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Faculteit Geneeskunde en Levenswetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

Effects of inhibition of adipose tissue lipolysis during exercise on glycaemic control and exercise performance in patients with type 2 diabetes mellitus

Hanne Martens

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen

PROMOTOR :
Prof. dr. Dominique HANSEN

COPROMOTOR :
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Hoeverkerkweg 28, 3660 Opglabbeek, 31/05/2018 H.M.

Research context

This master thesis is a part of the second master year of Rehabilitation Sciences and Physiotherapy at Hasselt University and fits in the research domain of rehabilitation of internal diseases.

Currently, diabetes is the most common metabolic disease worldwide and is approaching epidemic proportions globally. Worldwide, approximately 425 million people (*i.e.* 8.8% of world population) suffer from diabetes, in which type 2 diabetes mellitus (T2DM) accounts for 90% of the cases. The prevalence has been steadily rising in recent decades and is expected to increase even further at an alarming rate to 642 million worldwide (*i.e.* 9.9% of world population) in 2045 (International Diabetes Federation [IDF], 2015). An additional concern is the growing incidence of T2DM in younger adults and adolescents (Reinehr, 2013).

T2DM results in numerous potentially life threatening micro- and macrovascular complications (Forbes & Cooper, 2013). In 2015, T2DM accounted for 10.7% of global all-cause mortality in 20 to 79 year olds (IDF, 2017). Given these figures, it is needless to say that diabetes negatively affects quality of life and involves large healthcare spending (Trikkalinou, Papazafiropoulou, & Melidonis, 2017). Consequently, it is clinically important to early diagnose and treat T2DM in the most efficacious manner.

Nowadays, treatment of T2DM consists of lifestyle management, *i.e.* addressing dietary habits and physical activity behavior, mostly combined with pharmacological agents (*e.g.* oral blood-glucose lowering agents or exogenous insulin injections) (Olokoba, Obateru, & Olokoba, 2012). In this master thesis, exercise as a cornerstone of T2DM treatment will be further examined, in attempt to optimize treatment strategies for patients with T2DM.

This single master thesis consists of processing data obtained from a previous study, concerning a single bout of aerobic exercise in T2DM patients with partial inhibition of adipose tissue lipolysis, led by Prof. dr. Dominique Hansen and dr. Kenneth Verboven (*i.e*. part 1). Part 2 of this master thesis is part of an ongoing study that examines the effect of partial inhibition of adipose tissue lipolysis during cardiopulmonary exercise testing in T2DM patients. Here, the master student was involved in the recruitment of participants, data acquisition and data processing.

Reference list research context

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiol Rev, 93*(1), 137-188. doi:10.1152/physrev.00045.2011

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: a review of current trends. *Oman Med J, 27*(4), 269-273. doi:10.5001/omj.2012.68

Reinehr, T. (2013). Type 2 diabetes mellitus in children and adolescents. *World J Diabetes, 4*(6), 270-281. doi:10.4239/wjd.v4.i6.270

Trikkalinou, A., Papazafiropoulou, A. K., & Melidonis, A. (2017). Type 2 diabetes and quality of life. *World J Diabetes, 8*(4), 120-129. doi:10.4239/wjd.v8.i4.120

International Diabetes Federation [Website]. Consulted in April 2018 via https://www.idf.org/

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Abstract

Background: In type 2 diabetes mellitus (T2DM), hypertriglyceridemia is associated with insulin resistance. A single bout of endurance exercise with inhibition of adipose tissue lipolysis (using acipimox) results in a more pronounced short-term improvement in glycaemic control, compared to exercise alone. However, its effect on postprandial periods remains unknown. Furthermore, the effect of acipimox on exercise performance has not been investigated previously.

Objectives: *Part 1*: To examine parameters of glycaemic control after a single bout of endurance exercise using acipimox concomitantly, with emphasis on the postprandial phase. *Part 2:* To assess the effect of acipimox on exercise performance during cardiopulmonary exercise testing (CPET), to determine whether heartrate-based (or other known methods for intensity determination) endurance exercise is suitable when using acipimox concomitantly.

Participants: *Part 1*: 14 non-insulin dependent T2DM patients. *Part 2:* 13 non-insulin dependent T2DM patients.

Measurements: *Part 1*: Plasma glucose, insulin, free fatty acids (FFAs) and oral glucose tolerance test (OGTT). *Part 2:* aerobic (VT1) and anaerobic (VT2) threshold, heart rate (HR), oxygen uptake capacity (VO_2) and workload capacity (W).

Results: *Part 1:* endurance exercise with acipimox intake is associated with decreased plasma FFAs, insulin and glucose levels up to 3 h after the second meal of the day and lower postprandial glucose excursion, compared to endurance exercise alone. Day-after OGTT response is not affected. *Part 2:* CPET with acipimox results in a tendency towards lower $\%VO_{2peak}$ at VT1, whereas $\%VO_{2peak}$ at VT2 is unaffected. Heart rate, W_{peak} and VO_{2peak} are also unaffected by acipimox intake during CPET, compared to CPET with placebo.

Conclusion: *Part 1:* endurance exercise with acipimox intake results in lower post-exercise and postprandial plasma glucose and insulin levels and lower postprandial glucose excursion. *Part 2:* $\%VO_{2peak}$, $\%HR_{peak}$ and $\%W_{peak}$ are feasible methods for endurance exercise intensity determination in T2DM patients.

Introduction

Currently, diabetes is the most common metabolic disease worldwide and its prevalence is increasing at an alarming rate. This imposes a large personal, economic, social and healthcare burden and emphasizes the relevance of this widespread noncommunicable disease (International Diabetes Federation [IDF], 2015; World Health Organisation [WHO], 2017). Both obesity (which contributes to approximately 55% of cases of T2DM) and physical inactivity, which often coincide with ageing, are strongly and independently associated with diabetes and diabetes-related comorbidities. Therefore, development and progression of T2DM is often preventable through lifestyle adjustments (Colberg, 2012; Sullivan, Morrato, Ghushchyan, Wyatt, & Hill, 2005).

