Kinetics and utilization of lipid sources during acute exercise and acipimox

Birgitte Nellemann,1,2 Ebis Sondergaard,1 Jørgen Jensen,2 Steen Bønløkke Pedersen,1 Niels Jessen,1,3 Jens Otto Lunde Jørgensen,1 and Søren Nielsen1

1Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark; 2Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway; and 3Research Laboratory for Biochemical Pathology, Institute for Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark

Submitted 27 January 2014; accepted in final form 1 June 2014

Nellemann B, Sondergaard E, Jensen J, Pedersen SB, Jessen N, Jørgensen JO, Nielsen S. Kinetics and utilization of lipid sources during acute exercise and acipimox. Am J Physiol Endocrinol Metab 307: E199–E208, 2014. First published June 3, 2014; doi:10.1152/ajpendo.00043.2014.—Overweight is associated with abnormalities of lipid metabolism, many of which are reversed by exercise. We investigated the impact of experimental antilipolysis and acute exercise on lipid kinetics and oxidation from VLDL-TG, plasma FFA, and “residual lipids” in overweight men (n = 8) using VLDL-TG and palmitate tracers in combination with muscle biopsies in a randomized, placebo-controlled design. Participants received placebo or acipimox on each study day (4 h of rest, 90 min of exercise at 50% V02max). Exercise suppressed VLDL-TG secretion significantly during placebo but not acipimox (placebo-rest: 64.2 ± 9.4; placebo-exercise: 48.3 ± 8.0; acipimox-rest: 55.2 ± 13.4; acipimox-exercise: 52.0 ± 10.9). Resting oxidation of VLDL-TG FA and FFA was significantly reduced during acipimox compared with placebo, whereas “residual lipid oxidation” increased significantly [VLDL-TG oxidation (placebo: 18 ± 3 kcal/h; acipimox: 11 ± 2 kcal/h), FFA oxidation (placebo: 14 ± 2 kcal/h; acipimox: 4.0 ± 0.5 kcal/h), and residual lipid oxidation (placebo: 3 ± 5 kcal/h; acipimox: 14 ± 5 kcal/h)]. Additionally, during exercise on both placebo and acipimox, oxidation of VLDL-TG and FFA increased, but the relative contribution to total lipid oxidation diminished, except for FFA, which remained unchanged during acipimox. Residual lipid oxidation increased significantly during exercise in both absolute and relative terms. Changes in selected cellular enzymes and proteins provided no explanations for kinetic changes. In conclusion, suppressed FFA availability blunts the effect of exercise on VLDL-TG secretion and modifies the contribution of lipid sources for oxidation.

Differences cannot differentiate between tissue oxidation and storage, and IML oxidation has usually been calculated indirectly by subtracting plasma FFA oxidation from total lipid oxidation measured by indirect calorimetry (14, 43).

Regulation of VLDL-TG metabolism includes both substrate availability and endocrine control (28), and the additional effects of exercise are not fully understood. Only two studies have investigated the acute effects of exercise on VLDL-TG kinetics, with conflicting results (27, 34). Furthermore, the overall increase in lipid oxidation during exercise cannot be accounted for by increased FFA flux, indicating oxidation of other lipid sources (15). In lean subjects, the increase in lipid oxidation is not accounted for by VLDL-TG oxidation, and VLDL-TG secretion is indeed suppressed (34). However, increased VLDL-TG turnover may be associated with greater VLDL-TG oxidation (35, 37).

The aim of this study was to investigate the impact of combining experimental antilipolysis with acute exercise on plasma FFA and VLDL-TG kinetics to determine the effects of substrate availability on lipid oxidation during exercise. We hypothesized that reduced FFA availability potentiates exercise-induced suppression of VLDL-TG secretion and increases the contribution of VLDL-TG to total lipid oxidation. In addition, we examined potential molecular mechanisms behind the expected shift in substrate utilization. We studied these questions in overweight, untrained men by using contemporary tracer approaches, indirect calorimetry, and muscle biopsies.

METHODS

This study was approved by the Central Denmark Region Committees on Health Research Ethics, and informed consent was obtained from all participants.

Participants. Eight healthy, untrained, overweight (BMI >26 kg/m2), nonsmoking men who used no medication were recruited randomly through a local advertisement. Before inclusion, a normal blood count, chemistry panel, and electrocardiogram were documented, and a maximal oxygen capacity (V02max) test was performed.

Protocol. Participants were examined twice 4–5 wk apart. In connection with each study day they received four tablets at t = 720, −600, −120, and 120 min. The tablets were identical and contained either placebo (PLA) or acipimox (ACI, 250 mg) (38); the order was randomized and double-blinded by the Hospital Pharmacy. Weight-maintaining diets (55% carbohydrate, 15% protein, and 30% fat) were calculated by a clinical dietician and provided by the hospital kitchen during the 3 days preceding the metabolic study day.

