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Adipose tissue lipolytic inhibition enhances the glucoregulatory properties of exercise in type 2 diabetes patients

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Abstract

Aims - Exercise combined with adipose tissue lipolytic inhibition augments intramuscular lipid and glycogen use in type 2 diabetes patients. The present study investigates the impact of adipose tissue lipolytic inhibition during exercise on subsequent postprandial glycemic control in type 2 diabetes patients.

Methods - Fourteen male type 2 diabetes patients (age 65 ± 2 years, HbA_{1c} $6.7 \pm 0.1\%$ (50 ± 2 mmol/mol)) participated in a double-blind placebo-controlled randomized cross-over study in which subjects performed endurance-type exercise after being administered 250 mg of an nicotinic acid analog (acipimox; ACP) or a placebo (PLA). A control experiment was included in which no exercise was performed (CON).

Results - 60 min of endurance-type exercise (at 45% W_{peak}) did not significantly lower circulating plasma glucose and insulin excursions in PLA when compared with CON ($P=0.300$). Acipimox administration strongly reduced circulating plasma FFA concentrations during exercise ($P<0.001$). Circulating plasma glucose and insulin excursions were substantially lower during 7.5 h of recovery from exercise (*i.e.* postprandial) in ACP when compared with either CON ($P=0.041$ and $P=0.002$, respectively) or PLA ($P=0.009$ and $P=0.001$, respectively).

Conclusions - Collectively, exercise with adipose tissue lipolytic inhibition reduces postprandial blood glucose and insulin excursions and, as such, further improves glycemic control in male type 2 diabetes patients.

Keywords – exercise; metabolism; physiology; sedentary living

Study registration number - NTR4710

1. Introduction

In the treatment of type 2 diabetes, tight glycemic control is important to reduce the likelihood of developing cardiovascular disease or other diabetes-related complications (de Vegt, 1999; Meigs, Nathan, D'Agostino & Wilson, 2002). Despite the use of blood glucose-lowering medication, postprandial blood glucose excursions remain prevalent throughout the day in most type 2 diabetes patients (Bonora et al., 2006). Postprandial hyperglycemia contributes substantially to the glycation of hemoglobin (Woerle et al., 2007) and forms an early sign of metabolic abnormalities before developing type 2 diabetes (Weyer, Bogardus, Mott & Pratley, 1999). Adjuvant strategies are warranted to attenuate blood glucose excursions throughout the day and to improve glycemic control in type 2 diabetes patients (International Diabetes Federation (IDF), 2017). Along with proper dietary counseling and blood glucose-lowering medication, exercise forms another cornerstone in type 2 diabetes treatment (Colberg et al., 2010; American Diabetes Association (ADA), 2017). It has been postulated that the glucoregulatory properties of each individual exercise session are of key importance to achieving long-term glycemic control targets (van Dijk & van Loon, 2015). A single bout of exercise has been shown to enhance insulin sensitivity and improve glycemic control for up to 48 h (van Dijk et al., 2013; Perseghin et al., 1996; Mikines, Sonne, Tronier & Galbo, 1989). Despite the use of different treatment strategies, many type 2 diabetes patients do not meet glycemic targets (ADA, 2017) and as these patients mostly suffer from poor exercise tolerance (Colberg et al., 2010), we need to develop strategies that can further increase the capacity of exercise and exercise training to reduce postprandial blood glucose and insulin excursions throughout the day and improve whole-body glucose tolerance (Praet & van Loon, 2007).

Disturbances in fatty acid handling play an essential role in the development or progression of peripheral insulin resistance in type 2 diabetes (Stinkens, Goossens, Jocken & Blaak, 2015). Impairments in the buffering capacity of the adipose tissue (Stinkens et al., 2015), as well as

skeletal muscle lipid mobilization and oxidation (Goossens et al., 2016; Blaak, 2017) contribute to elevated plasma free fatty acids (FFA). Excess availability of plasma derived FFA results in ectopic lipid deposition in insulin sensitive tissues such as liver, pancreas, heart and skeletal muscle tissue (Stinkens et al., 2015). These metabolic disturbances cause a state of metabolic inflexibility (Corpeleijn, Saris & Blaak, 2009), further promoting lipid deposition in skeletal muscle tissue as opposed to their mobilization and oxidation, thereby increasing peripheral insulin resistance (Samuel & Shulman, 2016).