T2DM is characterized by insulin resistance, declining insulin production and eventually pancreatic β-cell failure (Olokoba et al., 2012). Moreover, in individuals with obesity and T2DM, over-expansion of existing fat cells and elevated basal adipose tissue lipolysis results from excess calorie/saturated fat intake. Consequently, excess lipids are released into the blood stream and are subsequently stored in, for example, the liver and skeletal muscle tissue (McGarry, 2002; Phielix & Mensink, 2008; Sears & Perry, 2015). In addition, impaired lipid oxidation at the level of the skeletal muscle has been reported in the obese and diabetic state (Goodpaster & Sparks, 2017; Kraegen & Cooney, 2008). Consequently, accumulation of triglycerides (and resulting elevated levels FFAs) disrupts or modulates peripheral insulin signaling which might result in β-cell dysfunction (Parhofer, 2015). In addition, FFAs generate muscle lipid metabolites which impair insulin signaling directly (Delarue & Magnan, 2007). Furthermore, in T2DM patients, the skeletal muscle shows a phenotype of metabolic inflexibility; impaired capacity to upregulate muscle lipid oxidation during periods of high lipid supply (*i.e.* postprandial) results in increased ectopic fat accumulation and subsequently in peripheral insulin resistance (Galgani, Moro, & Ravussin, 2008). It is concluded that hypertriglyceridemia and metabolic inflexibility are major contributors to the development of insulin resistance and thus disturbed glucose homeostasis. Therefore, targeting adipocyte lipolysis (*e.g.* by acipimox, a nicotinic acid analog) might be a promising therapeutic strategy for treating insulin resistance in the obese and diabetic state (Morigny, Houssier, Mouisel, & Langin, 2016).

Treatment of T2DM is aimed at optimizing glycaemic control in order to prevent development and progression of micro- and macrovascular complications. Exercise therapy is an important cornerstone in T2DM management as it improves whole-body metabolic health by inducing adaptations at the level of the skeletal muscle (Laaksonen et al., 2005). Both a single bout of endurance- and resistance-type exercise acutely improve whole-body insulin sensitivity and/or oral glucose tolerance for up to 48h. Therefore, both exercise modalities are of therapeutic use in T2DM management (Praet & van Loon, 2009). Current exercise prescriptions include a combined moderate/vigorous endurance (3-4 h weekly) and strength (2-3 days per week) training program, with a program duration of at least 3 months and a frequency of 4 to 5 times a week (Hansen et al., 2018). This induces metabolic adaptations (*i.e.* improved mitochondrial function, increased mitochondrial biogenesis, increased expression of glucose transporter proteins,…), resulting in prolonged improvement in metabolic flexibility and insulin sensitivity (Praet & van Loon, 2009; Stanford & Goodyear, 2014).

Despite the demonstrated favorable effects of exercise training, scientists are continuously searching for more effective interventions to optimize glycaemic control (which is periodically measured by blood glycated hemoglobin (HbA_{1c})). What has yet to be explored is to what extent inhibition of adipose tissue lipolysis promotes the effect of exercise. For example, it has been demonstrated that a single bout of endurance exercise with temporal inhibition of adipose tissue lipolysis (using acipimox) in male T2DM patients results in a more pronounced short-term improvement in glycaemic control markers (measured acutely in the fasted state), as compared to endurance exercise alone (Akanji, Osifo, Kirk, & Hockaday, 1993; L. J. van Loon et al., 2005).

In this study, the purpose is to further examine the course of parameters of glycaemic control after a single bout of endurance exercise with temporal inhibition of adipose tissue lipolysis (using acipimox), with emphasis on the postprandial phase (which has not been explored previously), since postprandial glucose excursion is a main contributor to overall glycaemic control and thus is an important measure to address in T2DM management (Madsbad, 2016). In addition, we aim to assess the effect of acipimox on exercise performance during CPET, to determine whether heartrate-based (or other known methods for intensity determination) endurance exercise is suitable when using acipimox concomitantly.

Subjects and methods

Part 1

Subjects

A total of 18 male T2DM patients on blood-glucose lowering medication were recruited to participate in this study. Patients were included based upon the following inclusion criteria: HbA_{1C} >6.5% (>48mmol/mol), aged 45-75 years, body mass index (BMI) 27.5-35 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, orthopaedic symptoms that would interfere with exercise, or involvement in an exercise training or caloric restriction program within one year prior to the current study. This study was approved by the local medical ethical committee (Jessa Hospital and Hasselt University, Hasselt, Belgium on 11/06/2013), and was performed conform to the standards set by the latest revision (2013) of the Declaration of Helsinki. After careful explanation about the nature and risk of the experimental procedures, all subjects gave their written informed consent before participating in the study (study registration number NTR4710).

Screening and testing

Following a 2-day cessation of blood-glucose lowering medication intake, subjects arrived at the laboratory at 08.00 AM after an overnight fast. 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 20W). VO_2 measurements were performed continuously (Jaeger Oxycon, Erich Jaeger GmbH, Germany), and VO_{2peak} was compared with normal age- and gender-related VO_{2peak} (expressed through $\%VO_{2peak}$ predicted) (Fairbarn et al., 1994). HR was monitored continuously using a 12-leads electrocardiogram. All subjects cycled until exhaustion. The test was ended when subjects were no longer able to maintain a cycling frequency of 55 revolutions

per minute (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.

Study design

Subjects participated in a randomized (double-blind, placebo-controlled) cross-over trial consisting of two conditions, interspersed by one week between treatments. Subjects were randomly assigned to a condition in which a 60' endurance-type exercise bout was performed exactly 60' after oral administration of a nicotinic acid derivate (acipimox, ACP) or after placebo intake (PLA). Subjects were subsequently followed for 7.5 h in the laboratory. 22 hours after cessation of exercise, subjects returned to the laboratory for an OGTT.

Study protocol

On day 1 of each intervention period, subjects arrived at the laboratory at 08.00 AM following an overnight fast. An intravenous catheter was inserted in an antecubital vein for blood sampling purposes, followed one hour later 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 analog acipimox (Nedios, Altana Pharma, Hoofddorp, The Nederlands). One hour after placebo or acipimox administration, subjects performed 60' of endurance-type exercise on an electronically braked bike at 45% of their individual W_{peak}. An overview of the study protocol is provided in Figure 1.

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 every 30 or 60 min after consumption of the first and second standardized meal in EDTA-containing tubes and immediately centrifuged at 1000 g for 5 min at 4°C. Plasma aliquots were frozen in liquid nitrogen and stored at -80° until analysis. On day 2, subjects returned to the laboratory at 08.00 AM following an overnight fast (≥ 10h) 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.

Blood chemistry

Plasma glucose (A11A01667, Cobas Pentra semiautomatic analyser, Roche, Basel, Switzerland) and insulin (HI-14K, Human Insulin specific RIA Kit, Millipore, Billerica, MA, USA) concentrations were assessed *in duplo* in each sample.