Metabolic study day. Participants were admitted to the research unit the evening before and remained in bed during the study period. Only tap water was allowed. The study started at 0800 and included a 4-h basal period (t = 0–240 min), followed by 90 min (t = 240–330 min) of bicycling at 50% of individual VO2max. One catheter was placed in the antecubital vein for infusion of [1-14C]VLDL-TG (primed with

OVERWEIGHT IS ASSOCIATED WITH proatherogenic abnormalities of lipid metabolism. The alterations reflect reduced insulin suppression of both hepatic very low-density lipoprotein triglyceride (VLDL-TG) secretion and adipose tissue lipolysis, leading to greater plasma triglycerides (TG) and free fatty acid (FFA) concentrations (30). Many of these abnormalities are reversed by physical exercise (21). During low- to moderate-intensity exercise, fasting lipid oxidation accounts for ~50% of energy expenditure (EE) (25, 33, 46), and the major sources are plasma FFA, intramyocellular lipids (IML), and VLDL-TG fatty acids (FA). Plasma FFA oxidation (19), measured isotonically (15), increases rapidly during exercise. Conversely, measurements of VLDL-TG oxidation are methodologically challenging (24) and have been calculated mostly from regional A-V concentration differences (19); however, these

http://www.ajpendo.org 0193-1849/14 Copyright © 2014 the American Physiological Society E199

Address for reprint requests and other correspondence: S. Nielsen, Dept. of Endocrinology and Internal Medicine, Aarhus University Hospital, Nørrebrogade 44, 8000 Aarhus C, Denmark (e-mail: nielsen.soren@dadlnet.dk).
20% bolus) and [9,10-3H]palmitate. Another was placed in a contralateral dorsal hand vein and kept heated to ensure arterialized blood. Samples for [14C]VLDL-TG-specific activity (SA) were obtained at t = 0, 210, 220, 230, 240, 300, 310, 320, and 330 min and for palmitate concentration and SA at t = 30, 60, 70, 80, 90, 270, 300, 310, 320, and 330 min. Breath samples were collected at t = 0, 210, 220, 230, 240, 300, 310, 320, and 330 min for determination of 14CO2 SA. Indirect calorimetry was performed at t = 180–210 min and every 15 min during exercise to ensure O2 expenditure at 50% of VO2max and to determine CO2 production rate (VCO2). Muscle biopsies were obtained from musculus vastus lateralis at t = 150 min and immediately after exercise (t = 330 min).

[1-14C]VLDL-TG tracer preparation. One week before the study days, an 80-ml blood sample was obtained aseptically after a 12-h fast, and VLDL-TG labeling was performed as described previously (11), with minor modifications. In brief, in a sterile test tube, plasma was sonicated with [1-14C]triolein (Perkin-Elmer, Waltham, MA) for 2 h at 5°C. Thereafter, the solution was transferred to sterile test tubes, covered with sterile saline (1.006 g/cm3), and ultracentrifuged (50.3 Ti rotor; Beckman Instruments, Palo Alto, CA) at 40,000 rpm for 18 h at 10°C. The supernatant was collected with a sterile Pasteur pipette, filtered, and stored at 5°C until use. A small sample was cultured to ensure sterility.

VLDL-TG concentration and SA. VLDL-TG was separated from 3–5 ml of plasma by ultracentrifugation, as described above, and the fraction secured by tube-slicing 1.5 cm from the top. A small portion was used for measurement of TG content (COBAS, c111; Roche, Basel, Switzerland) and calculation of VLDL-TG concentration. The exact volume was recorded, scintillation fluid was added, and 14C was measured by dual-channel scintillation counting to <2% counting error.

Breath 14CO2 SA. Breath samples were collected in breath bags (Wagner Analysen Technik), and the air passed through a hyamine solution for trapping of CO2, as described previously (34). Scintillation fluid was added and counted to <2% counting error.

Palmitate kinetics. Systematic palmitate flux and oxidation were measured using an isotope dilution technique, with a 1-h constant infusion of [9,10-3H]palmitate (0.3 pmol/min) (Department of Clinical Physiology and Nuclear Medicine, Aarhus University Hospital, Aarhus, Denmark) in each period. Palmitate concentration and SA were measured by HPLC, using [3H]palmitate as internal standard. Basal palmitate flux was calculated as [3H]palmitate infusion rate (disintegrations/min) divided by the steady-state palmitate SA (disintegrations/min 106 3H/106 mol). During exercise, Steele’s equation fitted for non-steady-state data was used after spline fitting of the palmitate concentration and SA (16, 47). Data were insufficient in one participant.