One of the mechanisms by which exercise is believed to improve insulin sensitivity is the mobilization and oxidation of the intramuscular lipid depots (Wojtaszewski et al., 2003), which is largely determined by circulating plasma FFA concentrations (van Loon et al., 2005a; van Loon et al., 2005b). The elevated plasma FFA concentrations in obese or type 2 diabetes patients inhibit the hydrolysis of intramuscular lipid stores (van Loon et al., 2005b). Of interest, administration of the nicotinic acid analog acipimox has previously been shown to increase intramuscular triglyceride use during exercise in type 2 diabetes patients, reduce intramuscular lipid contents and lower circulating insulin concentrations in type 2 diabetes patients (van Loon et al., 2005b). We hypothesize that adipose tissue lipolytic inhibition during endurance-type exercise will help to further improve postprandial glycemic control in type 2 diabetes patients. To test our hypothesis, we determined the impact of a single bout of moderate-intense endurance-type exercise with or without adipose tissue lipolysis inhibition (acipimox administration) on subsequent postprandial blood glucose and insulin excursions throughout the day in male type 2 diabetes patients.

2. Materials and Methods

2.1 Subjects

A total of 18 male type 2 diabetes patients on blood glucose-lowering medication were recruited in this study (Table 1). Sample size calculations were based on previous work (van Loon *et al.*, 2005b), with an effect size of 0.9, an alpha error probability of 0.017 and a power of 0.8 and taking into account potential drop-outs. Patients were included based upon the following inclusion criteria: blood glycated hemoglobin (HbA_{1c}) >6.5% (>48 mmol/mol), aged 45-75 y, BMI 27.5-35.0 kg/m², sedentary lifestyle (<2 h sports related activities per week) and Caucasian ethnicity. Exclusion criteria were: exogenous insulin therapy, self-reported coronary artery, pulmonary, renal or gastric disease, orthopedic symptoms that would interfere with exercise, or involvement in an exercise training or caloric restriction program within one year prior to the current study. Due to lack of motivation, four subjects withdrew from the study, leaving 14 male type 2 diabetes patients participating in the study (Supplemental Fig. 1). All subjects were either overweight or obese, showed a relative low exercise tolerance (VO_{2peak} $78 \pm 4\%$ of predicted norm which was based on normal age- and gender-related VO_{2peak} (Fairbairn *et al.*, 1994)) and maximal cycling power output (absolute W_{peak} 162 ± 12 W). Except for one patient on monotherapy (DPP-4 inhibitor), all patients were treated with metformin ($n=11$) and/or other treatments including lipid lowering ($n=7$), antiplatelet ($n=6$), blood pressure lowering ($n=7$) or additional glucose lowering ($n=8$) therapy. Subjects' characteristics are summarized in Table 1. This study was approved by the local medical ethical committee (Jessa Hospital and Hasselt University, Hasselt, Belgium), and the study was performed in accordance with the standards set by the latest revision (2013) of the Declaration of Helsinki. After careful explanation about the nature and risks of the experimental procedures, all subjects gave their written informed consent before participating in the study (study registration number NTR4710).

2.2 Screening and testing

After an overnight fast, subjects arrived at the laboratory at 08.00 AM and a fasting blood sample was obtained for blood HbA_{1c} concentration (Hi-Auto A1c Analyzer, Menarini Diagnostics, Florence, Italy). Subjects underwent an evaluation of body composition by a dual x-ray absorptiometry scan (DEXA, Lunar DPXL, WI, USA), followed by a maximal cardiopulmonary exercise test on a cycle ergometer (eBike Basic, General Electric GmbH, Bitz, Germany) to assess peak oxygen uptake capacity (VO_{2peak}) and workload capacity (W_{peak}), using a 1-min work stage protocol (starting workload of 40 Watt (W), incremental workload of 20 W). VO₂ measurements were performed continuously (Jaeger Oxycon, Erich Jaeger GmbH, Germany), and VO_{2peak} was compared with normal age- and gender-related VO_{2peak} (expressed as % VO_{2peak} predicted). Heart rate (HR) was monitored continuously using a 12-lead electrocardiogram. All subjects cycled until exhaustion. The test was ended when subjects were no longer able to maintain a cycling frequency of 55 rpm or higher. Peak exercise effort was confirmed when respiratory gas exchange ratio (RER) was ≥ 1.10 , in combination with dyspnea, leg and/or general fatigue.

2.3 Study design

Subjects participated in a randomized (double-blind, placebo-controlled) cross-over trial consisting of three conditions, interspersed by one week between treatments. Subjects were randomly assigned (Supplemental Fig. 1) to either a non-exercise condition (no exercise with placebo intake, CON), or to conditions in which a 60-min endurance-type exercise bout was performed exactly 60 min after oral administration of a nicotinic acid analog (acipimox, ACP) or a placebo (PLA). Subjects were subsequently followed for 7.5 h in the laboratory. Twenty-two hours after cessation of exercise subjects returned to the laboratory for an oral glucose tolerance test (OGTT) (Supplemental Fig. 2).