Glucose variability was defined by standard deviation (SD) calculations: $SD=\sqrt{\frac{\sum(x_i-\bar{x})^2}{k-1}}$ (x_i=individual observation, $\bar{x}$=mean of observations, k=number of observations) (Siegelaar, Holleman, Hoekstra, & DeVries, 2010). To enable comparison between postprandial periods, total area under the curve (AUC) calculations by trapezoidal rule were used for plasma glucose and insulin (Wolff, 2017). AUC1 represents the first postprandial period (which includes time points 180 to 390min), whereas AUC2 represents the postprandial period after the second meal (which includes time points 420 to 630min).

Medication, food intake and habitual physical activity

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 three days prior to each visit. In addition, subjects recorded their dietary intake over three days prior to day 1 and copied their diet prior to the subsequent visit.

Statistical analysis

Non-parametric testing was applied, *i.e.* data ware analyzed via matched pairs, Wilcoxon signed-rank test for between group comparisons, as for comparison between postprandial periods. All data were analyzed in JMP Pro 13 (JMP, Version *13*. SAS Institute Inc., Cary, NC, 1989-2007). Significance was set at the 0.05 level of confidence.

Part 2

Subjects

A total of 16 male T2DM patients were selected to participate in this study. Inclusion criteria were: HbA_{1c} >6.5% (48 mmol/mol), aged 40-70 years, BMI 25-37.5 kg/m² and sedentary lifestyle (<2 h sports related activities per week). Exclusion criteria were: exogenous insulin therapy or self-

reported coronary artery, pulmonary, renal or gastric disease or orthopaedic symptoms that could possibly interfere with exercise. This study was approved by the local medical ethical committee (Jessa Hospital and Hasselt University, Hasselt, Belgium on 22/02/2017), and the study was performed conform to 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: 16.89/REVA16.08).

Study design

Subjects participated in a randomized (double-blind, placebo-controlled) cross-over trial consisting of two conditions, interspersed by at least one week between treatments. Subjects arrived at the lab after an overnight fast (>10h). During the first visit, a blood sample was taken for HbA_{1c} determination**.** Patients received a single capsule containing either 250 mg acipimox (ACP) (Nedios, Altana Pharma, Hoofddorp, The Netherlands) or a placebo (PLA) capsule (gelatin capsule containing starch). Subjects were instructed to cease glucose-lowering medication on test days. Subjects recorded their dietary intake the day prior to the first test day and copied their evening meal prior to the subsequent visit.

Maximal cardiopulmonary exercise test

Volume, ambient air and gas calibration was performed before every measurement. Subjects performed a maximal cardiopulmonary exercise test on a cycle ergometer (Cortex 3000, CORTEX Biophysik GmbH, Germany), starting 1 h after acipimox/placebo intake. VO_{2peak} and W_{peak} were assessed, using a 1-min work stage protocol (starting workload of 40W, incremental workload of 20W). VO_2 (ml/min) was continuously measured. RER was measured by indirect calorimetry. Time to aerobic/anaerobic threshold was set at time to RER =0.92 and RER =1.00, respectively. Heart rate was monitored continuously using a 12-lead electrocardiogram (ECG). All subjects were encouraged to cycle 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 RER ≥1.10, in combination with dyspnea, leg or general fatigue.

Blood chemistry

A venous blood sample (4ml) was collected in an EDTA tube for blood HbA1c concentration determination (Hi-Auto A1c Analyzer, Menarini Diagnostics, Florence, Italy).

Statistics

Non-parametric testing was applied, *i.e.* data ware analyzed via matched pairs, Wilcoxon signed-rank test for between group comparisons. All data were analyzed in JMP Pro 13 (JMP, Version *13*. SAS Institute Inc., Cary, NC, 1989-2007). Significance was set at the 0.05 level of confidence.

Results

Part 1

Subjects' characteristics

Due to lack of motivation, four subjects withdrew from the study, resulting in 14 subjects with T2DM. All were men aged 65 years, having a BMI of 30.2 kg/m², with a known disease duration of 10 years and HbA_{1c} of 6.7% (mean values). Subjects' characteristics are summarized in Table 1. Except for flushing (redness of the skin), no side-effects were experienced by the subjects after ACP administration.

Plasma FFA

Plasma FFA concentrations were significantly lower in ACP as compared to PLA from 1 h after administration (start of exercise) up to 8 h after administration (whole postprandial period 1 and 1.5 h after the second meal) ($p<0.05$). Plasma FFA concentrations are shown in Figure 2.

Plasma insulin

Plasma insulin concentrations were significantly lower in ACP as compared to PLA from cessation of exercise (2 h after acipimox/placebo intake) and this difference remained extant for 7.5 h (up to 3 h after second meal) ($p<0.05$). No difference in postprandial peak plasma insulin concentration was found between groups ($p=0.71$). There was also no difference in postprandial insulin excursion (*i.e.* difference between highest and lowest value) between the two treatment conditions ($p=0.67$). Plasma insulin concentrations are shown in Figure 3.

AUC1 ($p=0.003$) and AUC2 ($p=0.002$) of ACP were significantly lower as compared to AUC1 and AUC2 of PLA, respectively. The effect of acipimox on postprandial insulin levels was similar for both postprandial period ($p=0.15$).

Plasma glucose

Plasma glucose concentrations were significantly lower in ACP as compared to PLA in the period delimited by cessation of exercise and first standardized meal and again 1 h after the first meal up to 3 h after the second meal ($p<0.05$). There was no difference in postprandial peak plasma glucose concentration between groups ($p=0.24$). Plasma glucose concentrations are shown in Figure 4.

AUC1 (p=0.02) and AUC2 (p=0.003) were significantly lower in ACP as compared to resp. AUC1 and AUC2 of PLA. The effect of acipimox on AUC was not more pronounced in any postprandial period (p=0.76).

Glucose excursion (*i.e.* difference between highest and lowest value) was significantly lower in ACP, compared to PLA, during the postprandial period (p= 0.007). Postprandial glucose variability showed a tendency towards lower SD values in ACP (p=0.09).

OGTT

There was no significant difference in OGTT values (*i.e.* glucose/insulin levels) between groups the day after the training session (p>0.05). Glucose and insulin levels, obtained from OGTT, are shown in Figure 5.