Plasma palmitate oxidation rate was measured using the rate of 3H2O production vs. time (t = 30–90 min, t = 270–330 min), as described elsewhere (15). In brief, the slope of the 3H2O production curve was divided by palmitate SA to calculate palmitate oxidation. The palmitate oxidation was converted to its total FFA equivalent curve was divided by palmitate SA to calculate palmitate oxidation. The palmitate oxidation was converted to its total FFA equivalent.

Indirect calorimetry. EE and respiratory exchange ratio (RER) were measured by indirect calorimetry at baseline (Deltatrac monitor; Datex Instrumentarium, Helsinki, Finland) and during exercise (Oxycon Pro; Erich Jaeger). Net lipid and glucose oxidation were calculated after correction for protein oxidation (8) measured from urine nitrogen. Before being assayed, the samples were freeze-dried for 48 h and dissected free of fat, blood, and connective tissue under a stereomicroscope.

Intramuscular lipid. Freeze-dried muscle (2–3 mg) was saponified in 250 μl of 30% ethanolic KOH and neutralized with MgCl2, followed by a glycerol assay (no. TR0100; Sigma, St. Louis, MO). One sample had markedly increased lipid content (>3-fold) and perlipin A expression compared with the other samples, and therefore, it was suspected to be contaminated with extracellular lipids. Hence, the sample was excluded from the cellular lipid analysis.

Intramuscular glycojen. Freeze-dried muscle tissue (1–2 mg) was hydrolyzed in 100 μl of 1.8 M HCl for 2.5 h at 100°C and then neutralized with 30 μl of NaOH. The free glycosyl units were assayed by spectrophotometry with a hexokinase-dependent assay (3).

Protein expression and phosphorylation. Proteins were purified from freeze-dried muscle (5–6 mg) and homogenized in ice-cold buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM Na2PO4, 30 mM NaF, 1 mM Na3VO4, 10 mM EDTA, 2.5 mM Benznidamid, and 0.5 μl/10 mg of muscle tissue, P-8340 inhibitor cocktail; Sigma). The homogenate was gently stirred at 4°C for 15 min before being centrifuged for 20 min at 4°C to remove insoluble material. The supernatant was aspirated and kept at –80°C until analysis. Protein concentration was assessed by chemiluminescence (LumiGLO reagent and peroxide; Cell Signaling Technology). Phosphospecific antibodies against phosphorylated (p)-AMP-activated protein kinase (AMPK)-Thr172, p-glycogen synthase (GS)-Ser473, p-Akt substrate of mTOR (p-mTOR), p-Akt-Thr308, p-hormone-sensitive lipase (HSL)-Ser663, and Ser669 were from Cell Signaling Technology, and p-acetyl-CoA carboxylase (ACC)-Ser79 was from Millipore. Antibodies against perlipin A, AMPK, GS, Akt, and HSL were from Cell Signaling Technology, antibody against AS160 was from Upstate, and antibody against GLUT4 was from Millipore. Because of technical difficulties we were unable to quantify ACC expression, and therefore, ACC phosphorylation on Ser79 was expressed as a ratio to β-actin expression (antibody from Cell Signaling Technology) instead.

Gene expression. Gene expression was analyzed with real-time reverse transcriptase polymerase chain reaction (RT-PCR), where complementary DNA was constructed using a random hexamer primer as described by the manufacturer (Verso cDNA kit from WVR, Herlev, Denmark). PCR master mix was added, and real-time quantification of genes was performed by SYBR green real-time RT-PCR assay (KAPA SYBR Fast Universal kit from Ken-En-Tec, Taastrup, Denmark) in 384 well formats in a LightCycler (68298) from Roche Roche Applied Science (Mannheim, Germany).

The following pairs were designed using QuantPrime (1): angioptin-like-4 (ANGPTL4), TATGCACCCTCTGCTCCTCC-GAGATG-GGACGGCAATGGT; lipoprotein lipase (LPL), GCATTACCAGGT-TCGCC-CTCCCATCATGTTGAAACTACC; diacylglycerol acyltransferase (DGAT)1, TTTAGCAACTACCTGGTGACAT-ACCGGCGATTGGTCAGTCA; DGAT2, ACTGGAAACAACACAAAGATGTT-GTCTCAGGAATGAGCCACAG; CD36, TCTGTGCTTGT- TAACCCCAA-GCCGGTTGGAAGCTGTGTA; β2-microglobulin, GAGGCTATCCCAGTACCTAC-AAGTGCGATGATGAC; cDNA with specific primers was amplified in separate tubes and the increase in fluorescence measured in real time. All samples were amplified in duplicate, and no product was observed in control tubes, where no reverse transcriptase had been added.

