Title

Kinetics and utilization of lipid sources during acute exercise and acipimox

Running title:

VLDL, exercise and acipimox

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ABSTRACT

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 (4h rest, 90min exercise at 50% VO2max). 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 were significantly reduced during acipimox compared with placebo, whereas “residual lipid oxidation” increased significantly; VLDL-TG oxidation (placebo: 18±3, acipimox: 11±2 kcal×h⁻¹), FFA oxidation (placebo: 14±2, acipimox: 4±0.5 kcal×h⁻¹) and “residual lipid” oxidation (placebo: 3±5, acipimox: 14±5 kcal×h⁻¹). 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.

Key words: very low-density lipoprotein, exercise, antilipolysis, lipid oxidation
INTRODUCTION

Overweight is associated with pro-atherogenic 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 isotopically(15), increases rapidly during exercise. Conversely, measurements of VLDL-TG oxidation are methodologically challenging(24) and have mostly been calculated from regional A-V concentration differences(19), which, however, 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, however 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 using contemporary tracer approaches, indirect calorimetry, and muscle biopsies.

**METHODS**

Ethics Committee approval and informed consent was obtained from all participants.

**Participants**

Eight healthy, untrained, overweight (BMI>26 kg/m²), non-smoking men who used no medication were randomly recruited through local advertisement. Before inclusion a normal blood count, chemistry panel, and electrocardiogram were documented, and a maximal oxygen capacity (VO₂max) test was performed.

**Protocol**

Participants were examined twice 4-5 weeks 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 250 mg (ACI)(38); the order was randomized and double-blinded by the Hospital Pharmacy. Weight-maintaining diets (55% carbohydrate, 15% protein, 30% fat) were calculated by a clinical dietician and provided by the hospital kitchen during the 3 days preceding the metabolic study days.
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 8.00 and included a four-hour 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 20% bolus) and [9,10-3H]palmitate. Another was placed in a contralateral dorsal hand vein and kept heated to ensure arterialized blood. 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 30,60,70,80,90,270,300,310,320, and 330 min. Breath samples were collected at 0,210,220,230,240,300,310,320,330 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 study days an 80 ml blood sample was obtained aseptically after a 12-hour fast and VLDL-TG labeling performed as previously described(11) with minor modifications. In brief, in a sterile test tube plasma was sonicated with [1-14C]triolein (PerkinElmer, Waltham, MA) for 2h at 5°C. Hereafter the solution was transferred to sterile test tubes, covered with sterile saline (1.006 g×cm-3), and ultracentrifuged (50.3 Ti rotor, Beckman Instruments, Inc., PaloAlto, CA) at 40,000 rpm for 18h 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 approximately 3 ml 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) and calculation of VLDL-TG concentration. The exact volume was recorded, scintillation fluid added, and \(^{14}\)C measured by dual-channel scintillation counting to \(<2\%\) counting error.

**Breath \(^{14}\)CO\(_2\) SA.** Breath samples were collected in breath bags (Wagner Analysen Technik) and the air passed through a hyamine solution for trapping of \(\text{CO}_2\) as described previously(34). Scintillation fluid was added and counted to \(<2\%\) counting error.

**Palmitate kinetics.** Systemic palmitate flux and oxidation were measured using isotope dilution technique with one-hour constant infusion of \([9,10-^{3}\text{H}]\)palmitate (0.3 \(\mu\text{Ci} \times \text{min}^{-1}\)) (Department of Clinical Physiology and Nuclear Medicine, Aarhus University Hospital, Denmark) in each period. Palmitate concentration and SA were measured by HPLC using \(^2\text{H}_{31}\)-palmitate as internal standard. Basal palmitate flux was calculated as \(^3\text{H}\)-palmitate infusion rate (dpm\(\times\)min\(^{-1}\)) divided by the steady-state palmitate SA (dpm\(\times\)\(\mu\text{mol}\)\(^{-1}\)). 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 was insufficient in one participant.

Plasma palmitate oxidation rate was measured using the rate of \(^3\text{H}_2\text{O}\) production versus time (T=30-90 min, T=270-330 min) as described elsewhere(15).

In brief, the slope of the \(^3\text{H}_2\text{O}\) production curve was divided by palmitate SA to calculate palmitate oxidation. The palmitate oxidation was converted to its total FFA equivalent using a palmitate fraction estimation of 29% of total FFA. Data was insufficient in one additional participant (n=6).