Part 2

Subjects' characteristics

Three out of sixteen subjects dropped out of the study due to error in measurement, cardiac arrhythmia (shown on EGC during test) or feeling unwell before CPET was finished. Data from the remaining 13 subjects were used for analysis. All were men aged 59 years, having a BMI of 30.9 kg/m², with a known disease duration of 8 years and HbA_{1c} of 7.1% (mean values). Subjects' characteristics are summarized in Table 2. Except for flushing, no side-effects were experienced by the subjects after ACP administration.

RER_{peak}

There was no difference in RER_{peak} (p=0.37) between conditions. In all, except for 1 CPET in PLA (RER_{peak}=1.04), RER≥1.10 was reached to confirm maximal effort.

Ventilatory thresholds, W_{peak}, VO_{2peak}

VT2 was reached at similar $\%VO_{2peak}$ between conditions (p=0.41). However, ACP showed a tendency towards lower $\%VO_{2peak}$ at VT1 (mean difference of 4.4%, p=0.06). $\%VO_{2peak}$ at aerobic/anaerobic threshold is shown in Figure 6.

VT1 was reached earlier in ACP, as compared to PLA (mean difference of 36sec, p=0.03). There was no significant difference in time to VT2 between conditions (p=0.11). Time to ventilatory thresholds is shown in Figure 7.

W_{peak} (p=0.28) and VO_{2peak} (p=0.19) did not differ between conditions.

Heart rate

There was no significant difference in heart rate when aerobic (p=0.38) or anaerobic (p=0.55) threshold was reached between groups. HR_{peak} (p=0.67) and HR after 2' recovery (p=0.41) were also similar in both groups. Heart rates during CPET are shown in Figure 8.

Discussion

In the present thesis, administration of acipimox combined with aerobic exercise resulted in a decrease in plasma FFAs, insulin and glucose levels up to 3 h after the second meal of the day, lower postprandial glucose excursion and a tendency towards lower glucose variability. The effect of the intervention was not more pronounced in either postprandial period. Peak plasma insulin and glucose did not differ with acipimox administration. Furthermore, no difference in day-after OGTT insulin and glucose levels was found. CPET with acipimox intake resulted in faster aerobic threshold attainment, whilst anaerobic threshold was reached at similar points in time, compared to CPET with placebo. Also, a tendency towards lower $\%VO_{2peak}$ at VT1 was found with acipimox, whereas $\%VO_{2peak}$ at VT2 remained unaffected. No difference in heart rate at VT1, VT2 or after 2' recovery was found. HR_{peak}, W_{peak} and VO_{2peak} were also unaffected by acipimox intake during CPET, compared to CPET with placebo.

The fact that plasma FFAs were lower in ACP is a direct consequence of acipimox administration, as it is known for its antilipolytic effect by inhibition of hormone-sensitive lipase in adipocytes and thus lowers FFA availability (Claus et al., 2005). Consequently, intramuscular triacylglycerol oxidation rates and endogenous carbohydrate use can be elevated in order to compensate (L. J. van Loon et al., 2005).

This shift in energy substrate possibly contributes to lower plasma insulin and glucose levels as observed in ACP. Administration of acipimox, combined with aerobic exercise, in male T2DM subjects has been previously investigated by L. J. C. van Loon et al. (2005) and Akanji et al. (1993). Our results are comparable to the findings of these studies; a single bout of endurance exercise, combined with acipimox administration, improves markers of glycaemic control (*i.e.* plasma insulin and glucose). In addition, we concluded that obtained effects remained extant up to 7.5 h after cessation of exercise (*i.e.* 3 h after 2nd meal). We hypothesized that the effect would be more pronounced in the first postprandial period, compared to the second, as the effect of acipimox would gradually decrease. This was not the case, potentially due to the fact that the provided meals were not similar in composition; the first meal contained more carbohydrates and the second more fat. Further examination to clarify this finding is suggested.

Peak insulin and glucose levels did not differ between groups. However, it should be noted that many of the cellular processes that occur with hyperglycemic spikes also occur with hypoglycemia. Therefore, interpretation of (postprandial) glucose excursion and variability measures (expressed through SD), rather than peak levels alone, might be more clinically relevant (Hirsch, 2015). Despite the fact that the difference in postprandial SD between groups was non-significant, the tendency towards lower SD values in ACP might be of interest, especially when combined with the observation that the differences between postprandial peak and nadir values (*i.e.* glucose excursion) did demonstrate a significant difference between groups in favour of ACP. This interest results from the fact that glycaemic variability, independent of HbA_{1c} levels, has been associated with the development and progression of micro- and macrovascular complications in T2DM patients (Nalysnyk, Hernandez-Medina, & Krishnarajah, 2010).

We think it is plausible to suggest that an exercise program combined with acipimox results in an indisputable long-term improvement in glucose variability. We suggest acipimox administration only on training days, because flushing might be disadvantageous in regard to compliance, as it can be an unpleasant (yet harmless) side effect. However, chronic administration of acipimox has not previously been investigated. We conclude that administration of acipimox might be clinically relevant in regard to T2DM management, provided no allergic reaction to acipimox occurs.

Current exercise prescriptions define a moderate to vigorous aerobic training intensity, which approximately corresponds to VT2 (Colberg et al., 2016). Since time to VT2, $\%VO_{2peak}$ at VT2 and VO_{2peak} were unaffected by acipimox, training intensity in T2DM patients can be determined based on $\%VO_{2peak}$. Other methods for determination of training intensity, such as $\%W_{peak}$ or $\%HR_{peak}$, also seem to be feasible when using acipimox, since no difference in W_{peak} and HR_{peak} was found during CPET with/without acipimox (Hansen, Stevens, Eijnde, & Dendale, 2012). A limitation of the study is that resting heart rate was not measured. Consequently, the applicability of the Karvonen method (*i.e.* % heart rate reserve) in T2DM patients when using acipimox cannot be examined (Hansen et al., 2012).

With acipimox, lipid availability is lowered, which in turn might affect substrate selection and/or metabolic flexibility (L. J. C. van Loon et al., 2005). Hereby, T2DM patients might shift to

carbohydrate as energy substrate earlier during exercise, which might cause earlier VT1 attainment and a tendency towards lower $\%VO_{2peak}$ at VT1. The effect of acipimox on heart function has previously been investigated by Wolf et al. (2016). They concluded that inhibition of adipose tissue lipolysis is associated with a rapid depletion of myocardial lipid stores and reduced systolic heart function (*i.e.* decreased ejection fraction). Consequently, adipose tissue lipolysis is required to maintain systolic heart function. We conclude that future research is required to clarify the effect of acipimox on heart function.