2.4 Study protocol

On day 1 of each intervention period, subjects arrived at the laboratory at 08.00AM following an overnight fast. An intravenous catheter was inserted in an antecubital vein for blood sampling purposes, one hour later followed by the oral administration of a placebo capsule (gelatin capsule containing starch) or a capsule of identical size and color containing 250 mg of the nicotinic acid analog acipimox (Nedios, Altana Pharma, Hoofddorp, The Netherlands). Except for flushing, no side-effects were experienced by the subjects after ACP administration. One hour after placebo or acipimox administration, subjects performed 60 min of endurance-type exercise on an electronically braked bike at 45% of their individual W_{peak} (PLA or ACP) or remained sedentary (CON). Heart rate (HR) was monitored continuously (Polar, Oy, Finland), whereby exercise elicited a HR of 112 ± 4 and 116 ± 4 $bts \cdot min^{-1}$ in PLA and ACP, respectively ($P > 0.05$). Energy expenditure during both exercise bouts was estimated (heart rate based) to be 1.4 ± 0.1 MJ (American College of Sports Medicine (ACSM), 2014).

Subjects next consumed three standardized meals during the day in the laboratory at 30 min (total energy: 2203 kJ (545 kcal); 18 g fat; 64 g carbohydrates; 25 g protein), 270 min (total energy: 2480 kJ (595 kcal); 36 g fat; 36 g carbohydrates; 27 g protein) and 510 min (total energy: 3292 kJ (788 kcal); 42 g fat; 71 g carbohydrates; 27 g protein) after cessation of exercise with *ad libitum* water consumption. Venous blood samples were collected in EDTA-containing tubes at rest and during exercise (every 30 min) and during both postprandial phases (every 30 min in the first two hours and every 60 min during the following two hours after consumption of the standardized meals) (Supplemental Fig. 2). Blood samples were immediately centrifuged at 1,000 g for 5 min at 4°C. Plasma aliquots were frozen in liquid nitrogen and stored at -80°C until analysis. On day 2, subjects returned to the laboratory at 08.00 AM following an overnight fast (≥ 10 h) for an OGTT (starting at 22 h after completion of the exercise bout), in which blood glucose and insulin concentrations were assessed every 30 min after the ingestion of 75 g glucose (dextrose monohydrate) dissolved in 250 mL water.

2.5 Blood chemistry

Plasma glucose (A11A01667, Cobas Pentra semiautomatic analyzer, Roche, Basel, Switzerland), insulin (HI-14K, Human Insulin specific RIA Kit, Millipore, Billerica, MA, USA), FFA (NEFA HR(2) R1 set 434-91795 and Nefa HR(2) R2 set 436-91995 Wako, Neuss, Germany), triglyceride (A11A01640 (Roche), ABX Pentra Triglycerides CP, HORIBA ABX), and lactate (NAD 10127990001 and LDH 10127876001, Cobas Pentra semiautomatic analyzer, Roche, Basel, Switzerland) concentrations were assessed in duplicate in each sample.

2.6 Medication, food intake and habitual physical activity

Except for the screening visit, medication intake remained unchanged during the entire study period and was taken on the morning of each experimental visit (during the first standardized meal on day 1 and prior to the OGTT on day 2). Subjects maintained their normal habitual physical activity level and diet, but refrained from exhaustive physical activity and any alcohol intake three days prior to each visit. In addition, subjects recorded their dietary and caffeine (if any) intake over three days prior to day 1 and copied their diet and caffeine intake prior to each subsequent visit.

2.7 Statistical analysis

Data are presented as means \pm s_x. Shapiro-Wilk test indicated no normal distribution of data. To compare blood parameters (interval data) between different conditions, Friedman tests (for three related samples) were performed. In case of a significant main effect, post-hoc Wilcoxon signed ranks tests (for two related samples) were performed corrected for multiple testing (Bonferroni). Total areas under the curve (tAUC) of these blood parameters was calculated by the trapezoid method before and during exercise (08.00-10.30 AM; tAUC₀₋₁₅₀) and for a total of 7.5 h after exercise (11.00 AM - 06.30 PM; tAUC₁₈₀₋₆₃₀). In addition, tAUC during OGTT

on day 2 was calculated. Statistical significance was set at $P < 0.05$ (two-tailed) for main effect and $P < 0.017$ (two-tailed) for two related samples. SPSS 22 for Windows was used to perform all calculations (IBM Corporation, Armonk, NY, USA).

