(\frac{C_2 - C_1}{t_2 - t_1} \right) \quad (5)$$

where F is the infusion rate ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$); V = distribution volume for palmitate or glucose (40 and 160 ml/kg, respectively); C_1 and C_2 are the palmitate or glucose concentrations (mmol/l) at times 1 (t_1) and 2 (t_2), respectively, and E_2 and E_1 are the plasma palmitate or glucose enrichments (TTR) at t_1 and t_2 , respectively. $^{13}\text{CO}_2$ production ($\text{Pr}^{13}\text{CO}_2$, mol/min) from the infused palmitate tracer was calculated as

$$\text{Pr}^{13}\text{CO}_2 = (\text{TTR}_{\text{CO}_2} \cdot \dot{V}\text{CO}_2)/(k \cdot \text{Ar}) \quad (6)$$

where TTR_{CO_2} is the breath $^{13}\text{C}/^{12}\text{C}$ ratio at a given time point, $\dot{V}\text{CO}_2$ is carbon dioxide production (l/min), k is the volume of 1 mol of CO_2 (22.4 l/min), and Ar is the fractional ^{13}C label recovery in breath CO_2 , observed after the infusion of labeled acetate (45) and calculated as

$$\text{Ar} = ((\text{TTR}_{\text{CO}_2} \cdot \dot{V}\text{CO}_2)/(k \cdot 2F)) \quad (7)$$

where F is infusion rate of [1,2- ^{13}C]acetate (mol/min). Plasma palmitate oxidation (R_{ox} , mol/min) can subsequently be calculated as

$$R_{\text{ox}} \text{ palmitate} = R_d \text{ palmitate} \cdot (\text{Pr}^{13}\text{CO}_2/F \cdot 16) \quad (8)$$

where R_d palmitate is the rate of palmitate disappearance (mol/min); F is the palmitate infusion rate (mol/min), and 16 is the number of carbon atoms in palmitate. Total plasma FFA oxidation was calculated by dividing palmitate oxidation rates by the fractional contribution of plasma palmitate to total plasma FFA concentration. Muscle-derived TG use was estimated by subtracting plasma FFA oxidation from total fat oxidation. However, it should be noted that the indirect stable isotope methodology does not differentiate between muscle- or lipoprotein-derived TG use. However, the contribution of lipoprotein-derived TG oxidation to total energy expenditure is assumed to be of relatively minor quantitative importance, especially in an overnight fasted state (52).

In a previous study, where we applied both [U- ^{13}C]- and [6,6- $^2\text{H}_2$]glucose tracers (19) during moderate intensity exercise, it was shown that the percentage of plasma glucose R_d that was oxidized varied between 96 and 100%. Therefore, plasma glucose oxidation rate during exercise was calculated as

$$R_{\text{ox}} \text{ plasma glucose} = R_d \text{ plasma glucose} \quad (9)$$

Muscle glycogen oxidation was calculated by subtracting plasma glucose oxidation from total carbohydrate oxidation.

Muscle sample analyses. Muscle samples were freed from any visible nonmuscle material and rapidly frozen in liquid nitrogen. About 15 mg of each muscle sample were frozen in liquid nitrogen-cooled isopentane and embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands). Multiple serial sections (5 μm) from biopsy samples were thaw mounted together on uncoated, precleaned glass slides for each subject and stained with Oil Red O together with immunolabeled cellular constituents by use of the protocol described before (53–55). The applied epifluorescence technique represents a semiquantitative method that can be used to compare fiber type-

specific intramyocellular lipid content between muscle cross sections (53–55).

About 15–20 mg of each muscle biopsy sample were freeze-dried, dissected free from visible blood and connective tissue, and powdered. About 10 mg of muscle powder were then extracted with 0.5 M perchloric acid containing 1 mM EDTA. After centrifugation, the supernatant was neutralized with 2.2 M KHCO_3 and used for determination of muscle phosphocreatine, free creatine (13), citrate (2), lactate (13), glycogen, glucose 6-phosphate, free carnitine, acetylcar-nitine, long-chain fatty acylcarnitine, long-chain fatty acyl-CoA, CoASH, and acetyl-CoA (5) concentrations. Except for muscle lac-tate, glycogen, and citrate, all these concentrations were corrected for muscle total creatine content within each subject.

Another piece of muscle sample (20 mg) was ground to a fine powder under liquid nitrogen and homogenized in 200 μl of ice-cold buffer, as described previously (8). For measurement of AMP-acti-vated protein kinase (AMPK) activity, muscle lysates containing 100 μg of protein were immunoprecipitated using 10 μg of sheep anti- α 1-plus 10 μg of anti- α 2-AMPK coupled to protein G-Sepharose before a standard AMPK assay was performed using AMARA-peptide (AMARAASAAA-LARRR) as a substrate (12). HSL Ser⁵⁶³ and Ser⁵⁶⁵ phosphorylation, acetyl-CoA carboxylase (ACC) Ser²²¹ phos-phorylation, and total ACC expression were measured in muscle lysates containing 20 μg of protein by SDS-PAGE followed by Western blotting onto nitrocellulose (64). Membranes were probed with affinity-purified antibodies as follows: anti-HSL phospho-Ser⁵⁶³ (0.2 $\mu\text{g}/\text{ml}$), anti-HSL phospho-Ser⁵⁶⁵ (0.06 $\mu\text{g}/\text{ml}$), anti-ACC phos-pho-Ser²²¹ (1 $\mu\text{g}/\text{ml}$). Antibody binding was detected using the LiCor Odyssey IR imaging system, which uses near-infrared (IR) fluorescent dyes for detection of proteins. Briefly, membranes were incubated with donkey anti-sheep IgG labeled with IRDye 680 (Molecular Probes) for 1 h before being imaged. ACC expression was determined using streptavidin labeled with IRDye 800 (Rockland Immuno-chemicals). The sheep anti- α 1, anti- α 2, and phosphospecific ACC antibodies have been described previously (14, 64). Sheep phos-phospecific HSL antibodies against Ser⁵⁶⁵ and Ser⁵⁶³ were raised against synthetic peptides CTESMRRSVSpEAALAQP and CT-ESMRRSpVSEAAALQP, respectively (residues 556–571 of rat skel-et al muscle HSL with cysteine at the NH_2 terminus for coupling), using methods previously described (47).