A limitation of the present thesis concerns glucose variability measurement. There is no gold standard available and when using a time interval of 30 min to 1 h in measuring blood glucose levels, as performed in this study, certain peaks and nadirs will be overlooked, simply because they occur between two measurements. This lowers the accuracy of the glucose variability measure (expressed through SD) (note: this also applies to the glucose excursion measurement). Therefore, calculating SD from continuous glucose measurement data seems preferable, yet from a practical point of view difficult to achieve (Siegelaar et al., 2010). In regard to generalization of obtained results, it should be noted that no women were included to participate. Furthermore, medication that might alter heart rate was an exclusion criteria, although many T2DM patients use such medication (*e.g.* β-blockers), because of the association between T2DM and cardiopulmonary diseases (Bertoluci & Rocha, 2017). A limitation of both studies included in this thesis, is the fact that a limited sample size was used. Therefore, the studies have limited power and results should be interpreted with caution. Further research to this subject with a larger sample size is suggested.

In conclusion, administration of acipimox in an overnight fasted state effectively suppresses adipose tissue lipolysis (shown by lowered plasma FFAs) during exercise, subsequent recovery and postprandial phase. This is associated with lower post-exercise and postprandial plasma glucose and insulin levels. Furthermore, lower postprandial glucose excursion and a tendency towards lower glucose variability is reported. CPET with acipimox intake in the fasted state results in earlier aerobic threshold attainment, whilst time to anaerobic threshold is unaffected. Also, a tendency towards lower $\%VO_{2peak}$ at VT1 is reported with acipimox, whereas $\%VO_{2peak}$ at VT2 is not affected by acipimox. Furthermore, VO_{2peak}, W_{peak}, heart rate at aerobic/anaerobic threshold,

HR_{peak} and HR after 2’ recovery are unaffected by acipimox. Consequently, $\%VO_{2peak}$, $\%HR_{peak}$ and $\%W_{peak}$ are feasible methods for endurance exercise intensity determination in T2DM patients.

Reference list

Akanji, A. O., Osifo, E., Kirk, M., & Hockaday, T. D. R. (1993). THE EFFECTS OF CHANGES IN PLASMA NONESTERIFIED FATTY-ACID LEVELS ON OXIDATIVE-METABOLISM DURING MODERATE EXERCISE IN PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES-MELLITUS. *Metabolism-Clinical and Experimental, 42*(4), 426-434. doi:10.1016/0026-0495(93)90098-9

Bertoluci, M. C., & Rocha, V. Z. (2017). Cardiovascular risk assessment in patients with diabetes. *Diabetol Metab Syndr, 9*, 25. doi:10.1186/s13098-017-0225-1

Claus, T. H., Lowe, D. B., Liang, Y., Salhanick, A. I., Lubeski, C. K., Yang, L., . . . Clairmont, K. B. (2005). Specific inhibition of hormone-sensitive lipase improves lipid profile while reducing plasma glucose. *J Pharmacol Exp Ther, 315*(3), 1396-1402. doi:10.1124/jpet.105.086926

Colberg, S. R. (2012). Physical activity: the forgotten tool for type 2 diabetes management. *Front Endocrinol (Lausanne), 3*, 70. doi:10.3389/fendo.2012.00070

Colberg, S. R., Sigal, R. J., Yardley, J. E., Riddell, M. C., Dunstan, D. W., Dempsey, P. C., . . . Tate, D. F. (2016). Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association. *Diabetes Care, 39*(11), 2065-2079. doi:10.2337/dc16-1728

Delarue, J., & Magnan, C. (2007). Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care, 10*(2), 142-148. doi:10.1097/MCO.0b013e328042ba90

Fairbarn, M. S., Blackie, S. P., McElvaney, N. G., Wiggs, B. R., Pare, P. D., & Pardy, R. L. (1994). Prediction of heart rate and oxygen uptake during incremental and maximal exercise in healthy adults. *Chest, 105*(5), 1365-1369.

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiol Rev, 93*(1), 137-188. doi:10.1152/physrev.00045.2011

Galgani, J. E., Moro, C., & Ravussin, E. (2008). Metabolic flexibility and insulin resistance. *Am J Physiol Endocrinol Metab, 295*(5), E1009-1017. doi:10.1152/ajpendo.90558.2008

Goodpaster, B. H., & Sparks, L. M. (2017). Metabolic Flexibility in Health and Disease. *Cell Metab, 25*(5), 1027-1036. doi:10.1016/j.cmet.2017.04.015

Hansen, D., Niebauer, J., Cornelissen, V., Barna, O., Neunhauserer, D., Stettler, C., . . . Dendale, P. (2018). Exercise Prescription in Patients with Different Combinations of Cardiovascular Disease Risk Factors: A Consensus Statement from the EXPERT Working Group. *Sports Med*. doi:10.1007/s40279-018-0930-4

Hansen, D., Stevens, A., Eijnde, B. O., & Dendale, P. (2012). Endurance exercise intensity determination in the rehabilitation of coronary artery disease patients: a critical re-appraisal of current evidence. *Sports Med, 42*(1), 11-30. doi:10.2165/11595460-000000000-00000

Hirsch, I. B. (2015). Glycemic Variability and Diabetes Complications: Does It Matter? Of Course It Does! *Diabetes Care, 38*(8), 1610-1614. doi:10.2337/dc14-2898

Kraegen, E. W., & Cooney, G. J. (2008). Free fatty acids and skeletal muscle insulin resistance. *Curr Opin Lipidol, 19*(3), 235-241. doi:10.1097/01.mol.0000319118.44995.9a

Laaksonen, D. E., Lindstrom, J., Lakka, T. A., Eriksson, J. G., Niskanen, L., Wikstrom, K., . . . Uusitupa, M. (2005). Physical activity in the prevention of type 2 diabetes: the Finnish diabetes prevention study. *Diabetes, 54*(1), 158-165.

Madsbad, S. (2016). Impact of postprandial glucose control on diabetes-related complications: How is the evidence evolving? *J Diabetes Complications, 30*(2), 374-385. doi:10.1016/j.jdiacomp.2015.09.019

McGarry, J. D. (2002). Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes, 51*(1), 7-18.