3. Results

3.1 Plasma FFA and triglycerides

In the fasted state, plasma FFA concentrations increased in both CON and PLA over time ($P = 0.001$ and $P = 0.001$, respectively), with a greater increase following the onset of exercise (PLA) when compared with CON ($P = 0.022$) (Fig. 1A). Acipimox administration prevented the fasting- (CON) and exercise-induced (PLA) rise in circulating plasma FFA concentrations, resulting in lower plasma FFA concentrations when compared with both CON and PLA ($P < 0.001$) (Fig. 1A). Overall $tAUC_{0-150}$ for the plasma FFA concentrations differed significantly between treatments ($P_{\text{treatment}} < 0.001$) (Table 2). After exercise, plasma FFA concentrations temporarily remained significantly lower in ACP when compared with both CON (until consumption of the second meal) ($P = 0.004$) and PLA (until 90 min after the second meal) ($P = 0.002$) (Fig. 1A). Overall $tAUC_{180-630}$ for the total postprandial plasma FFA concentrations differed significantly between treatments ($P_{\text{treatment}} = 0.002$) (Table 2). Plasma triglyceride concentrations slightly increased over time and did not show any differences between treatments in the fasted ($P_{\text{treatment}} = 0.789$) or postprandial state ($P_{\text{treatment}} = 0.458$) (Fig. 1B; Table 2).

3.2 Plasma glucose, insulin and lactate

Plasma glucose concentrations (Fig. 2A) and $tAUC_{0-150}$ (Table 2) were not significantly different between treatments during exercise ($P_{\text{treatment}} = 0.607$ and $P_{\text{treatment}} = 0.607$, respectively).

Plasma insulin concentrations (Fig. 2B) and $tAUC_{0-150}$ (Table 2) were not significantly different between treatments during exercise ($P_{\text{treatment}}=0.751$ and $P_{\text{treatment}}=0.223$, respectively). ACP did not affect exercise-induced plasma lactate levels when compared with PLA ($P=0.683$), while plasma lactate concentrations were higher during exercise in PLA ($P=0.001$) and ACP ($P=0.001$) treatments when compared with CON (Fig. 2C).

Following exercise, postprandial plasma glucose concentrations were substantially lower in ACP when compared with PLA ($P_{\text{treatment}}=0.011$). This resulted in overall significantly different plasma glucose $tAUC_{180-630}$ between treatments, with lower plasma glucose concentrations in ACP when compared with PLA ($P=0.009$) and a strong trend for lower plasma glucose concentrations in ACP when compared to CON ($P=0.041$) (Table 2). Postprandial peak plasma glucose concentrations (measured during the entire postprandial timeframe) did not differ between treatments ($P_{\text{treatment}}=0.526$) (Table 2). Following exercise, ACP substantially reduced postprandial plasma insulin excursions when compared with CON ($P=0.002$) or PLA ($P=0.001$) ($P_{\text{treatment}}<0.001$), resulting in an overall significantly lower insulin $tAUC_{180-630}$ between treatments (Table 2). In addition, postprandial peak plasma insulin concentrations (measured during the entire postprandial timeframe) were substantially reduced in ACP compared with CON and PLA ($P_{\text{treatment}}=0.013$) (Fig. 2B; Table 2). Postprandial plasma lactate concentrations did not differ between treatments ($P_{\text{treatment}}=0.395$) (Fig. 2C).

3.3 Oral glucose tolerance test

The OGTT performed 22 h after exercise showed no differences between CON, ACP and PLA with respect to fasting plasma glucose ($P_{\text{treatment}}=0.526$) and fasting plasma insulin

concentrations ($P_{\text{treatment}}=0.751$), peak plasma glucose ($P_{\text{treatment}}=0.807$) and insulin concentrations ($P_{\text{treatment}}=0.807$) or in their corresponding tAUC_{0-120} ($P_{\text{treatment}}=0.607$ and $P_{\text{treatment}}=0.931$, respectively) between treatments (Table 2).

4. Discussion

In the present study, we show that lowering plasma FFA availability by inhibiting adipose tissue lipolysis during a single bout of endurance-type exercise attenuated the postprandial rise in circulating blood glucose and insulin concentrations in male type 2 diabetes patients, when compared with exercise performed without adipose tissue lipolytic inhibition. These glucoregulatory benefits of combining exercise with adipose tissue lipolytic inhibition persisted for at least 7.5 hours after cessation of exercise but were no longer present 24 hours after cessation of exercise.

Patients with type 2 diabetes generally suffer from exercise intolerance and muscle weakness (Colberg et al., 2010), which was also valid for the patients investigated (Table 1). The applied 60 min bout of moderate-intensity exercise performed at 45% W_{max} (which was in accordance to the most recent clinical guidelines for type 2 diabetes treatment (ADA, 2017)) represented an intense effort for these sedentary patients, despite the fact that the absolute workload was merely 73 ± 6 W. Notwithstanding the subjective effort, the exercise bout (PLA) did not significantly improve postprandial glycemic control when compared to the non-exercise control trial (CON). Regardless of these somewhat unexpected findings, the current results clearly substantiate our opinion that strategies are warranted to further augment the glucoregulatory properties of exercise and exercise training.