Statistics. All data are expressed as means \pm SE. To compare tracer kinetics, substrate utilization rates, IMTG contents, and/or plasma metabolite concentrations over time between trials, a two-way re-peated-measures analysis of variance (ANOVA) was applied. Scheffé's post hoc test was applied in case of a significant *F*-ratio to locate specific differences. For non-time-dependent variables, a Student's *t*-test for paired observations was used. Significance was set at the 0.05 level of confidence.

RESULTS

Tracer kinetics. Plasma FFA and glucose concentrations at rest, exercise, and postexercise recovery under normal fasting conditions (CON) and reduced plasma FFA availability (LFA) are shown in Fig. 2. Because plasma concentrations varied over time in the LFA and/or CON trial, non-steady-state Steele equations were applied to calculate tracer kinetics. Plasma glucose R_a and R_d were significantly higher during exercise compared with resting values in both the CON and LFA trials (Table 1), with R_a and R_d being increased over time during exercise (Fig. 3A; $P < 0.001$). No differences in plasma glucose kinetics were observed between trials. In the CON trial, plasma palmitate R_a , R_d , and R_{ox} were significantly higher during exercise compared with resting values (Table 1), with R_a , R_d , and R_{ox} being increased over time (Fig. 3B; $P < 0.001$). In the LFA trial, plasma palmitate R_a and R_d were

similar at rest and during exercise, with only R_{ox} being signif-icantly greater during exercise compared with resting values ($P < 0.001$; Table 1). No changes in plasma palmitate R_a , R_d , and R_{ox} were observed over time (Fig. 3B). Both at rest and during exercise, plasma palmitate R_a , R_d , and R_{ox} were sub-stantially lower in the LFA trial than in the CON trial ($P < 0.005$; Table 1 and Fig. 3).

Plasma metabolite concentrations. Plasma FFA, glycerol, TG, glucose, and lactate concentrations are shown in Fig. 2. Plasma FFA concentrations in the CON trial increased throughout the exercise period, reaching peak levels at 15 min after cessation of exercise. Thereafter, plasma FFA concentra-tions declined but remained well above preexercise resting levels ($P < 0.0001$). In contrast, plasma FFA concentrations in the LFA trial decreased during exercise but increased gradually toward preexercise levels during recovery ($P < 0.0001$). At all time points, plasma FFA concentrations were significantly lower in the LFA than in the CON trial ($P < 0.001$). Plasma glycerol concentrations increased significantly during exercise and decreased during postexercise recovery in both trials ($P < 0.001$). At all time points, free glycerol concentrations were significantly lower in the LFA than in the CON trial ($P < 0.0001$). Plasma TG decreased gradually during exercise in both trials ($P < 0.0001$), with a tendency to increase during recovery in the CON trial. Nevertheless, plasma TG levels were not significantly different between trials. Plasma glucose concentrations significantly declined during exercise in both trials ($P < 0.0001$), with no further changes during recovery. Plasma lactate concentrations had increased well above preex-ercise resting levels within the first 15 min of exercise, after which plasma lactate concentrations declined throughout the remaining experimental time in both trials ($P < 0.0001$). However, plasma lactate concentrations during recovery were significantly greater in the LFA than in the CON trial ($P < 0.05$).

Substrate utilization. Energy expenditure and substrate source utilization rates at rest are shown in Fig. 4A. Energy expenditure was similar between trials and averaged 5.54 ± 0.23 and 5.26 ± 0.24 kJ/min, respectively. However, substrate source utilization significantly differed between trials ($P < 0.05$). Total fat oxidation rate averaged 0.09 ± 0.01 and 0.07 ± 0.01 g/min, contributing 64 ± 4 and $53 \pm 4\%$ to energy expenditure, in the CON and LFA trials, respectively ($P < 0.01$). The apparent difference in total fat oxidation was en-tirely accounted for by a $38 \pm 7\%$ reduction in plasma FFA oxidation rate in the LFA compared with the CON trial (0.04 ± 0.004 and 0.07 ± 0.006 g/min, respectively; $P < 0.01$). In contrast, estimated IMTG oxidation rates were substantially augmented in the LFA compared with the CON trial (0.029 ± 0.005 vs. 0.017 ± 0.006 g/min, respectively; $P < 0.05$). Carbohydrate oxidation rates were significantly higher in the LFA compared with the CON trial, and averaged 0.15 ± 0.01 and 0.12 ± 0.02 g/min, respectively.

In the subsequent exercise trial, the applied 50% W_{max} workload averaged 194 ± 7 W, corresponding to $59 \pm 1\%$ of $\dot{V}O_{2max}$. Energy expenditure and endogenous substrate source utilization during exercise are illustrated in Figs. 4B and 5. In the CON trial, total fat oxidation rate increased significantly during exercise and was matched by a decrease in total carbo-hydrate oxidation ($P < 0.001$). The increase in total fat oxidation rate during exercise in the CON trial, which occurred

despite the progressive decline in IMTG- plus lipoprotein-derived TG oxidation, was entirely accounted for by an increase in plasma FFA oxidation rate ($P < 0.001$; Fig. 5, A and B). The decrease in total carbohydrate oxidation rate during

exercise in the CON trial was fully accounted for by the gradual decrease in muscle glycogen use, whereas plasma glucose oxidation rates significantly increased over time ($P < 0.0001$; Fig. 5, C and D). In contrast to the CON trial, total fat