Calculations. VLDL-TG secretion rate (μmol/min) = F/SA, where F is the [1-14C]VLDL-TG tracer infusion rate, and SA is the steady-state SA for the last half-hour of the basal and exercise periods. VLDL-TG clearance rate (ml/min) = VLDL-TG secretion rate/CVLDL-TG, where CVLDL-TG is the average VLDL-TG concentration in each steady-state period. Fractional VLDL-TG oxidation (% = [14CO2 SA × VO2]/k × Ar × Fi), where k is the volume of 1 mol CO2 at 20°C and 1 atmospheric pressure (22.4 l mol–1), and Ar is the acetate recovery factor of 0.56 at rest (32) and 0.98 during exercise (39). Total VLDL-TG oxidation...
RESULTS

Clinical data are shown in Table 1. Participants received an ACI dose of 2.4–2.9 mg/kg body wt.

Participants were untrained with $\dot{V}O_2$ max at 3,182 ± 111 ml/min (33.6 ± 1.4 ml $O_2$-kg$^{-1}$-min$^{-1}$). $\dot{V}O_2$ during exercise was similar on each study day, averaging ≈50% of $\dot{V}O_2$ max: PLA, 1,619 ± 43.7 ml/min; ACI, 1,609 ± 49.3 ml/min ($P = 0.75$). The workloads on each study day were also similar: PLA, 85 ± 4 W; ACI, 88 ± 3 W ($P = 0.3$).

Hormones and metabolites. Plasma total TG concentration increased slightly during exercise in both groups, whereas VLDL-TG concentrations remained unchanged (Fig. 1). There were no significant differences between study days in total TG or VLDL-TG. As expected, basal and exercise FFA levels were reduced during ACI compared with PLA (Fig. 1C). Exercise increased FFA concentration significantly in both groups. GH levels were higher during ACI and increased significantly on both days during exercise (Fig. 1D). Insulin levels were significantly lower during ACI than PLA (Fig. 1E), and exercise promoted a comparable, significant decrease in both groups. Basal glucose concentrations were similar on the 2 study days but decreased significantly during exercise in the ACI group compared with baseline and compared with PLA (Fig. 1F).

VLDL-TG kinetics. Basal VLDL-TG secretion and clearance rates were similar on the 2 study days. Exercise decreased VLDL-TG secretion rate ($P < 0.001$, RM-ANOVA; Fig. 2A); however, there was no difference in the decrease in PLA and ACI. VLDL-TG secretion adjusted for FFM showed the same pattern [VLDL-TG secretion (μmol·kg FFM$^{-1}$·min$^{-1}$): PLA basal, 0.97 ± 0.14; PLA exercise, 0.83 ± 0.2; ACI basal, 0.74 ± 0.12; ACI exercise, 0.78 ± 0.16, $P < 0.001$, RM-ANOVA]. Exercised decreased VLDL-TG clearance rate ($P = 0.002$, RM-ANOVA; Fig. 2B); however, there was no difference in the decrease between treatments.

Basal VLDL-TG oxidation rate was significantly lower during ACI than during PLA ($P = 0.03$; Fig. 2C). Exercise was associated with a significant increase in oxidation rate ($P = 0.004$, RM-ANOVA). There was no significant difference in the increase between treatments. Basal fractional VLDL-TG oxidation was similar in the two groups. Exercise was also associated with a significant increase in fractional VLDL-TG oxidation rate ($P < 0.001$, RM-ANOVA); however, there was no significant difference between study days [fractional VLDL-TG oxidation (%): PLA basal, 62.7 ± 8.9; PLA exercise, 103.5 ± 10.9; ACI basal, 44.6 ± 3.7; ACI exercise, 90.6 ± 9.4].

Palmitate kinetics. As expected, palmitate concentration followed the same pattern as total FFA (Fig. 3A). Both basal and exercise palmitate fluxes were significantly reduced during ACI compared with PLA ($P < 0.001$) (Fig. 3B) and significantly greater during exercise compared with basal. Moreover, during exercise a similar decrease (≈25%) in palmitate flux was observed ($P < 0.001$, RM-ANOVA). Basal palmitate oxidation was significantly lower during ACI compared with PLA ($P = 0.004$). Exercise was associated with a significant increase in palmitate oxidation (RM-ANOVA, $P = 0.04$; Fig. 3C). The increase was significantly greater during PLA compared with ACI. Post hoc testing revealed significant increases during both PLA ($P < 0.001$) and ACI ($P = 0.002$).