Morigny, P., Houssier, M., Mouisel, E., & Langin, D. (2016). Adipocyte lipolysis and insulin resistance. *Biochimie, 125*, 259-266. doi:10.1016/j.biochi.2015.10.024

Nalysnyk, L., Hernandez-Medina, M., & Krishnarajah, G. (2010). Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. *Diabetes Obes Metab, 12*(4), 288-298. doi:10.1111/j.1463-1326.2009.01160.x

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: a review of current trends. *Oman Med J, 27*(4), 269-273. doi:10.5001/omj.2012.68

Parhofer, K. G. (2015). Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia. *Diabetes Metab J, 39*(5), 353-362. doi:10.4093/dmj.2015.39.5.353

Phielix, E., & Mensink, M. (2008). Type 2 diabetes mellitus and skeletal muscle metabolic function. *Physiol Behav, 94*(2), 252-258. doi:10.1016/j.physbeh.2008.01.020

Praet, S. F., & van Loon, L. J. (2009). Exercise therapy in type 2 diabetes. *Acta Diabetol, 46*(4), 263-278. doi:10.1007/s00592-009-0129-0

Reinehr, T. (2013). Type 2 diabetes mellitus in children and adolescents. *World J Diabetes, 4*(6), 270-281. doi:10.4239/wjd.v4.i6.270

Sears, B., & Perry, M. (2015). The role of fatty acids in insulin resistance. *Lipids Health Dis, 14*, 121. doi:10.1186/s12944-015-0123-1

Siegelaar, S. E., Holleman, F., Hoekstra, J. B., & DeVries, J. H. (2010). Glucose variability; does it matter? *Endocr Rev, 31*(2), 171-182. doi:10.1210/er.2009-0021

Stanford, K. I., & Goodyear, L. J. (2014). Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle. *Adv Physiol Educ, 38*(4), 308-314. doi:10.1152/advan.00080.2014

Sullivan, P. W., Morrato, E. H., Ghushchyan, V., Wyatt, H. R., & Hill, J. O. (2005). Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. *Diabetes Care, 28*(7), 1599-1603.

Trikkalinou, A., Papazafiropoulou, A. K., & Melidonis, A. (2017). Type 2 diabetes and quality of life. *World J Diabetes, 8*(4), 120-129. doi:10.4239/wjd.v8.i4.120

van Loon, L. J., Manders, R. J., Koopman, R., Kaastra, B., Stegen, J. H., Gijsen, A. P., . . . Keizer, H. A. (2005). Inhibition of adipose tissue lipolysis increases intramuscular lipid use in type 2 diabetic patients. *Diabetologia, 48*(10), 2097-2107. doi:10.1007/s00125-005-1889-x

van Loon, L. J. C., Thomason-Hughes, M., Constantin-Teodosiu, D., Koopman, R., Greenhaff, P. L., Hardie, D. G., . . . Wagenmakers, A. J. M. (2005). Inhibition of adipose tissue lipolysis increases intramuscular lipid and glycogen use in vivo in humans. *American Journal of Physiology-Endocrinology and Metabolism, 289*(3), E482-E493. doi:10.1152/ajpendo.00092.2005

Wolf, P., Winhofer, Y., Krssak, M., Smajis, S., Harreiter, J., Kosi-Trebotic, L., . . . Krebs, M. (2016). Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. *Nutr Metab Cardiovasc Dis, 26*(5), 387-392. doi:10.1016/j.numecd.2016.03.012

Wolff, K. (2017). *Basic Pharmacokinetics of Substance Misuse*.

International Diabetes Federation [Website]. Consulted in April 2018 via https://www.idf.org/

World Health Organisation [Website]. Consulted in April 2018 via http://www.who.int/en/

Appendices

Table 1: Subjects' characteristics part 1 (*n*=14)

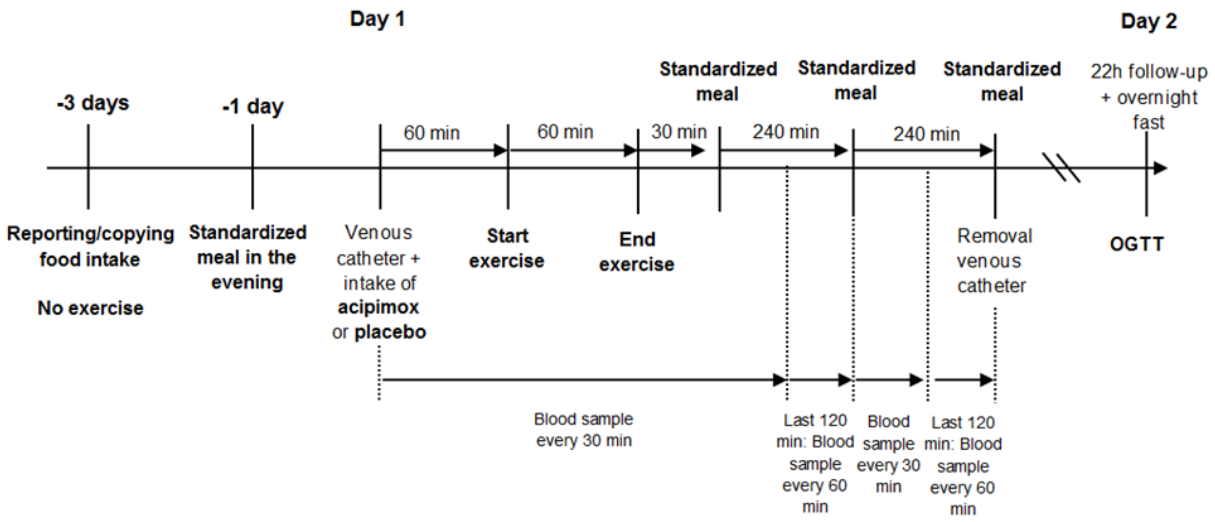
characteristic	mean	Range
Age (years)	65	52-75
Height (cm)	175	166-186
Weight (kg)	92.7	69.6-116.0
BMI (kg/m²)	30.2	28.2-35.3
Fat mass (kg)	29.7	16.2-45.4
Ltm (kg)	60.0	48.5-71.9
Fat%	31.7	23.0-38.7
HbA_{1c} (%)	6.7	6.1-7.8
VO_2/kg (ml/kg/min)	36.3	18.9-52.3
$\%VO_{2pred}$	78.9	46.3-114.2
HR_{peak} (bpm)	144	102-171
W_{peak} (Watt)	162	90-240
W/kg	2.7	1.4-3.9
Disease duration (years)	10	3-25
W during experiment (Watt)	73	40-108
Total VO_2 cycling (ml/kg)	729	477-995
CE cycling (kcal)	337	236-455