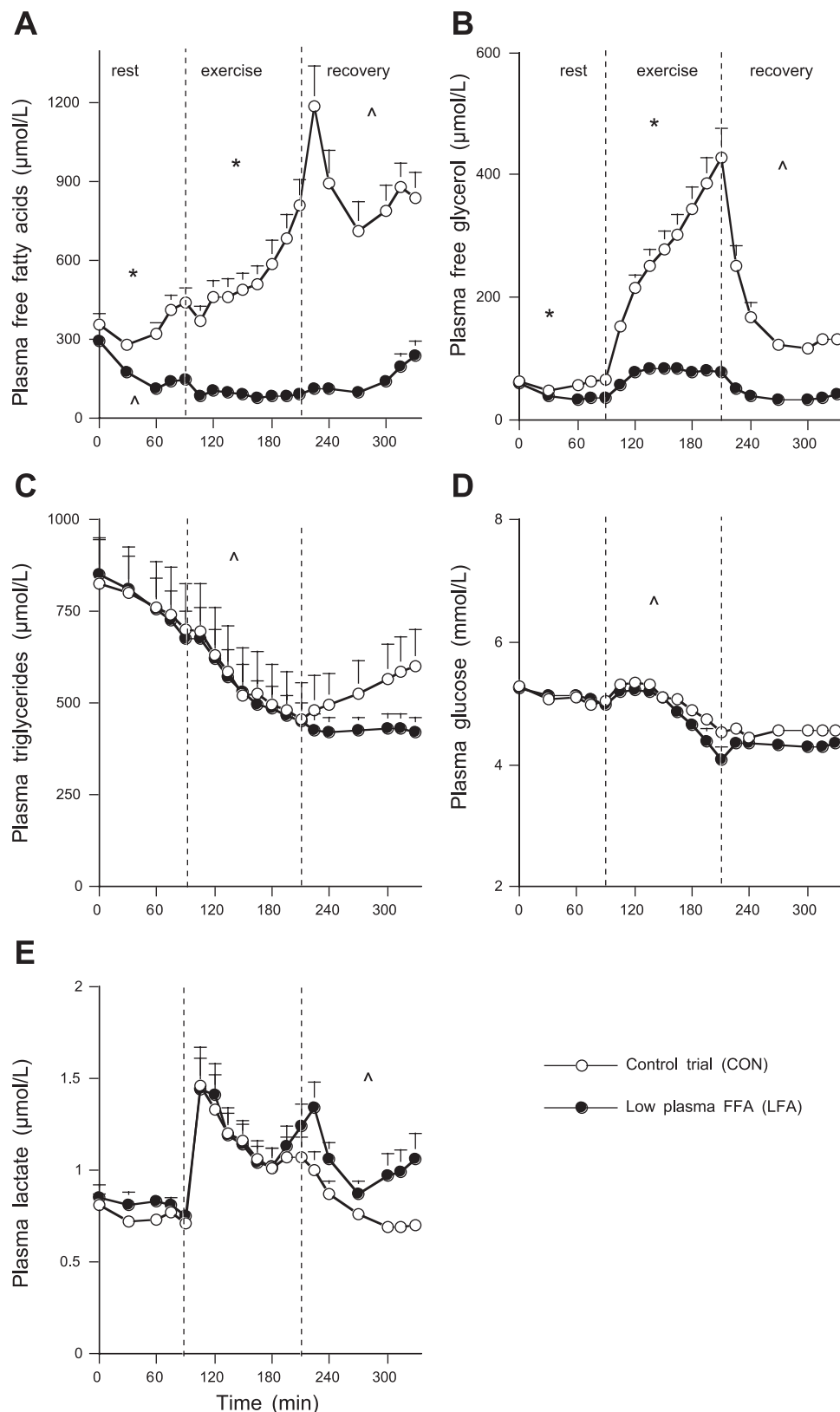


Fig. 2. Plasma metabolite concentrations at rest, during prolonged submaximal exercise, and during 2 h of postexercise recovery in CON and LFA trials. Data provided are means \pm SE. *Significant increase over time within resting, exercise, and/or postexercise recovery period; ^significant decrease over time within resting, exercise, and/or postexercise recovery period ($P < 0.05$).

Table 1. Tracer kinetics at rest and during exercise

	Control Trial (CON)	Low FFA Availability (LFA)
Rest		
Palmitate		
R_a	2.34 ± 0.22	1.41 ± 0.15†
R_d	2.30 ± 0.22	1.40 ± 0.14†
R_{ox}	0.81 ± 0.09	0.45 ± 0.06†
% $R_{a\ ox}$	34.3 ± 1.78	31.3 ± 1.81
Glucose		
R_a	15.8 ± 1.0	15.2 ± 0.7
R_d	16.0 ± 1.0	15.8 ± 0.7
Exercise		
Palmitate		
R_a	5.28 ± 0.57*	1.52 ± 0.09†
R_d	5.23 ± 0.56*	1.51 ± 0.09†
R_{ox}	4.80 ± 0.48*	1.20 ± 0.08*†
% $R_{a\ ox}$	92.0 ± 3.04*	79.7 ± 2.90*†
Glucose		
R_a	31.8 ± 1.8*	32.4 ± 3.3*
R_d	33.0 ± 1.9*	33.9 ± 3.4*

Values are expressed as means ± SE; $n = 8$ subjects. Tracer kinetics as calculated at rest and averaged during 2 h of exercise at 50% W_{max} . R_a , rate of appearance; R_d , rate of disappearance; R_{ox} , rate of oxidation ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$); % $R_{a\ ox}$, % R_a palmitate oxidized. *Significantly different from resting values ($P < 0.001$); †significantly different between trials ($P < 0.01$).

and carbohydrate oxidation rates remained stable during exercise in the LFA trial. Total fat oxidation rates were significantly lower in the LFA trial compared with the CON trial ($P < 0.0001$). This was entirely due to lower plasma FFA oxidation rates in the LFA trial ($P < 0.0001$; Fig. 5A). Estimated IMTG oxidation rates remained stable throughout exercise in the LFA trial and did not decline as shown in the CON trial ($P < 0.0001$). The latter resulted in significantly greater muscle-derived TG use in the LFA compared with the CON trial (Fig. 5B). Carbohydrate oxidation rates were substantially higher in the LFA compared with the CON trials ($P < 0.001$). Plasma glucose oxidation rates increased continuously during exercise ($P < 0.0001$) and were similar to those observed in the CON trial (Fig. 5C). Therefore, the greater

carbohydrate oxidation rate in the LFA trial was entirely attributed to the greater muscle glycogen use in the LFA trial (Fig. 5D), which gradually declined during exercise similarly to the observations in the CON trial ($P < 0.0001$).