Previous work has shown that the pool of intramuscular lipids, which are being linked to increased peripheral insulin resistance (Samuel & Shulman, 2016) are not mobilized during exercise when circulating plasma FFA concentrations are elevated (exceeding 300-500

$\mu\text{mol}\cdot\text{L}^{-1}$), especially in persons with obesity and type 2 diabetes mellitus (van Loon, 2004). Using acipimox to specifically and partially block adipose tissue lipolysis (Tunaru et al., 2003), we previously showed in healthy athletes (van Loon et al., 2005a) as well as type 2 diabetes patients (van Loon et al., 2005b) that the exercise-induced increase in circulating plasma FFA concentrations can be prevented. These findings are confirmed in the present study, from which it can be expected that under these conditions exercise stimulates the use of intramuscular lipid as well as glycogen pools (van Loon et al., 2005a; van Loon, 2004; Watt et al., 2004), thereby increasing peripheral insulin sensitivity and, as such, improving glycemic control (Wojtaszewski et al., 2000). In the previous studies we focused on assessing the impact of acipimox administration on endogenous substrate source utilization (van Loon et al., 2005a; van Loon, 2005b). In contrast, in the present study we focus on the impact on the postprandial blood glucose and insulin excursions and, as such, overall glycemic control throughout the day. However, acipimox-induced changes in substrate oxidation could not be verified as no indirect calorimetry measurements were performed in the present study.

Exercise in combination with adipose tissue lipolytic inhibition (ACP) substantially and temporarily reduced subsequent postprandial circulatory glucose ($-11\pm 3\%$) and insulin ($-25\pm 4\%$) responses for up to 7.5 h after cessation of exercise when compared to PLA (Table 2). This indicates that adipose tissue lipolytic inhibition during low- to moderate-intense exercise can strongly increase insulin action, resulting in lower postprandial blood glucose excursions with less circulating insulin and making this type of combinational exercise as effective as exercise bouts of either higher intensity (Di Pietro, Dziura, Yeckel & Neufer, 2006) or longer duration (Dube, Allison, Rousson, Goodpaster & Amati, 2012). Still, it remains unclear whether the volume of endurance exercise affects glycemic control, or whether the different exercise characteristics modulate the impact of exercise on glycemic control (van Dijk & van Loon, 2015). The benefits of exercise with adipose tissue lipolytic inhibition on postprandial

glycemic control were evident for the remainder of the day after cessation of exercise, an important finding with respect to the importance of postprandial plasma glucose excursions in predicting secondary diabetes complications (Cavelot et al., 2011). However, at 24 h after exercise, we no longer observed greater whole-body glucose tolerance in ACP or PLA treatments, as no differences in circulatory plasma glucose or insulin responses were observed following the OGTT (Table 2). Therefore, the need for structured exercise or increased physical activity on a daily basis should be emphasized in this sedentary population.

In the present study, combining moderate-intense endurance-type exercise with adipose tissue lipolytic inhibition (ACP) was shown to improve postprandial glycemic control more effectively when compared with both the exercise only treatment (PLA) as well as the non-exercise control treatment (CON). Thus, combining exercise with adipose tissue lipolytic inhibition is postulated to be a more effective interventional strategy to augment the glucoregulatory benefits of exercise intervention in type 2 diabetes treatment. Opposed to the combination therapy, one could suggest that adipose tissue lipolytic inhibition might be used without implementing exercise. However, the study design does not allow us to evaluate the impact of acipimox without concomitant exercise. Still, the chronic use of nicotinic acid (or its analogs) without concomitant exercise is contraindicated. Previous work has shown that more prolonged use of acipimox can eventually lead to tolerance development as indicated by unaffected or even increased circulatory plasma FFA concentrations (Wang, Basinger, Neese, Christiansen & Hellerstein, 2000; Morigny, Houssier, Mouisel & Langin, 2016; Davoren et al., 1998; Vaag & Beck-Nielsen, 1992; Saloranta et al., 1993). We hypothesize that intermittent use of acipimox, applied prior to exercise, might form an appropriate therapeutic strategy to increase intramuscular lipid and glycogen utilization in skeletal muscle, thereby further enhancing peripheral insulin sensitivity and, as such, improve postprandial glycemic control in patients with type 2 diabetes.

This combinational approach seems a promising alternative to enhance the clinical benefits of exercise therapy with equal effort of the patient in the long term, although one should be cautious about the potential side-effects of this combined therapy when applied chronically. In the present study we focus on the surplus benefits of acipimox use with exercise and its effects on postprandial glucose tolerance. The combinational strategy may be applied until skeletal muscle lipid turnover is restored properly, allowing a more balanced use of intra- and extramuscular lipid pools at rest and during exercise. However, future work should be performed to explore the more prolonged benefits of combining low-intensity exercise training with adipose tissue lipolytic inhibition on glycemic control and metabolic health in various diabetes subpopulations.