**Indirect calorimetry.** Energy expenditure 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 urea excretion during each period.

**Body composition.** Dual-energy x-ray absorptiometry (DEXA) scan was performed (QDR-2000, Hologic, Marlborough, MA) to determine fat free mass (FFM), fat mass, and fat percentage.

**Biopsies.** Muscle biopsies were obtained under sterile conditions and local anesthesia using a Bergströms cannula 15 cm above the lateral corner of patella. The tissue was immediately cleansed from visible fat and blood and snap frozen in liquid nitrogen. Before assaying the samples were freeze dried for 48h 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 ethanolic KOH 30% and neutralized with MgCl₂ followed by a glycerol assay (#TR0100, Sigma, St. Louis, MS, USA). One sample had markedly increased lipid content (>3-fold) and perilipin A expression compared to the other samples and therefore suspected to be contaminated with extracellular lipids. Hence, the sample was excluded from the cellular lipid analysis.

**Intramuscular glycogen.** Freeze dried muscle tissue (1-2 mg) was hydrolysed in 100 μl 1.8 M HCl for 2.5h at 100°C, and then neutralized with 30 μl 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) by homogenization in ice-cold buffer (50mM HEPES pH7.4, 150mM NaCl, 10mM Na₄P₂O₇, 30mM NaF, 1mM Na₃VO₄, 10mM EDTA, 2.5mM Benzamidin, 0.5µl/10mg muscle tissue Sigma P-8340 inhibitor cocktail). The
homogenate was gently swirled at 4°C for 15 min before being centrifuged for 20 minutes 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). Phospho-specific antibodies against pAMPKthr$^{172}$, pGSser$^{641}$, pAS160ser$^{588}$ and thr$^{642}$, pAKTthr$^{308}$, pHSLser$^{563}$ and ser$^{660}$ were from Cell Signaling, and pACCser$^{79}$ was from Millipore. Antibodies against Perilipin A, AMPK, GS, AKT, and HSL were from Cell Signaling, antibody against AS160 was from Upstate, and antibody against Glut4 was from Millipore. Due to technical difficulties we were unable to quantify ACC expression, and ACC phosphorylation on ser$^{79}$ was therefore expressed as a ratio to β-actin expression (antibody from Cell Signaling) instead.

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

The following pairs were designed using QuantPrime(1):

**ANGPTL4:** TAGTCCACTTCGCTCTCCC-GAGATGGCCCAGCCAGTT

**LPL:** GCATTACCCAGTGTCG-CCTCCATCCAGTTGATAAACC

**DGAT1:** TTCAGCACTACCGTGGCATC-ACCGGGCATTGCTCAAGATCAG

**DGAT2:** ACTGGAACACACCCAAGAAAGGTG-AGTCTCGAAAGTAGCGCCACAC
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

\[
VLDL-TG \text{ secretion rate (μmol×min}^{-1}) = \frac{F}{SA}
\]

Where F is the [1-14C]VLDL-TG tracer infusion rate, and SA is the steady state SA the last half hour of the basal and exercise periods.

\[
VLDL-TG \text{ clearance rate (ml×min}^{-1}) = \frac{VLDL-TG \text{ secretion rate}}{C_{VLDL-TG}}
\]

Where \( C_{VLDL-TG} \) is the average VLDL-TG concentration in each steady state period

\[
\text{Fractional VLDL-TG oxidation (％)} = \frac{[^{14}CO_2SA \times VCO_2]}{[\kappa \times Ar \times F]}
\]

Where \( \kappa \) is the volume of 1mol 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).

\[
\text{Total VLDL-TG oxidation (μmol×min}^{-1}) = \text{fractional VLDL-TG oxidation} \times VLDL-TG \text{ secretion rate}
\]

Statistics. Data are mean±SEM. A p-value <0.05 was considered significant. Basal comparisons were performed using Students t-test or Wilcoxon’s test. Comparison between study days of the overall effects of exercise was performed using two-way analysis of variance for repeated measurements (RM-ANOVA) with factors for treatment and period. Student-Newman-Keul was used for post-hoc testing. SigmaPlot 11.0 software was used.
RESULTS

Clinical data are shown in table 1. Participants received an acipimox dose of 2.4-2.9 mg per kg body weight. Participants were untrained with VO_{2max} at 3182±111 ml×min^{-1} (33.6±1.4 ml O_{2}×kg^{-1}×min^{-1}). VO_{2} during exercise was similar on each study day averaging ≈50% of VO_{2max}: PLA 1619±43.7 ml×min^{-1}, ACI 1609±49.3 ml×min^{-1} (p=0.75). The workloads on each study day were also similar: PLA 85±4 watts, ACI 88±3 watts (p=0.3).