BMI: body mass index, Ltm: lean tissue mass, HbA_{1c}: blood glycated hemoglobin, VO_2: oxygen uptake, HR: heart rate, W: workload, CE: caloric expenditure

Table 2: Subjects' characteristics part 2 (*n*=16)

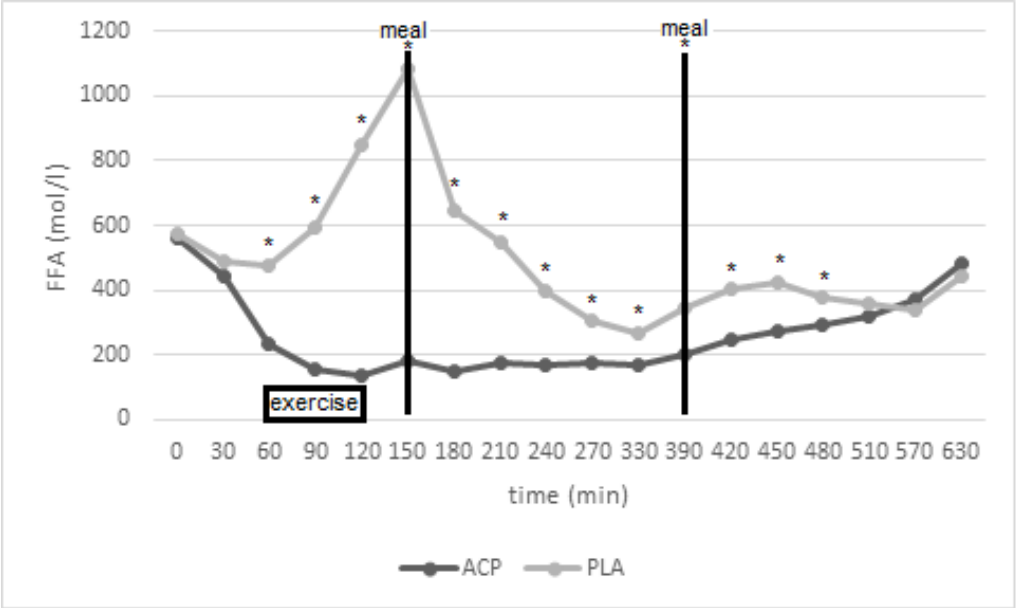
Characteristic	Mean	Range
Age (years)	59	47-74
Weight (kg)	95.5	67-119
Height (cm)	175	165-187
BMI (kg/m²)	30.9	23.3-36.0
Disease duration (years)	8	2-17
HbA_{1c} (mmol/mol)	54.8	41-78
HbA_{1c} (%)	7.2	5.9-9.3

BMI: body mass index, HbA_{1c}: blood glycated hemoglobin



OGTT: oral glucose tolerance test

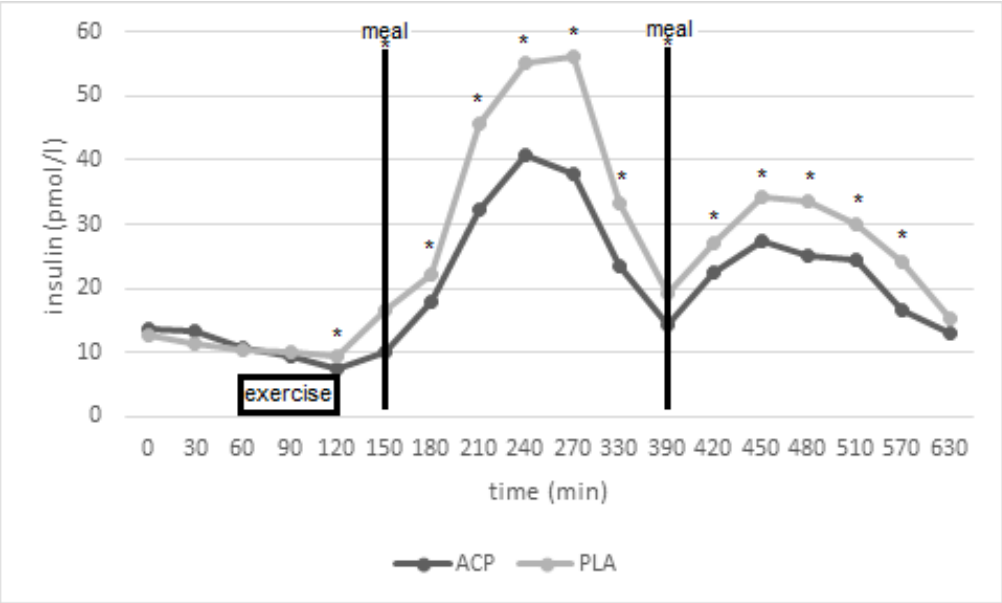
Figure 1: Study protocol part 1



FFA: free fatty acid

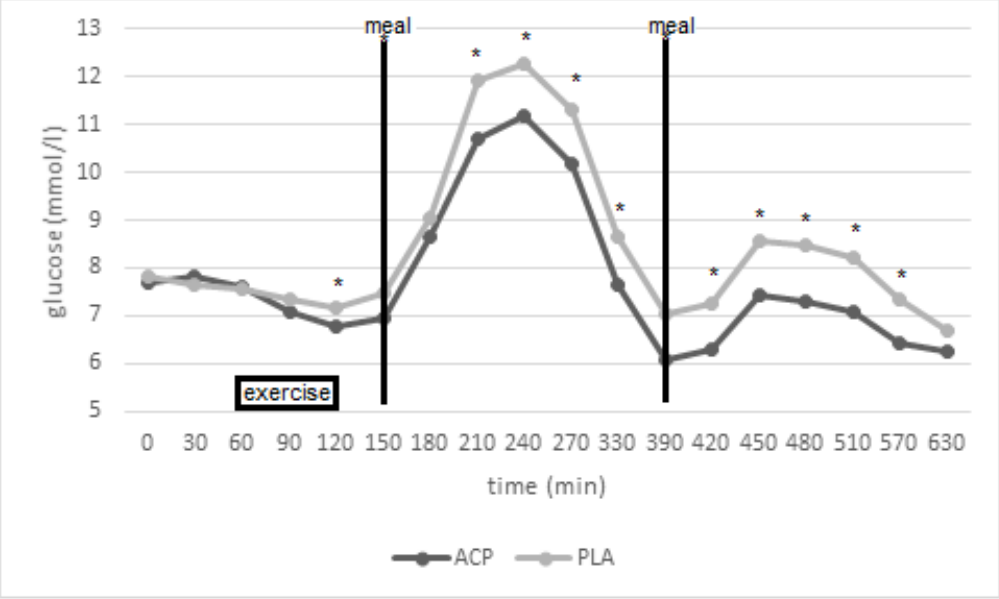
*p<0.05 for difference between conditions