Average substrate source utilization, calculated over the second hour of exercise, is illustrated in Fig. 4B. Total fat oxidation averaged 0.61 ± 0.04 and 0.39 ± 0.03 g/min, representing 43 ± 4 and $28 \pm 2\%$ of total energy expenditure in the CON and LFA trials, respectively ($P < 0.001$). In the CON trial, plasma FFA oxidation provided $30 \pm 3\%$ of total energy expenditure, with muscle and plasma lipoprotein-derived TG use contributing $13 \pm 1\%$. With low plasma FFA availability in the LFA trial, plasma FFA oxidation was substantially impaired, providing only $9 \pm 1\%$ of energy expenditure. In contrast, IMTG oxidation was significantly augmented in the LFA trial, providing $19 \pm 2\%$ of total energy expenditure. Total carbohydrate oxidation rates averaged 1.98 ± 0.13 and 2.54 ± 0.10 g/min in the CON and LFA trials, respectively ($P < 0.01$). The greater carbohydrate oxidation rate in the LFA trial was fully attributed to an increase in muscle glycogen use ($P < 0.01$), whereas plasma glucose oxidation rates did not differ between trials.

Resting energy expenditure was significantly increased in both trials during postexercise recovery compared with preexercise values (6.1 ± 0.3 and 5.7 ± 0.2 kJ/min in the CON and LFA trials, respectively). In the CON trial, this was entirely attributed to a greater total fat oxidation rate compared with preexercise resting values, with a concomitant decrease in carbohydrate use ($P < 0.001$). In the LFA trial, the higher energy expenditure during recovery was fully accounted for by a greater total carbohydrate oxidation rate. In line with preexercise measurements, absolute as well as relative fat oxidation rates were significantly higher in the CON trial compared with the LFA trial during recovery ($P < 0.001$).

Muscle tissue analysis. Muscle tissue analysis for IMTG content using semiquantitative fluorescence microscopy on Oil Red O-stained muscle cross sections showed a significant decline in type I muscle fiber lipid content in both trials ($P < 0.01$; Fig. 6). The net decline in type I fiber lipid content was

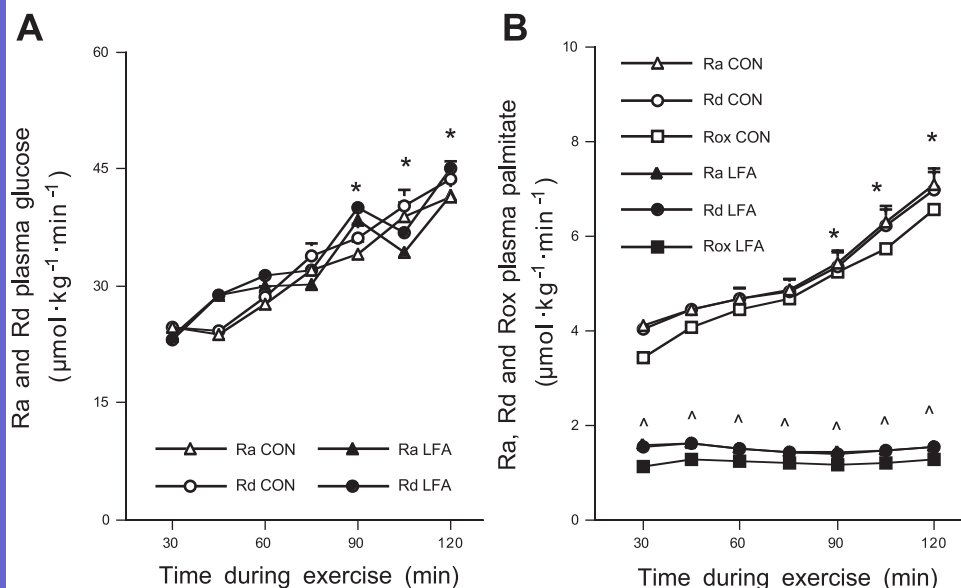


Fig. 3. Plasma glucose rate of appearance (R_a) and disappearance (R_d) (A) and plasma palmitate R_a , R_d , and rate of oxidation (R_{ox}) (B) during prolonged submaximal endurance exercise under normal fasting conditions (CON) and following administration of a peripheral lipolytic inhibitor (LFA) (in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Plasma glucose R_a and R_d substantially increased during exercise in both trials ($P < 0.0001$). Plasma palmitate R_a , R_d , and R_{ox} significantly increased during exercise in the CON trial ($P < 0.0001$), whereas in the LFA trial R_a , R_d , and R_{ox} remained stable over time at a substantially lower value ($P < 0.0001$). Data are means ± SE. *Significantly higher compared with values at $t = 30$ min within each trial ($P < 0.05$); ^significant difference in R_a , R_d , and R_{ox} between trials ($P < 0.0001$).