Hormones and Metabolites

Plasma total TG concentration increased slightly during exercise in both groups, whereas VLDL-TG concentrations remained unchanged (figure 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 (figure 1c). Exercise increased FFA concentration significantly in both groups. GH levels were higher during ACI and increased significantly on both days during exercise (figure 1d). Insulin levels were significantly lower during ACI than PLA (figure 1e) and exercise promoted a comparable significant decrease in both groups. Basal glucose concentrations were similar on the two study days but decreased significantly during exercise in the ACI group compared with baseline and compared with PLA (figure 1f).

VLDL-TG kinetics

Basal VLDL-TG secretion and clearance rates were similar on the two study days. Exercise decreased VLDL-TG secretion rate (p<0.001, RM-ANOVA) (figure 2a); however, there was no difference in the decrease between 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]. Exercise decreased VLDL-TG clearance rate (p=0.002, RM-ANOVA) (figure 2b); however, there was no difference in the decrease between treatments.

Basal VLDL-TG oxidation rate was significantly lower during ACI than PLA (p=0.03) (figure 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 (figure 3a). Both basal and exercise palmitate fluxes were significantly reduced during ACI compared with PLA (p<0.001) (figure 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 than PLA (p=0.004). Exercise was associated with a significant increase in palmitate oxidation (RM-ANOVA, p=0.04) (figure 3c). The increase was significantly greater during PLA compared with ACI. Post-hoc testing revealed significant increases both during 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
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 PLA (table 2, p=0.05).

For comparison VLDL-TG and plasma FFA oxidation rates (μmol×min⁻¹) in figure 2c were converted to kcal×hour⁻¹ (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 IML was not different between study days and remained unchanged during exercise (p=0.33, RM-ANOVA) (figure 4a). Basal intramuscular glycogen content was not significantly different between PLA and ACI but decreased significantly during exercise (p=0.001, RM-ANOVA) (figure 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 a ≈3 fold similar significant increase in phosphorylation (p<0.001, RM-ANOVA) (figure 5a). Basal phosphorylation of ACCser79 was not significantly different between PLA and ACI, whereas exercise was associated with a significant increase in phosphorylation (p=0.049, RM-ANOVA) (figure 5b). Post-hoc testing revealed the main-effect being during PLA (p=0.04). Correspondingly, basal GS phosphorylation of ser641 was increased during ACI compared with PLA (p=0.04). Exercise was associated with a significant similar decrease in both groups (p<0.001, RM-ANOVA) (figure 5c).

Basal phosphorylation level of HSL at ser563 and ser660 was not different between PLA and ACI. Exercise was associated with a significant increase of HSLser660 (p=0.03, RM-ANOVA) but not HSLser563 [pHSLser563 (% of basal): PLA basal 100±0; PLA exercise 130±25; ACI basal 109±11; ACI exercise 133±26, p=0.35, n=7]. The increase in phosphorylated HSLser660 was not significantly different between PLA and ACI [pHSLser660 (% of basal): PLA basal 100±0; 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 up regulation of
ANGPTL4 mRNA in both groups (p=0.01, RM-ANOVA) (figure 5d). Basal LPL mRNA was not significantly different between study days. However, exercise was associated with a significant similar up-regulation in both groups (p=0.03, RM-ANOVA) (figure 5e). CD36 mRNA expression tended to decrease during ACI both during 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 ser$^{588}$ tended to be lower during ACI than PLA (p=0.09) and increased similarly and significantly during exercise (p=0.049, RM-ANOVA); [pAS160ser$^{588}$ (% of basal): PLA basal 100±0; PLA exercise 150±32; ACI basal 168±34; ACI exercise 232±62]. Basal AS160 phosphorylation on thr$^{642}$, Akt phosphorylation of thr$^{308}$, 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; the effect was, however, 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, both during ACI and PLA. However, in relative terms only “residual lipid oxidation” increased its contribution to total lipid oxidation, whereas VLDL-TG and plasma FFA decrease, except for plasma FFA oxidation, which during ACI does not alter its relative contribution. Fourth, whereas
the exercise-induced plasma FFA oxidation during ACI only reach one-third of that observed during PLA, VLDL-TG FA oxidation increased to levels comparable to 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. Acipimox increases GH secretion in obese subjects(17), and exerts effects both during 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 acipimox 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 changes in plasma insulin and FFA availability, although somewhat attenuated during ACI. The present finding of a relative lipid contribution of $\approx 40-50\%$ 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 alone be explained by differences in the suppression of VLDL-TG secretion rate, 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 ≈60 to ≈100% in the present study compared with from ≈50 to ≈80% in lean subjects (34)). For comparison the suppression of VLDL-TG turnover was 64 to 48 μmol × min⁻¹ in the present study and 39 to 31 μmol × min⁻¹ in the lean subjects (34). So both factors contribute to explain the increase in oxidation rate during exercise, with fractional oxidation being proportionally more important than suppression in VLDL-TG secretion rate. An additional finding was that during ACI resting VLDL-TG FA oxidation was lower compared to 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 to 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 counter-regulatory response to reduced FFA availability. Conversely, ANGPTL4, an inhibitor of LPL activity (22)
which is regulated by FFA in human skeletal muscle(5), increased during exercise on
both study days.