In conclusion, inhibition of adipose tissue lipolysis during exercise reduces circulatory plasma FFA concentration and transiently lowers subsequent postprandial blood glucose and insulin excursions throughout the remainder of the day in male type 2 diabetes patients. These findings introduce the combined use of exercise with adipose tissue lipolytic inhibition as an effective interventional strategy to augment exercise-induced improvements in glycemic control in type 2 diabetes patients.

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Table 1 Subjects' characteristics

| Variable | |
|--|-------------|
| <i>n</i> | 14 |
| Age, y | 64 ± 1 |
| Body height, m | 1.74 ± 0.01 |
| Body weight, kg | 92.6 ± 3.3 |
| BMI, kg*(m ²) ⁻¹ | 30.2 ± 0.9 |
| Body fat, % | 31 ± 1 |
| Fat free mass, kg | 60.0 ± 2.1 |
| HbA1c, % | 6.7 ± 0.1 |
| HbA1c, mmol/mol | 50 ± 2 |
| Years since diagnosis | 10 ± 2 |
| VO ₂ peak, mL*min ⁻¹ *kg ⁻¹ (FFM) | 36.3 ± 2.0 |
| W _{max} , W*kg ⁻¹ (FFM) | 2.7 ± 0.1 |
| Maximal heart rate, beats*min ⁻¹ | 144 ± 4 |
| Maximal RER | 1.17 ± 0.01 |
| Metformin (<i>n</i>) | 11 |
| Sulfonylurea (<i>n</i>) | 2 |
| DPP-4 inhibitor (<i>n</i>) | 6 |
| GLP-1 receptor agonist (<i>n</i>) | 1 |
| Statin (<i>n</i>) | 7 |
| Antiplatelet (<i>n</i>) | 6 |
| Calcium antagonist (<i>n</i>) | 3 |
| ACE inhibitor (<i>n</i>) | 3 |
| Angiotensin-2 receptor antagonist (<i>n</i>) | 1 |
| Diuretics (<i>n</i>) | 1 |
| Beta blocker (<i>n</i>) | 3 |

Data are mean ± s_x. HbA1c, blood glycated hemoglobin; VO₂peak, whole-body peak oxygen uptake; BMI, body mass index; FFM, fat free mass; RER, respiratory exchange ratio.

Table 2 Plasma glucose, insulin, free fatty acid, triglyceride and lactate concentrations during different treatments before and after exercise

| | Treatment | | | P _{TREATMENT} |
|---|------------------------------------|--------------------|---------------------|------------------------|
| | Control (no exercise + placebo) | Exercise + placebo | Exercise + acipimox | |
| Fasting and during exercise | | | | |
| tAUC glucose (mmol/L/0-150min) | 1094 ± 56 | 1119 ± 65 | 1096 ± 61 | 0.607 |
| tAUC insulin (nmol/L/0-150min) | 11 ± 0.9 | 11 ± 1 | 10 ± 1 | 0.223 |
| tAUC lactate (mmol/L/0-150min) | 177 ± 16 | 314 ± 30 * | 305 ± 20 * | <0.001 |
| tAUC triglycerides (mmol/L/0-150min) | 210 ± 36 | 207 ± 28 | 217 ± 38 | 0.789 |
| tAUC free fatty acids (mmol/L/0-150min) | 80 ± 7 | 97 ± 9 * | 40 ± 11 *# | <0.001 |
| Postprandial during the day | | | | |
| tAUC glucose (mmol/L/180-450min) | 3794 ± 172 | 3946 ± 183 | 3500 ± 124 # | 0.011 |
| peak glucose (mmol/L) | 11.7 ± 0.6 | 12.2 ± 0.6 | 11.1 ± 0.6 | 0.526 |
| tAUC insulin (nmol/L/180-450min) | 106 ± 13 | 103 ± 13 | 76 ± 7 *# | <0.001 |
| peak insulin (pmol/L) | 414 ± 93 | 388 ± 88 | 263 ± 36 *# | 0.013 |
| tAUC lactate (mmol/L/180-450min) | 827 ± 101 | 841 ± 77 | 790 ± 73 | 0.395 |
| tAUC triglycerides (mmol/L/180-450min) | 858 ± 134 | 817 ± 94 | 800 ± 126 | 0.458 |
| tAUC free fatty acids (mmol/L/180-450min) | 149 ± 16 | 170 ± 13 | 115 ± 16 *# | 0.002 |
| During OGTT | | | | |
| tAUC glucose (mmol/L/0-120min) | 1586 ± 83 | 1577 ± 93 | 1580 ± 86 | 0.607 |
| peak glucose (mmol/L) | 15.6 ± 0.8 | 15.5 ± 0.9 | 15.7 ± 0.8 | 0.807 |
| tAUC insulin (nmol/L/0-120min) | 31 ± 2 | 30 ± 3 | 31 ± 4 | 0.931 |
| peak insulin (pmol/L) | 361 ± 41 | 347 ± 41 | 368 ± 55 | 0.807 |