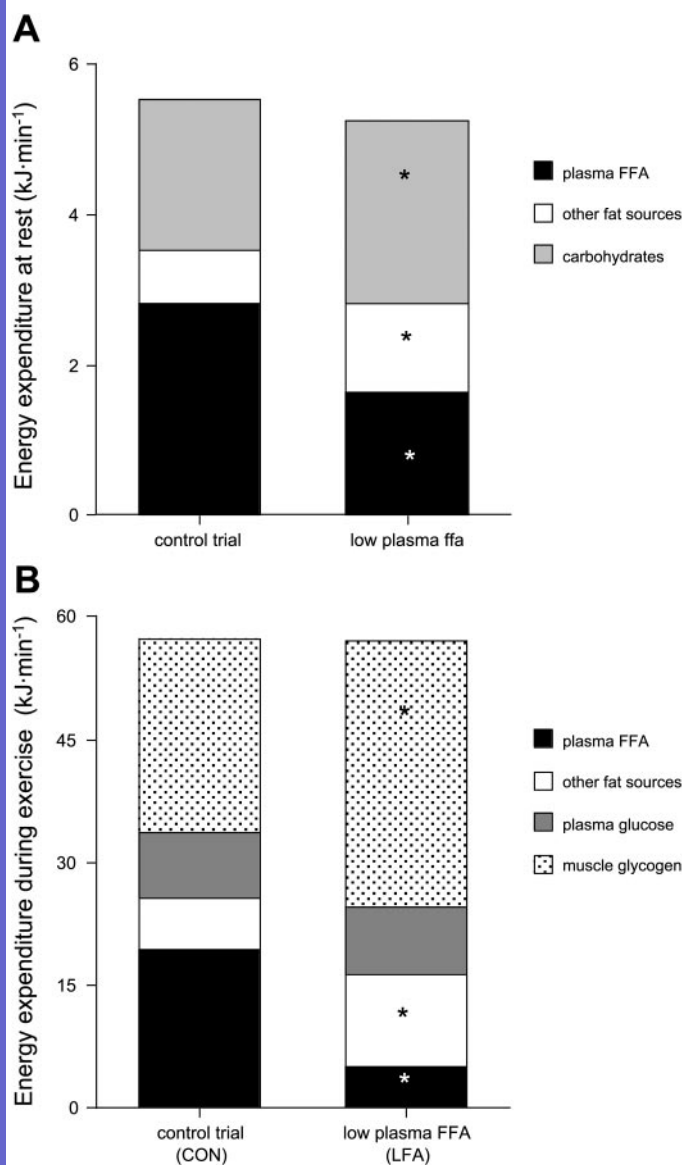


Fig. 4. Whole body substrate source utilization (in kJ/min) at rest (A) and during the 2nd h of exercise (B) in CON and LFA trials. *Significant difference in plasma FFA, other fat sources, total carbohydrate, and/or plasma glucose R_{ox} between trials ($P < 0.05$).

significantly greater in the LFA group than in the CON group (4.5 ± 0.7 and $2.6 \pm 0.7\%$ area lipid staining, respectively; $P < 0.05$). No changes in type II muscle fiber lipid content were observed between trials. However, within the LFA trial, type II fiber lipid content was significantly reduced after exercise ($P < 0.05$; Fig. 6B). The observed decreases in muscle lipid content were attributed to a significant decrease in both lipid droplet size and lipid droplet density (no. of droplets per area; $P < 0.01$). Mixed muscle metabolite concentrations, as determined by biochemical analyses, are provided in Table 2. Mixed muscle glycogen content decreased significantly in both trials ($P < 0.01$). The net difference between pre- and postexercise values averaged 305 ± 56 and 350 ± 31 mmol/kg in the CON and LFA trials, respectively (NS).

Skeletal muscle $\alpha 2$ -AMPK activity averaged 0.025 ± 0.005 and 0.025 ± 0.007 (rest), 0.025 ± 0.004 and 0.031 ± 0.006

(postexercise), and 0.031 ± 0.009 and 0.026 ± 0.007 U/mg (2-h recovery) in the CON and LFA trials, respectively. Total AMPK activity averaged 0.049 ± 0.009 and 0.040 ± 0.005 (rest), 0.041 ± 0.007 and 0.037 ± 0.011 (postexercise), and 0.049 ± 0.011 and 0.041 ± 0.003 U/mg (2-h recovery), respectively. No significant differences in $\alpha 2$ -AMPK and/or total AMPK activity were observed between the resting state, end of exercise, and 2-h recovery within or between groups. ACC phosphorylation status, expressed as the P-ACC/ACC ratio, averaged 1.55 ± 0.34 and 1.19 ± 0.23 (rest), 1.78 ± 0.51 and 2.12 ± 0.66 (postexercise), and 1.75 ± 0.51 and 1.45 ± 0.39 (2-h recovery) in the CON and LFA trials, respectively. ACC phosphorylation status tended to be higher at the end of exercise in the LFA trial ($P = 0.058$), but no significant differences were observed between trials. The phosphorylation status of HSL at Ser⁵⁶³ did not change over time and averaged 3.46 ± 0.55 and 2.97 ± 0.52 (rest), 3.27 ± 0.50 and 2.73 ± 0.53 (postexercise), and 3.98 ± 0.80 and 2.65 ± 0.62 (2-h recovery) in the CON and LFA trials, respectively. Furthermore, HSL phosphorylation at Ser⁵⁶⁵ also did not change over time and averaged 1.17 ± 0.08 and 1.30 ± 0.17 (rest), 0.99 ± 0.18 and 1.27 ± 0.233 (postexercise), and 1.19 ± 0.09 and 1.17 ± 0.29 (2-h recovery), respectively. The phosphorylation status of HSL at Ser⁵⁶³ or Ser⁵⁶⁵ did not differ between trials.

DISCUSSION

In the present study, acipimox administration is shown to effectively inhibit in vivo peripheral lipolysis, with plasma FFA R_a being substantially decreased at rest and during exercise compared with normal, fasting conditions (Table 1 and Figs. 2 and 3). The latter substantially reduced systemic FFA availability, with plasma concentrations remaining well below baseline level. As such, plasma FFA concentrations were substantially reduced in the LFA vs. the CON trial (Fig. 2). The reduction in FFA availability substantially increased the estimated muscle TG use at rest (Fig. 4A). During exercise, the absence of a progressive increase in plasma FFA availability in the LFA trial was accompanied by muscle TG use being sustained (Fig. 5), resulting in higher estimated muscle TG and glycogen oxidation rates (Fig. 4B). In agreement with the tracer data, a greater net decline in type I muscle fiber lipid content was observed in the LFA vs. CON trial (Fig. 6). Although the exact mechanisms responsible remain to be resolved, this study provides definite evidence that plasma FFA availability regulates muscle TG and glycogen use.

Nicotinic acid (analogs) has often been used to study the effects of adipose tissue lipolytic inhibition on in vivo metabolism (43, 50, 51, 58). Nicotinic acid inhibits lipolysis by binding to HM74 receptors in human adipose tissue (49). Binding to these receptors results in a suppression of intracellular cAMP levels, with a subsequent decrease in cAMP-dependent protein kinase activity, leading to a reduced association of HSL with its substrate in the lipid droplet of the adipocyte (6). These antilipolytic properties are specific for adipose tissue, as skeletal muscle does not express the HM74 receptor (49). Consequently, administration of nicotinic acid analogs like acipimox can be used to specifically inhibit adipose tissue lipolysis and reduce plasma FFA availability. In accordance with that, following acipimox administration we observed a massive reduction in plasma FFA R_a at rest and