Whereas 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
HSLser$^{660}$ phosphorylation, which was slightly greater during PLA. The apparent
lower increment during ACI was, however, associated with a marked increase in basal
oxidation rate and somewhat greater basal HSLser$^{660}$ 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 $\approx$50% of lipid oxidation being accounted for by
“residual lipid oxidation”. Therefore, we believe that “residual lipid oxidation”, as
estimated in the present study, mainly represent 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 towards increased glucose oxidation during exercise was reflected by
expected increases in phosphorylation status of the AMPK regulated phosphorylation
site on AS160 ser$^{588}$(45), as well as down regulation of GS phosphorylation. The
small non-significant decrease in whole-body lipid oxidation during ACI was
accompanied by a non-significant increase in phosphorylation of ACC. Fatty acid
oxidation is stimulated by phosphorylation of ACC(2). ACC regulates the production
of malonyl-CoA, an allosterical inhibitor of carnitine palmitoyltransferase 1 (CPT-1),
which controls transport of activated FA into the mitochondria(2). The trend to an increase in ACC phosphorylation during ACI could therefore 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 VO$_2$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. The AMPK phosphorylation pattern did therefore 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 approximately 100%. We suspect that, apart from the $^{14}$CO$_2$ trapped in the tricarboxylic acid cycle and bicarbonate pools, as corrected for by the acetate recovery factor, other intracellular pre-oxidative TG pools exist(15, 18) due to the four-hour tracer infusion period preceding exercise. Hence, labeled palmitate could be trapped in e.g. skeletal muscle, heart, and kidneys and released during exercise resulting in overestimation of fractional $[^{14}C]VLDL$-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 analogue is a well-established tool in human studies of antilipolysis(31, 41, 42), the observed effects could result from acipimox itself. Moreover, acipimox acutely improves insulin mediated glucose disposal irrespective of ambient FFA concentration. However,
effects on oxidation rates may be limited as acipimox preferentially improves non-
oxidative glucose disposal. Moreover, acipimox stimulates skin blood flow(7),
however, we do not suspect this to have major impact on the tracer kinetics as
acipimox 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. The major exercise-induced
increase in lipid oxidation is, however, due to “residual lipid oxidation”, an effect that
is further exaggerated when FFA availability is suppressed by acipimox. 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.
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DISCLOSURES

The authors have no disclosures.


**FIGURE LEGENDS**

**Figure 1: plasma metabolites**

Time course levels of TG (a), VLDL-TG (b), FFA (c), GH (d), insulin (e), and glucose (f). Black circles: PLA, white circles: ACI. Punctuated vertical line marks the exercise start. *p<0.001 compared to PLA basal, †p<0.001 compared to ACI basal, ‡p<0.001 compared to PLA exercise. Error bars: SEM.

**Figure 2: VLDL-TG metabolism**

Both VLDL-TG secretion (a) and clearance (b) are decreased during exercise; however, the exercise response is ameliorated on ACI. The VLDL-TG FA oxidation (c) is increased by exercise, and basal absolute oxidation rate is decreased basal on ACI. Black bars: basal, white bars: exercise. *p<0.001 compared to PLA basal, †p<0.001 compared to ACI basal. Error bars: SEM.