Data are expressed as means ± S_x-. *Significantly different as opposed to control condition (p<0.017). #Significantly different as opposed to exercise with placebo intake (p<0.017). tAUC, total area under the curve; OGTT, oral glucose tolerance test

6. Figure legends

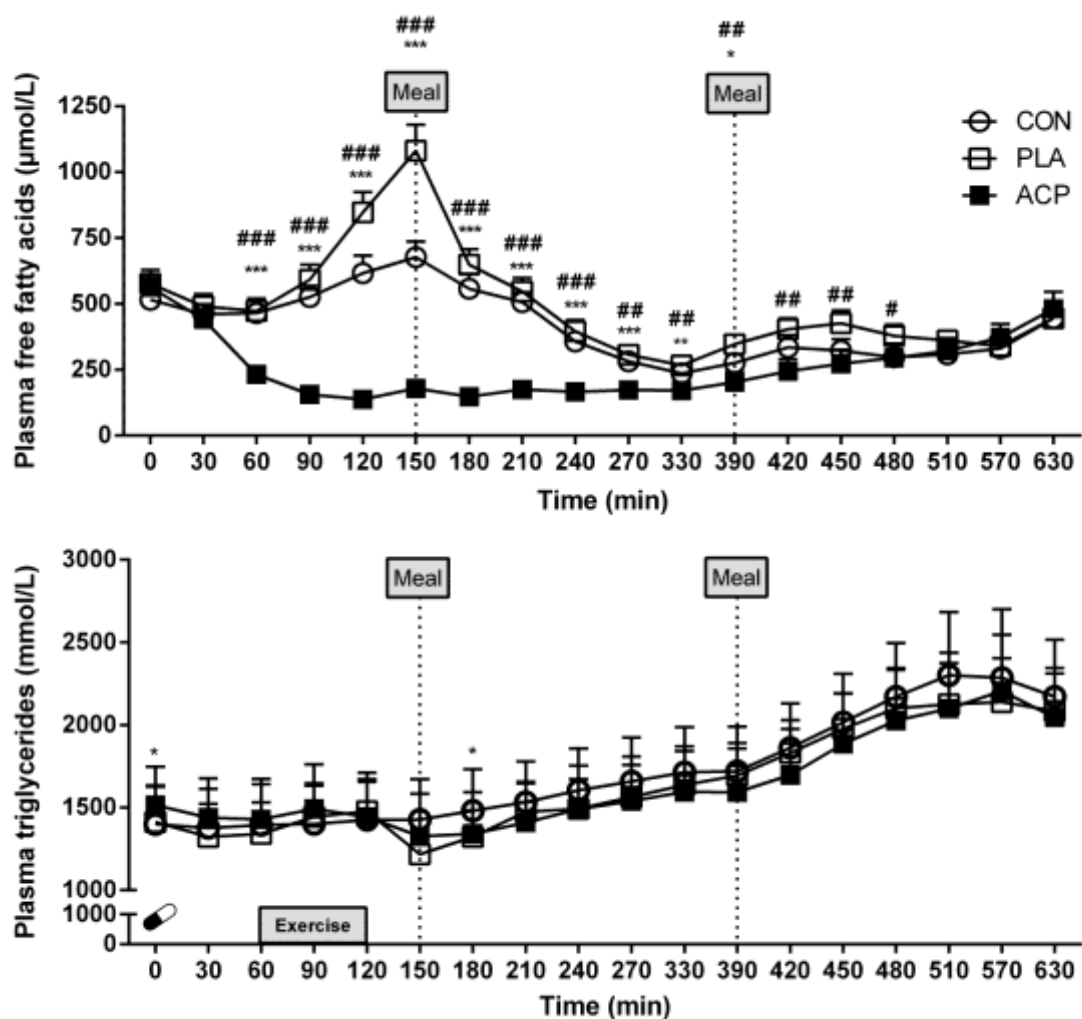


Figure 1. Plasma metabolite concentrations. Data represent means + s_x; *n* = 14. Plasma FFA (A) and plasma triglycerides (B) concentrations at rest, during endurance-type exercise (or the control condition) and in the subsequent postprandial state. * Significantly different between CON and ACP condition (* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001). # Significantly different between EX and ACP condition (# *P* < 0.05; ## *P* < 0.01; ### *P* < 0.001)