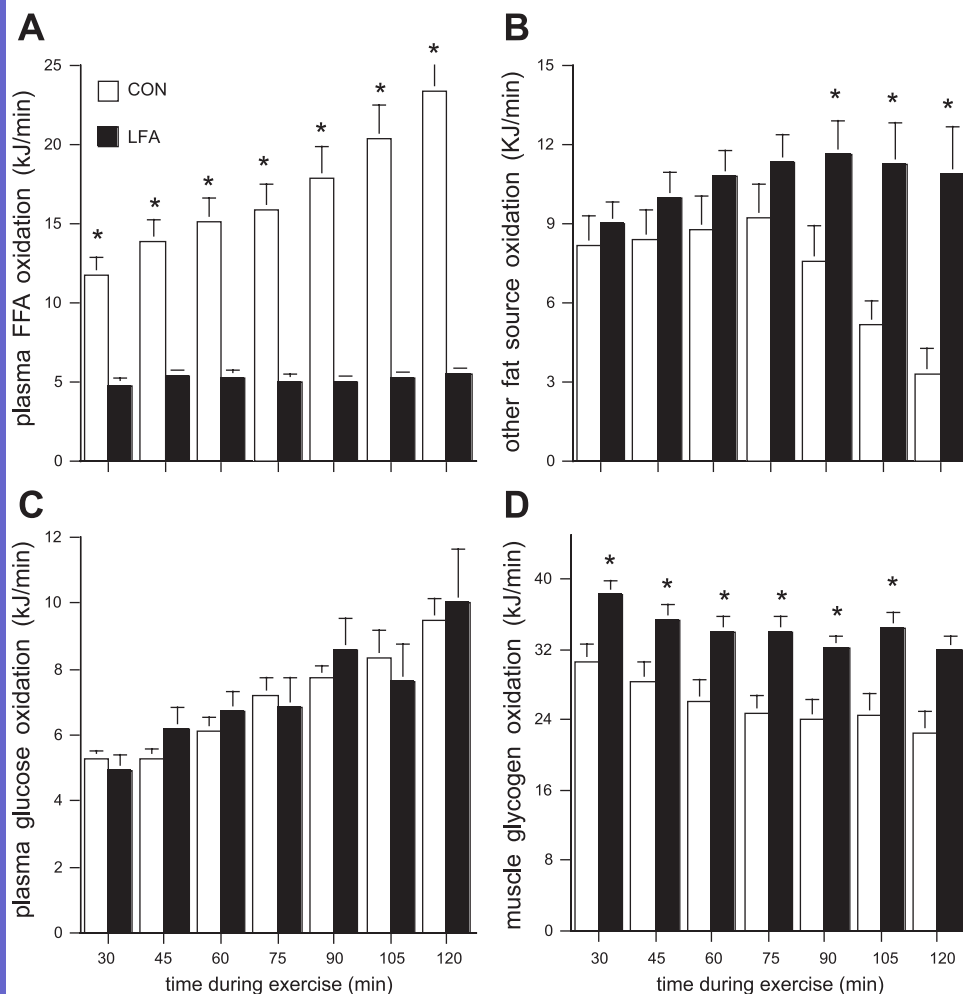


Fig. 5. Plasma FFA (A), other fat sources (B), plasma glucose (C), and muscle glycogen (D) R_{ox} over time during prolonged moderate-intensity exercise in CON and LFA trials. Data are means \pm SE. *Significantly different between trials ($P < 0.05$).

during exercise and subsequent recovery (Table 1), with FFA levels remaining well below baseline level (Fig. 2).

There has been much debate on the role of the IMTG pool as a substrate source at rest and/or during exercise (52, 57). Recently, we demonstrated that, besides plasma FFA, muscle-derived TG provides a substantial contribution to energy expenditure during exercise in trained males (54). In agreement, we demonstrated a $>60\%$ net decline in type I muscle fiber lipid content in vastus lateralis muscle following 120 min of exercise (54). Furthermore, we observed a strong, progressive decline in the oxidation rate of muscle (plus lipoprotein)-derived TG use during exercise. The latter correlated with the concomitant increase in plasma FFA R_a and oxidation (54). Those findings confirmed earlier estimations (40, 41) as well as more recent observations (56) and support the contention that the progressive increase in peripheral lipolytic rate and the subsequent increase in plasma FFA availability suppress IMTG mobilization and/or oxidation. In agreement, Watt et al. (58) recently reported a greater net decline in mixed muscle TG content following nicotinic acid administration in a 3-h exercise trial. In the present study, we extended their findings by investigating whether inhibition of adipose tissue lipolysis could stimulate IMTG use at rest and/or prevent the progressive decline in IMTG utilization rate in time during exercise, resulting in a greater net decline in fiber type-specific IMTG content.

At rest, the threefold lower plasma FFA availability in the LFA trial (Fig. 2) strongly reduced plasma FFA oxidation rates (Table 1). The latter was compensated by a significant increase in the use of other fat sources as well as higher carbohydrate oxidation rates (Fig. 4A). These other fat sources are generally assumed to reflect the use of muscle-derived TG. However, the applied stable isotope methodology does not allow differentiation between muscle- and lipoprotein-derived TG use. Although the contribution of lipoprotein-derived TG oxidation to total energy turnover is generally assumed to be of little quantitative importance in an overnight-fasted state (52), it should be noted that specific tissues likely contribute more substantially to the use of lipoprotein-derived TG. Furthermore, plasma lipoprotein-derived TG use may likely be increased during exercise conditions. Our data imply that, during resting conditions, IMTG turnover is augmented when plasma FFA availability is reduced. As we applied a $[6,6-^2H_2]$ glucose tracer, plasma glucose oxidation rates could not be quantified at rest. The latter is due to the fact that, in contrast to exercise conditions, glucose R_d does not match R_{ox} during resting conditions (19). However, plasma glucose R_a (hepatic glucose output) and R_d (whole body glucose uptake) were not affected by these short-term alterations in plasma FFA availability. The latter is in accord with previous reports on acipimox administration, which reported no change in plasma glucose R_d under basal resting conditions (43, 50).