Substrate oxidation. Basal RER was not different between study days but was higher on ACI than PLA during exercise. Exercise was associated with a similar significant increase in RER ($P < 0.001$, RM-ANOVA; Table 2). Basal EE was not different between the study days but increased similarly during exercise ($P < 0.001$, RM-ANOVA).

Basal lipid oxidation rate was not significantly different between the study days but was significantly lower during ACI than PLA during exercise (Table 2). As expected, exercise was associated with an increase in lipid oxidation ($P < 0.001$, RM-ANOVA), which was significantly greater during PLA compared with ACI ($P = 0.049$, RM-ANOVA). Post hoc testing revealed significant increases during both PLA ($P < 0.001$) and ACI ($P = 0.003$). The contribution of lipid oxidation to total EE at baseline was not different between study days, and exercise was not associated with significant changes in the contribution, although during exercise it tended to be lower during ACI than during PLA ($P = 0.05$; Table 2).

For comparison, VLDL-TG and plasma FFA oxidation rates (μmol/min) in Fig. 2C were converted to kilocalories per hour (Table 2). Basal lipid oxidation derived from lipid sources other than VLDL-TG and plasma FFA, i.e., “residual lipid oxidation,” was significantly greater during ACI than during PLA ($P = 0.03$). Moreover, exercise was associated with a significant increase in residual lipid oxidation ($P = 0.008$, RM-ANOVA); the increase was not significantly different between study days. The proportional contribution of VLDL-TG FA oxidation to total EE as well as total lipid oxidation at baseline was significantly lower during ACI than during PLA ($P = 0.01$). Moreover, exercise was associated with a significant decrease in the proportion ($P < 0.001$, RM-ANOVA). The decrease was significantly greater during PLA than during ACI ($P = 0.04$, RM-ANOVA). Post hoc testing revealed a significant decrease during both PLA ($P < 0.001$) and ACI ($P = 0.009$) [VLDL-TG contribution to total EE (%): PLA basal, 22.2 ± 3.0; PLA exercise, 5.6 ± 1.0; ACI basal, 14.4 ± 3.2; ACI exercise, 4.5 ± 0.9].

Intramuscular energy stores. Basal IMI was not different between study days and remained unchanged during exercise ($P = 0.33$, RM-ANOVA; Fig. 4A). Basal intramuscular glycogen content was not significantly different between PLA and...
ACI but decreased significantly during exercise \((P = 0.001, \text{RM-ANOVA})\) (Fig. 4B); the decrease was not different between study days.

**Skeletal muscle protein expression, phosphorylation, and gene expression.** Basal phosphorylation of the energy-sensing enzyme AMPK was not different between PLA and ACI, whereas exercise was associated with an approximately threefold similar significant increase in phosphorylation \((P < 0.001, \text{RM-ANOVA})\;\text{Fig. } 5\text{A}). Basal phosphorylation of ACC-Ser\(^{79}\) was not significantly different between PLA and ACI, whereas exercise was associated with a significant increase in phosphorylation \((P = 0.049, \text{RM-ANOVA}; \text{Fig. } 5\text{B})\). Post hoc testing revealed the main effect as being during PLA \((P = 0.04)\). Correspondingly, basal GS phosphorylation of Ser\(^{641}\) was increased during ACI compared with PLA \((P = 0.04)\). Exercise was associated with a significantly similar decrease in both groups \((P < 0.001, \text{RM-ANOVA}; \text{Fig. } 5\text{C})\).

Basal phosphorylation level of HSL at Ser\(^{563}\) and Ser\(^{660}\) was not different between PLA and ACI. Exercise was associated with a significant increase of HSL-Ser\(^{660}\) \((P = 0.03, \text{RM-ANOVA})\) but not HSL-Ser\(^{563}\) \([p\text{-HSL-Ser}^{563}\ (%\text{basal})]: \text{PLA basal, } 100 \pm 0; \text{PLA exercise, } 130 \pm 25; \text{ACI basal, } 109 \pm 11; \text{ACI exercise, } 133 \pm 26; P = 0.35, n = 7]\). The increase in phosphorylated HSL-Ser\(^{660}\) was not significantly different between PLA and ACI \([p\text{-HSL-Ser}^{660}\ (%\text{basal})]: \text{PLA basal, } 100 \pm 0;
Basal ANGPTL4 mRNA was not significantly different between PLA and ACI, whereas exercise was associated with a significant, similar upregulation in both groups ($P = 0.03$, RM-ANOVA; Fig. 5E). CD36 mRNA expression tended to decrease during ACI during both rest ($P = 0.05$) and exercise ($P = 0.08$, RM-ANOVA). Basal gene expression of DGAT1 and DGAT2 was not significantly different between study days and remained unchanged during exercise (data not shown).