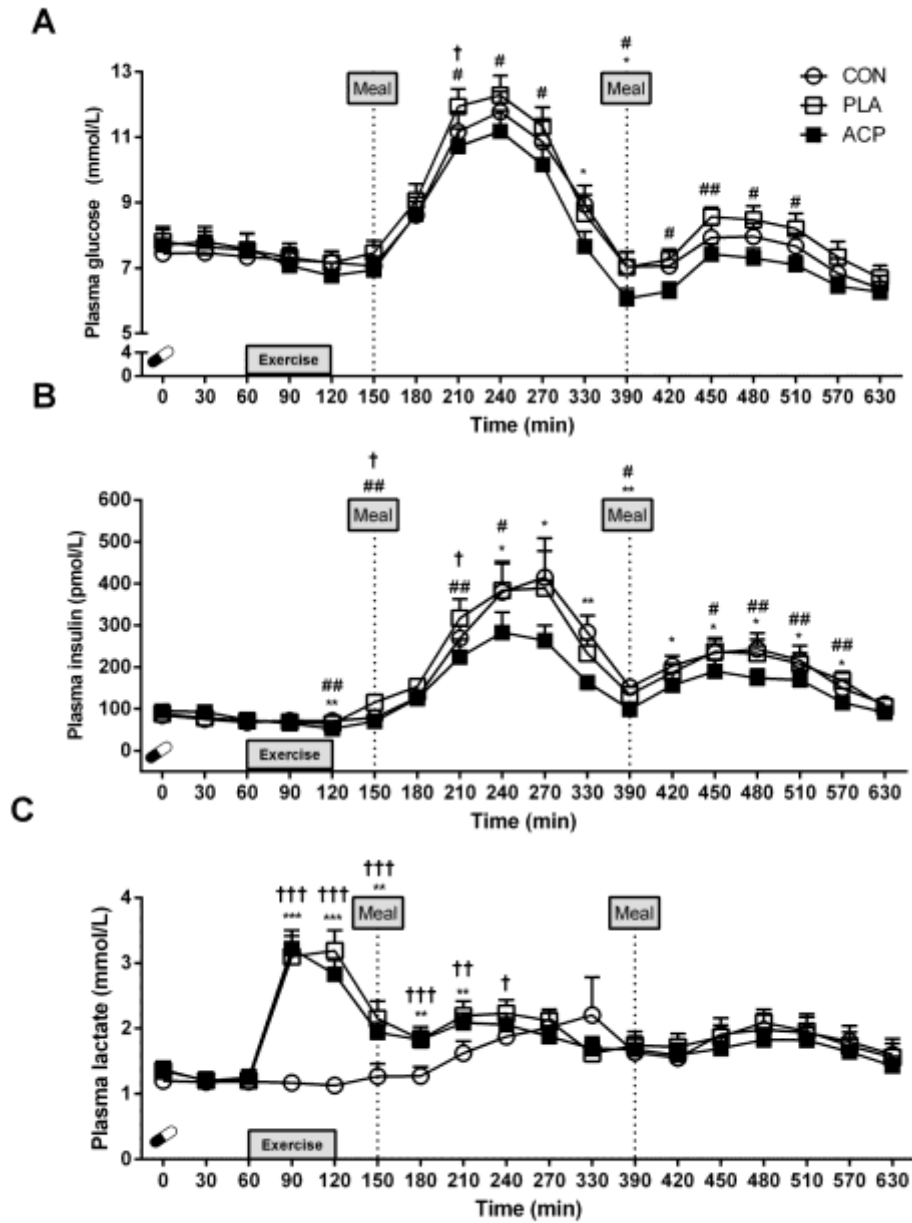


Figure 2. Plasma metabolite concentrations. Data represent means + s_x ; $n = 14$. Plasma glucose (A), plasma insulin (B) and plasma lactate (C) concentrations at rest, during endurance-type exercise (or the control condition) and in the subsequent postprandial state. * Significantly different between CON and ACP condition (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). # Significantly different between EX and ACP condition (# $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$). † Significantly different between CON and EX condition († $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$).

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Conflict of interest

The authors declare that there is no duality of interest associated with this manuscript.

Author Contributions

DH, KV, JWVD, KS and LM performed experiments. DH and KV performed statistical analysis and wrote the manuscript. AZ performed sample analyses and revised the manuscript. DH, LVL, JWVD designed the study. LM and KS performed experiments and revised the manuscript. LBV, LVL, DH, KV and JWVD contributed to data interpretation and editing of the manuscript. LVL, DH and KV are guarantors for the present work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have approved the final manuscript.