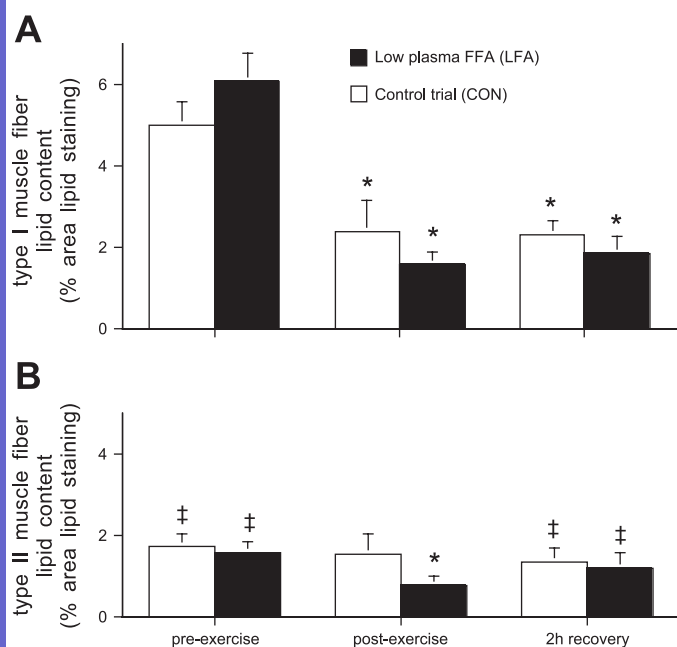


Fig. 6. Fiber type-specific intramyocellular lipid content (expressed as %area lipid stained) before exercise, immediately after exercise, and after 2 h of postexercise recovery as determined in type I (A) or type II (B) muscle fibers by use of semiquantitative fluorescence microscopy on Oil Red O-stained muscle cross sections. Data provided are means \pm SE. *Significantly lower vs. preexercise values; ‡significantly lower vs. type I muscle fibers ($P < 0.05$).

During exercise, we observed a progressive increase in total fat R_{ox} and a concomitant reduction in carbohydrate use under normal fasting conditions (CON). The greater fat R_{ox} was fully accounted for by a progressive increase in plasma FFA R_a , R_d , and R_{ox} and was accompanied by a decline in the use of muscle (and lipoprotein)-derived TG (Table 1 and Figs. 4 and 5). The latter is in agreement with others' (40, 41, 56, 58) as well as our recent observations (54), showing a progressive decline in IMTG use during prolonged moderate-intensity exercise under fasting conditions in healthy men. In the present study, acipimox administration effectively inhibited adipose tissue lipolysis, thereby preventing the exercise-induced increase in plasma

FFA R_a , R_d , and R_{ox} (Figs. 2 and 3). Consequently, no changes in substrate source utilization were observed throughout exercise in the LFA trial. As such, estimated IMTG R_{ox} did not decline but remained elevated throughout the entire exercise trial (Fig. 5B). This led to substantially greater estimated IMTG utilization rates during the latter stages of prolonged exercise in the LFA trial (Figs. 4 and 5). In agreement with the isotope tracer data, a greater net decline in type I muscle fiber lipid content was observed in the LFA trial (Fig. 6). Similar to the resting data, plasma glucose disposal during exercise was not affected by the acute changes in FFA availability (Table 1 and Figs. 3–5). As such, the higher carbohydrate R_{ox} in the LFA trial was entirely attributed to increased muscle glycogen use (Figs. 4 and 5). However, net changes in mixed muscle glycogen content in the biopsy samples did not significantly differ between trials and averaged 350 ± 31 and 305 ± 56 mmol/kg in the LFA and CON trials, respectively (Table 2).

Our second aim was to elucidate the mechanisms responsible for the shift in substrate use. We speculated that a reduction in plasma FFA availability stimulates carbohydrate use via the Randle cycle (36). A reduction in FFA availability could stimulate muscle glycogen use by stimulating pyruvate dehydrogenase complex (PDC) activation (via a reduction in the mitochondrial acetyl-CoA/CoASH ratio) and increasing glycolytic flux (via a lower citrate concentration, thereby disinhibiting phosphofructokinase). Furthermore, the higher glycolytic flux would lead to a greater decrease in glucose 6-phosphate concentration, which would relieve its inhibition on glycogen phosphorylase and hexokinase activity. The latter would accelerate muscle glycogenolysis and/or upregulate plasma glucose uptake, respectively. Some evidence to support this contention was observed, as muscle samples obtained after exercise showed significantly lower ratios of acetyl-CoA/CoASH and acetylcarnitine/carnitine in the LFA vs. CON trial (Table 2). Furthermore, skeletal muscle citrate and glucose 6-phosphate levels tended to be lower in the LFA trial. Whereas estimated muscle glycogen use was indeed elevated in the LFA trial, plasma glucose uptake did not differ between trials.

The greater IMTG use during exercise in the LFA trial was entirely attributed to the absence of the progressive decline in IMTG use, as was observed in the CON trial (Fig. 5B).

Table 2. Muscle metabolites

	Control Trial (CON)			Low FFA Availability Trial (LFA)		
	Preexercise	Postexercise	Recovery	Preexercise	Postexercise	Recovery
Free carnitine, mmol/kg	16.66 \pm 1.20	9.35 \pm 1.08*	13.53 \pm 1.71	17.29 \pm 0.57	13.12 \pm 0.69*†	17.46 \pm 0.75‡†
Acetylcarnitine, mmol/kg	4.01 \pm 0.72	11.23 \pm 1.06*	7.25 \pm 1.19‡	3.24 \pm 0.38	7.58 \pm 0.66*†	3.35 \pm 0.48‡†
LC-fatty acylcarnitine, mmol/kg	1.25 \pm 0.11	1.40 \pm 0.08	1.40 \pm 0.10	1.33 \pm 0.06	1.53 \pm 0.10	1.49 \pm 0.11
CoASH, μ mol/kg	104.7 \pm 7.9	76.4 \pm 8.8*	77.5 \pm 7.0*	97.4 \pm 6.9	92.5 \pm 5.0	103.8 \pm 7.4†
LC-fatty acyl-CoA, μ mol/kg	10.43 \pm 1.37	7.34 \pm 1.53	7.41 \pm 1.51	6.06 \pm 1.04†	6.00 \pm 0.76	5.05 \pm 0.82
Acetyl-CoA, μ mol/kg	15.78 \pm 2.21	34.34 \pm 5.40	27.51 \pm 6.08*	17.83 \pm 1.90	23.70 \pm 2.13	14.59 \pm 2.29‡
Acetyl-CoA/CoASH	0.15 \pm 0.02	0.50 \pm 0.09*	0.40 \pm 0.09	0.19 \pm 0.02	0.26 \pm 0.02†	0.15 \pm 0.03‡
Citrate, mmol/kg	1.15 \pm 0.09	2.12 \pm 0.29*	1.55 \pm 0.43‡	1.15 \pm 0.14	1.48 \pm 0.13	1.20 \pm 0.15
Glucose 6-phosphate, mmol/kg	2.58 \pm 0.59	1.86 \pm 0.37	1.69 \pm 0.32	3.92 \pm 1.06	1.44 \pm 0.46	2.92 \pm 0.93
Glycogen, mmol/kg	586 \pm 50	281 \pm 25*	290 \pm 36*	595 \pm 38	245 \pm 43*	268 \pm 43*
Lactate, mmol/kg	3.10 \pm 0.71	9.15 \pm 2.82	4.76 \pm 2.02	3.87 \pm 0.69	7.37 \pm 3.03	5.44 \pm 1.66