**Figure 3: palmitate metabolism**

Palmitate concentration (a) was not in steady state during exercise on either PLA or ACI neither was palmitate flux (b) on PLA, but on ACI steady state was achieved. Palmitate oxidation (c) was increased by exercise, and suppressed on ACI during both periods. Scatter plots: black circles: PLA, white circles: ACI. Punctuated vertical line marks the exercise start. Histogram: Black bars: basal, white bars: exercise. *p<0.001 compared to PLA basal, †p<0.001 compared to ACI basal. Error bars: SEM.

**Figure 4: intramuscular energy stores**

IML (a) was not affected by exercise, and not different between PLA and ACI. Intramuscular glycogen was significantly decreased by exercise, and there were no differences between PLA and ACI. Black bars: basal, white bars: exercise. *p<0.001 compared to PLA basal, †p<0.001 compared to ACI basal. Error bars: SEM.
Figure 5: intracellular protein phosphorylation and mRNA expression

Protein phosphorylation of AMPK ser\textsuperscript{172} (a), ACC ser\textsuperscript{79} (b), GS ser\textsuperscript{641} (c), data are expressed as the percentage change from CON basal which was considered the point of origin. mRNA expression of ANGPTL4 (d), LPL (e), and CD36 (f) expressed as the relation between the target gene and the housekeeping gene B2-microglobulin.

Black bars: basal, white bars: exercise. * p<0.001 compared to PLA basal, † p<0.001 compared to ACI basal, punctuated line p as stated. Error bars: SEM.
Table 1: Anthropometry. Data shown as mean ± SEM or median (range).

<table>
<thead>
<tr>
<th></th>
<th>n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>30 (23-46)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.9 ± 2.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 ± 0.02</td>
</tr>
<tr>
<td>BMI (kg×m⁻²)</td>
<td>29.1 ± 0.8</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>105.5 ± 2.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>27.8 ± 1.6</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>65.7 ± 2.0</td>
</tr>
<tr>
<td>Fat percent (%)</td>
<td>29.6 ± 1.3</td>
</tr>
</tbody>
</table>

Table 2: RER, EE, substrate oxidation (ox) rates. Plasma FFA and residual lipid ox rates were based on n=6, therefore the individual lipid source oxidation values do not add up to 100% or to total lipid oxidation. Data shown as mean ± SEM, * vs. PLA basal, † vs. ACI basal, ‡ vs. PLA exercise

<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>Energy expenditure (kcal × hour⁻¹)</td>
<td>79 ± 2</td>
<td>473 ± 13 *</td>
<td>73 ± 3</td>
<td>478 ± 14 †</td>
</tr>
<tr>
<td>Total lipid ox (kcal × hour⁻¹)</td>
<td>37 ± 5</td>
<td>209 ± 31 *</td>
<td>29 ± 3</td>
<td>141 ± 23 †‡</td>
</tr>
<tr>
<td>Total lipid ox (% of energy expenditure)</td>
<td>46 ± 5</td>
<td>44 ± 6</td>
<td>40 ± 3</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>VLDL-TG ox (kcal × hour⁻¹)</td>
<td>18 ± 3</td>
<td>24 ± 4 *</td>
<td>11 ± 2 *</td>
<td>21 ± 4 †</td>
</tr>
<tr>
<td>VLDL-TG ox (% of total lipid ox)</td>
<td>50 ± 5</td>
<td>13 ± 2 *</td>
<td>38 ± 9 *</td>
<td>19 ± 6 †</td>
</tr>
<tr>
<td>Plasma FFA ox (kcal × hour⁻¹)</td>
<td>14 ± 2</td>
<td>64 ± 14 *</td>
<td>4 ± 0.5 *</td>
<td>21 ± 7 ‡</td>
</tr>
<tr>
<td>Plasma FFA ox (% of 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 × hour⁻¹)</td>
<td>3 ± 5</td>
<td>128 ± 22 *</td>
<td>14 ± 5 *</td>
<td>107 ± 31 †</td>
</tr>
<tr>
<td>Residual lipid ox (% of 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 × hour⁻¹)</td>
<td>23 ± 4</td>
<td>205 ± 29 *</td>
<td>23 ± 2</td>
<td>321 ± 26 †‡</td>
</tr>
<tr>
<td>Protein ox (kcal × hour⁻¹)</td>
<td>19 ± 2</td>
<td>10 ± 2 *</td>
<td>21 ± 1</td>
<td>16 ± 2 ‡</td>
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</tbody>
</table>

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