Basal AS160 phosphorylation on Ser588 tended to be lower during ACI than during PLA ($P = 0.09$) and increased similarly and significantly during exercise ($P = 0.049$, RM-ANOVA) [p-AS160-Ser588 (% basal): PLA basal, 100 ± 0; PLA exercise, 150 ± 32; ACI basal, 168 ± 34; ACI exercise, 232 ± 62]. Basal AS160 phosphorylation of Thr642, Akt phosphorylation of Thr308, and GLUT4 expression were not significantly different between study days and remained unchanged during exercise (data not shown).

**DISCUSSION**

We assessed the effects of acute exercise with and without suppression of FFA availability on VLDL-TG kinetics and energy utilization in overweight men and report several novel findings. First, hepatic VLDL-TG FA secretion rate is suppressed during exercise; however, the effect was attenuated during short-term ACI treatment. Second, ACI blunted the basal contribution of VLDL-TG and plasma FFA to total lipid oxidation, whereas residual lipid oxidation increased markedly. Third, exercise increased oxidation of all lipid sources during both ACI and PLA. However, in relative terms only residual lipid oxidation increased its contribution to total lipid oxidation, whereas VLDL-TG and plasma FFA decreased, except for plasma FFA oxidation, which during ACI does not alter its relative contribution. Fourth, whereas the exercise-induced plasma FFA oxidation during ACI reached only one-third of that observed during PLA, VLDL-TG FA oxidation increased to levels comparable with placebo during exercise, averaging 10–15% of total lipid oxidation.

The decrease in VLDL-TG secretion and clearance rates during exercise corresponds with previous findings in lean subjects using similar methodology (34). We extend these findings to overweight men. The absolute secretion and clearance rates in the present study correspond with those reported previously in overweight subjects (13, 37) but contrast previous findings by Morio et al. (27), who reported increased VLDL-TG fractional catabolic rate during exercise. This may be explained by differences in exercise protocols, subject characteristics, and methodologies. Of note, exercise did not decrease during ACI. This is somewhat surprising, since elevating plasma FFA experimentally results in increased VLDL-TG secretion rate (23). One explanation may relate to the metabolic effects of GH (26), which is disproportionately increased during ACI. ACI increases GH secretion in obese subjects (17) and exerts effects during both rest and exercise (20). Thus, greater GH levels during ACI may increase lipid oxidation as well as insulin resistance in skeletal muscle, thereby counteracting the antilipolytic effect of ACI, including any substrate-driven effects on hepatic VLDL-TG secretion. It is also possible that the achieved reduction in plasma FFA was too small or too short to impact sufficiently on secretion rates. Therefore, we suggest that the suppressive impact of exercise on VLDL-TG secretion outweighs any stimulatory effects of

---

PLA exercise, 138 ± 37; ACI basal, 112 ± 15; ACI exercise, 127 ± 20; $n = 7$.

Basal ANGPTL4 mRNA was not significantly different between PLA and ACI, whereas exercise was associated with a significant, similar upregulation of ANGPTL4 mRNA in both groups ($P = 0.01$, RM-ANOVA; Fig. 5D). Basal LPL mRNA was not significantly different between study days. However, exercise was associated with a significant, similar upregulation in both groups ($P = 0.03$, RM-ANOVA; Fig. 5E). CD36 mRNA expression tended to decrease during ACI during both rest ($P = 0.05$) and exercise ($P = 0.08$, RM-ANOVA). Basal gene expression of DGAT1 and DGAT2 was not significantly different between study days and remained unchanged during exercise (data not shown).
changes in plasma insulin and FFA availability, although it is somewhat attenuated during ACI.

The present finding of a relative lipid contribution of \(~40\)–\(~50\)% to total EE during rest and exercise concurs with previous reports (25, 27). Conversely, the contribution of VLDL-TG FA to whole body lipid oxidation is controversial (10, 14, 19, 34, 42), mainly because VLDL-TG FA oxidation has proven difficult to measure. Hence, previous estimates have not adjusted for concomitant IML oxidation (14, 40, 43) or been limited to regional A-V difference measurements, which does not distinguish between TG oxidation and storage (19). In this study, we measured whole body VLDL-TG FA oxidation directly (11, 34). A novel finding is a significant exercise-induced increase in VLDL-TG oxidation. This differs from previous results in lean subjects, in whom VLDL-TG oxidation remained unchanged (34). The difference in change in VLDL-TG FA oxidation rate during exercise between lean and overweight subjects cannot be explained by differences in the suppression of VLDL-TG secretion rate alone but is also a result of greater fractional oxidation. In the present study, the fractional oxidation of the infused tracer increased to a greater extent \([from \sim 60 to \sim 100\% in the present study compared with from \sim 50 to \sim 80\% in lean subjects (34)]\). For comparison, the suppression of VLDL-TG turnover was 64 to 48 \(\mu\)mol/min in the present study and 39 to 31 \(\mu\)mol/min in the lean subjects (34). So both factors contribute to explain the
Table 2. RER, EE, and substrate ox rates