Values are means \pm SE; $n = 10$. Intramuscular free carnitine, acetylcarnitine, long-chain (LC) fatty acylcarnitine, CoASH, LC-fatty acyl-CoA, acetyl-CoA, citrate, glucose 6-phosphate (p), glycogen, and lactate concentrations (expressed per kg dry muscle wt) before and after exercise and after 2 h of postexercise recovery in CON and LFA. Except for citrate concentrations, all concentrations have been normalized to total muscle creatine content. *Significantly different from resting values within each trial ($P < 0.05$); †significantly different between trials ($P < 0.05$); ‡significantly different from postexercise values within each trial.

Because muscle TG mobilization by HSL (24) represents the rate-limiting step in IMTG lipolysis, we investigated which mechanism(s) could be responsible for the increased activation of HSL in the LFA trial. We speculated that the reduced availability of plasma FFA could affect AMPK activation and the phosphorylation status of HSL at Ser⁵⁶⁵. Furthermore, previous studies (58) have reported a greater increase in plasma epinephrine concentrations during exercise after nicotinic acid administration, which could stimulate HSL activation by the phosphorylation of HSL at Ser⁵⁶³, acting through PKA. In addition, long-chain fatty acyl-CoA concentrations have been shown to inhibit HSL allosterically in adipose tissue (24) and muscle homogenates (24). A lower plasma FFA availability could reduce intramuscular long-chain fatty acyl-CoA concentrations and reduce allosteric inhibition of HSL. Despite our efforts to unravel the mechanism(s) responsible for the greater IMTG use in the LFA trial, we could not find evidence to support any of these mechanisms. Neither AMPK and α 2-AMPK activity nor ACC phosphorylation status changed from rest to exercise and were not elevated after cessation of exercise in the LFA trial. These findings are in accord with Watt et al. (58), who also reported a greater net decline in mixed muscle lipid content following exercise when nicotinic acid was administered. In accord with the absence of changes in AMPK activity, no significant changes were observed in the phosphorylation status of ACC and the phosphorylation status of HSL at either Ser⁵⁶³ or Ser⁵⁶⁵. The latter findings are in line with recent observations by Roepstorff et al. (39) showing no changes in HSL phosphorylation at either Ser⁵⁶³ or Ser⁵⁶⁵ after 60 min of moderate-intensity exercise under normal (non-glycogen-depleted) conditions despite a substantial increase in plasma epinephrine concentration. Furthermore, we did not observe a greater decline in intramuscular fatty acyl-CoA concentrations in the LFA trial (Table 2). The latter does not necessarily prove that reduced long-chain acyl-CoA concentrations are not responsible for the greater IMTG use under reduced plasma FFA availability, as compartmentalization of the long-chain fatty acyl-CoA pool has been suggested to occur (18). Consequently, although we clearly show that reduced plasma FFA availability stimulates IMTG use in vivo, the exact mechanism responsible remains to be elucidated.

The present study shows that inhibition of adipose tissue lipolysis using a nicotinic acid analog elevates intramuscular lipid and glycogen use. These findings could be of clinical relevance for the prevention and/or treatment of skeletal muscle insulin resistance. Depletion of both intramuscular lipid and glycogen content are associated with the exercise-induced increase in skeletal muscle insulin sensitivity (52, 62). In agreement, interventions known to improve insulin sensitivity, like dietary lipid withdrawal (30), acute exercise (30, 54), and the use of thiazolidinediones (31) have all been associated with a concomitant reduction in IMTG content. Peroxisome proliferator-activated receptor- γ (PPAR γ) agonists, like the thiazolidinediones, improve insulin sensitivity partly through lipid retention in adipose tissue, thereby stimulating the redistribution of TG from skeletal muscle and liver back to adipose tissue (28). Nicotinic acid (analog) seems to act along the same paradigm. Similar to the metabolic consequences of excess muscle lipid accretion, increased muscle glycogen storage following overfeeding and/or inactivity has been reported to induce insulin resistance (29). In reverse, exercise stimulates

muscle glycogen use (23) and augments insulin sensitivity, even in an insulin-resistant state (35, 61). The depletion of the muscle glycogen stores is associated with this exercise-induced increase in insulin sensitivity (35, 61), which can be maintained for up to 48 h (60) depending on the rate of muscle glycogen repletion (7, 9). Consequently, combined exercise and pharmacological interventions that elevate intramuscular lipid and/or glycogen use are likely more effective to maximize the exercise-induced increase in insulin sensitivity. The latter could be even more relevant in an insulin-resistant state, in which intramuscular lipid (52) and glycogen (4) use seem to be impaired.

In conclusion, oral administration of a nicotinic acid analog effectively suppresses adipose tissue lipolysis both at rest and during exercise conditions. The subsequent reduction in plasma FFA availability stimulates intramuscular lipid oxidation both at rest and during exercise. Inhibition of adipose tissue lipolysis during exercise provides an effective mechanistic approach to increase intramuscular lipid and glycogen use.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert analytic assistance of Jos Stegen, Annemie Gijzen, Annita Rousseau, and Mia Meers as well as the enthusiastic support of the subjects who volunteered to participate in these trials. The monoclonal antibody A4.840 developed by Dr. Blau was obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the National Institute of Child Health and Human Development, and maintained by the University of Iowa, Department of Biological Science, Iowa City, IA 52242.

GRANTS

M. Thomason-Hughes and D. G. Hardie are supported by grants from Diabetes, UK. L. J. C. van Loon was supported by a grant from the Netherlands Organization for Scientific Research.

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