<table>
<thead>
<tr>
<th></th>
<th>PLA Basal</th>
<th>PLA Exercise</th>
<th>ACI Basal</th>
<th>ACI Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER</td>
<td>0.82 ± 0.02</td>
<td>0.87 ± 0.02*</td>
<td>0.84 ± 0.01</td>
<td>0.92 ± 0.02*</td>
</tr>
<tr>
<td>EE, kcal/h</td>
<td>79 ± 2</td>
<td>473 ± 13*</td>
<td>73 ± 3</td>
<td>478 ± 14*</td>
</tr>
<tr>
<td>Total lipid ox, kcal/h</td>
<td>37 ± 5</td>
<td>209 ± 31*</td>
<td>29 ± 3</td>
<td>141 ± 23*</td>
</tr>
<tr>
<td>Total lipid ox (%EE)</td>
<td>46 ± 5</td>
<td>44 ± 6</td>
<td>40 ± 3</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>VLDL-TG ox, kcal/h</td>
<td>18 ± 3</td>
<td>24 ± 4*</td>
<td>11 ± 2*</td>
<td>21 ± 4*</td>
</tr>
<tr>
<td>VLDL-TG ox (%total lipid oxidation)</td>
<td>50 ± 5</td>
<td>13 ± 2*</td>
<td>38 ± 9*</td>
<td>19 ± 6*</td>
</tr>
<tr>
<td>Plasma FFA ox, kcal/h</td>
<td>14 ± 2</td>
<td>64 ± 14*</td>
<td>4 ± 0.5*</td>
<td>21 ± 7*</td>
</tr>
<tr>
<td>Plasma FFA ox (%total lipid ox)</td>
<td>44 ± 9</td>
<td>30 ± 4*</td>
<td>13 ± 2*</td>
<td>15 ± 3*</td>
</tr>
<tr>
<td>Residual lipid ox, kcal/h</td>
<td>5 ± 5</td>
<td>128 ± 22*</td>
<td>14 ± 5*</td>
<td>107 ± 31*</td>
</tr>
<tr>
<td>Residual lipid ox (%total lipid ox)</td>
<td>0.3 ± 13</td>
<td>58 ± 5*</td>
<td>45 ± 13*</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Glucose ox, kcal/h</td>
<td>23 ± 4</td>
<td>205 ± 29*</td>
<td>23 ± 2</td>
<td>321 ± 26*</td>
</tr>
<tr>
<td>Protein ox, kcal/h</td>
<td>19 ± 2</td>
<td>10 ± 2*</td>
<td>21 ± 1</td>
<td>16 ± 2*</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE. RER, respiratory exchange ratio; EE, energy expenditure; ox, oxidation; PLA, placebo; ACI, acipimox; TG, triglycerides; FFA, free fatty acids. Plasma FFA and residual lipid ox rates were based on n = 6; therefore, the individual lipid source oxidation values do not add ≤100% or total lipid ox. *Vs. PLA basal; †vs. ACI basal; ‡vs. PLA exercise.

increase in oxidation rate during exercise, with fractional oxidation being proportionally more important than suppression in the VLDL-TG secretion rate. An additional finding was that, during ACI, resting VLDL-TG FA oxidation was lower compared with PLA. The mechanism could involve reduced facilitated transmembrane FA transport, as CD36 mRNA was somewhat suppressed during ACI. Of note, exercise ameliorated the difference in VLDL-TG FA oxidation by increasing it to a level comparable with PLA. We believe that this is the first study to demonstrate a clear whole body increase in VLDL-TG oxidation during exercise. Regarding VLDL-TG hydrolysis, we found a significant increase in LPL mRNA during ACI but not during PLA, which could represent a counterregulatory response to reduced FFA availability. Conversely, ANGPTL4, an inhibitor of LPL activity (22) that is regulated by FFA in human skeletal muscle (5), increased during exercise on both study days.

Although exercise increased oxidation of all lipid sources, the most pronounced increase was IML/residual lipid oxidation. The difference in the proportional increase between PLA and ACI was accompanied by an increase in HSL-Ser660 phosphorylation, which was slightly greater during PLA. However, the apparent lower increment during ACI was associated with a marked increase in basal oxidation rate and somewhat greater basal HSL-Ser660 phosphorylation. These novel findings collectively suggest that resting VLDL-TG FA and residual lipid oxidation are regulated differently. In cell studies, intramuscular TGs provide a large proportion of substrate for muscle lipid metabolism during electronic stimulated contractions (6), which supports our findings of ≈50% of lipid oxidation being accounted for by residual lipid oxidation. Therefore, we believe that residual lipid oxidation, as estimated in the present study, represents mainly oxidation of intracellular lipid stores.

Obviously, IML content does not assess the interaction between IML storage and oxidation but merely reflects the total content at the time of the biopsy. This may partly explain the unchanged IML and emphasizes the need for more direct methods to measure VLDL-TG FA and IML oxidation simultaneously. The shift in substrate utilization toward increased glucose oxidation during exercise was reflected by expected increases in phosphorylation status of the AMPK-regulated phosphorylation site on AS160-Ser588 (45) as well as down-regulation of GS phosphorylation. The small, nonsignificant decrease in whole body lipid oxidation during ACI was accompanied by a nonsignificant increase in phosphorylation of ACC. FA oxidation is stimulated by phosphorylation of ACC.

Fig. 4. Intramuscular energy stores. A: intramyocellular lipid was not affected by exercise and not different between PLA and ACI. B: intramuscular glycogen was significantly decreased by exercise, and there were no differences between PLA and ACI. Black bars, basal; open bars, exercise. *P < 0.001 compared with PLA basal; †P < 0.001 compared with ACI basal. Error bars, SE.
ACC regulates the production of malonyl-CoA, an allosterical inhibitor of carnitine palmitoyltransferase 1, which controls transport of activated FA into the mitochondria (2). Therefore, the trend toward an increase in ACC phosphorylation during ACI could be interpreted as a compensation for the reduced FFA availability. The ACC kinase AMPK that is sensitive to intracellular energy levels was, as expected, significantly activated during exercise. The marked increase in AMPK phosphorylation is probably caused by the prolonged exercise in the present study, since exercise at low intensities (40% of V\textsubscript{O2 max}) induces a substantial increase in AMPK phosphorylation if continued till exhaustion (44). Interestingly, ACI did not increase AMPK phosphorylation in the resting state, indicating that AMP and ATP levels in skeletal muscle were not affected by ACI. Therefore, the AMPK phosphorylation pattern did not completely match phosphorylation of ACC. This observation is in agreement with previous reports and has been explained by an allosteric activation of AMPK (4).

Fractional VLDL-TG oxidation during exercise increased to ~100%. We suspect that, apart from the \textsuperscript{14}CO\textsubscript{2} trapped in the tricarboxylic acid cycle and bicarbonate pools, as corrected for by the acetate recovery factor, other intracellular preoxidative TG pools exist (15, 18) due to the 4-h tracer infusion period preceding exercise. Hence, labeled palmitate could be trapped in, e.g., skeletal muscle, heart, and kidneys and released during

![Graphs and figures](AJP-Endocrinol Metab • doi:10.1152/ajpendo.00043.2014 • www.ajpendo.org)
exercise, resulting in overestimation of fractional \(^{14}\text{C}\)-VLDL-TG oxidation. We suspect tracer recycling to be a lesser problem, as recycled tracer generally plays a minor role in constant infusion protocols (36).

The study has limitations. Although nicotinic acid analog is a well-established tool in human studies of antilipolysis (31, 41, 42), the observed effects could result from ACI itself. Moreover, ACI acutely improves insulin-mediated glucose disposal irrespective of ambient FFA concentration. However, effects on oxidation rates may be limited, as ACI preferentially improves nonoxidative glucose disposal. Moreover, ACI stimulates skin blood flow (7); however, we do not suspect this to have a major impact on the tracer kinetics, as ACI has no effect on adipose tissue (9) or forearm blood flow (12, 29). Second, our results cannot be extended to women or other patient groups.

We conclude that exercise suppresses hepatic VLDL-TG secretion and increases VLDL-TG oxidation in overweight men. However, the major exercise-induced increase in lipid oxidation is due to residual lipid oxidation, an effect that is further exaggerated when FFA availability is suppressed by ACI. VLDL-TG FA oxidation increases during exercise to similar levels irrespective of FFA availability; however, the relative contribution remains markedly less during exercise compared with rest. Approaches allowing increased VLDL-TG oxidation are warranted to further increase lipid oxidation and promote removal of atherogenic particles.

ACKNOWLEDGMENTS
We thank Lone Kvist, Susanne Sørensen, Eva Schriver, Helle Zibrandtsen, and Astrid Bolling for excellent technical assistance.

GRANTS
This study was supported by grants from the Danish Medical Research Council and Novo Nordisk Foundation to S. Nielsen.

DISCLOSURES
The authors do not have disclosures, financial or otherwise, to declare.

AUTHOR CONTRIBUTIONS

REFERENCES