Figure 2: Plasma free fatty acid levels



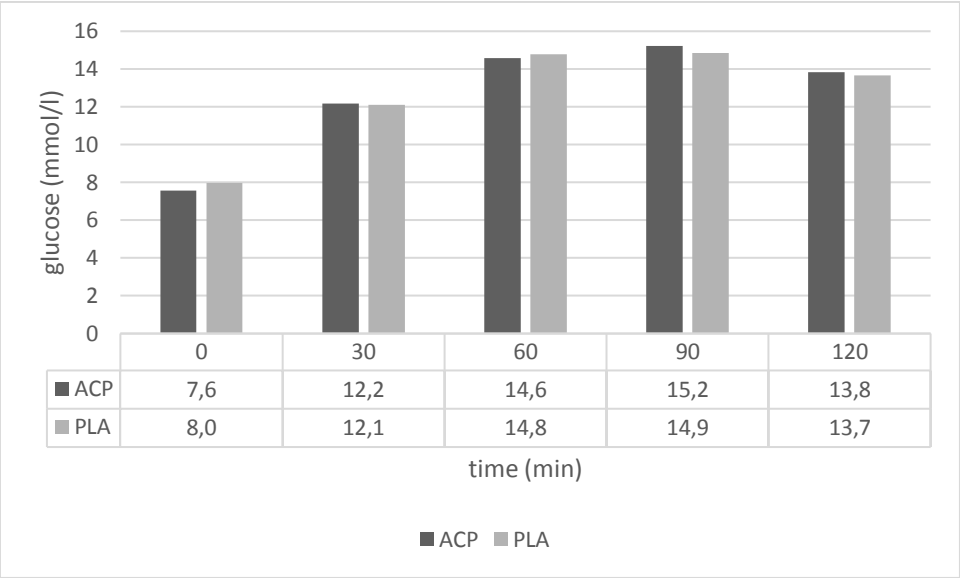
*p<0.05 for difference between conditions

Figure 3: Plasma insulin levels



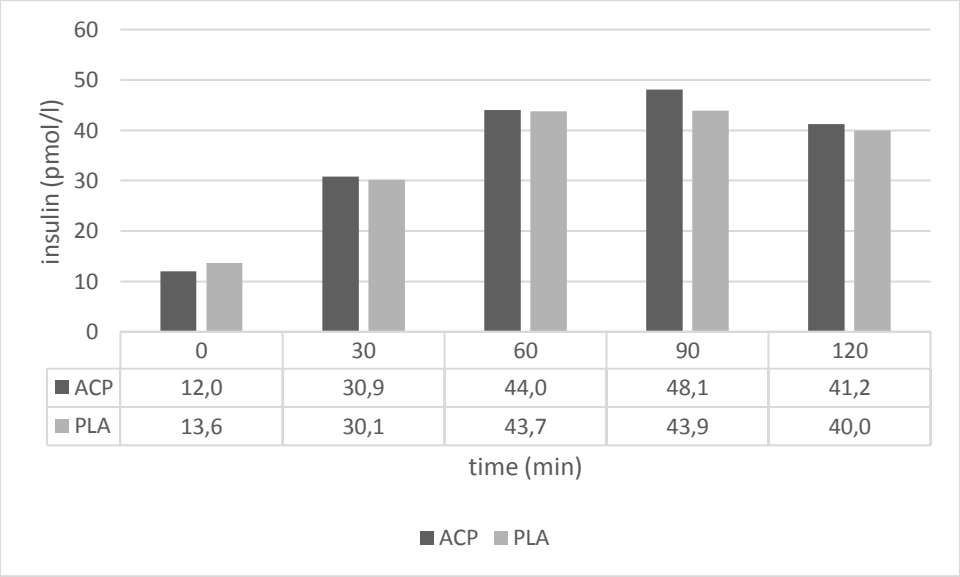
*p<0.05 for difference between conditions

Figure 4: Plasma glucose levels



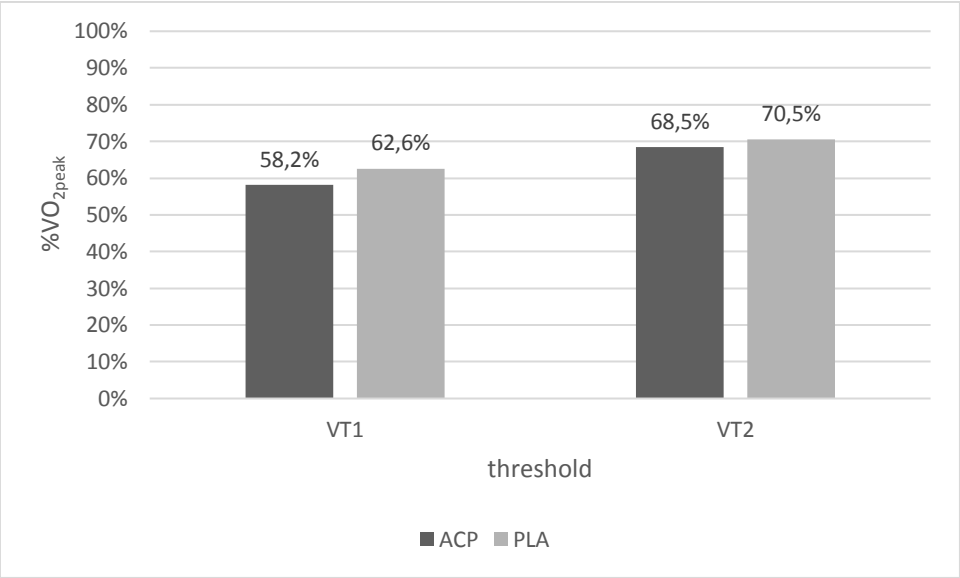
Expressed through mean values

Figure 5.1: Oral glucose tolerance test: glucose levels



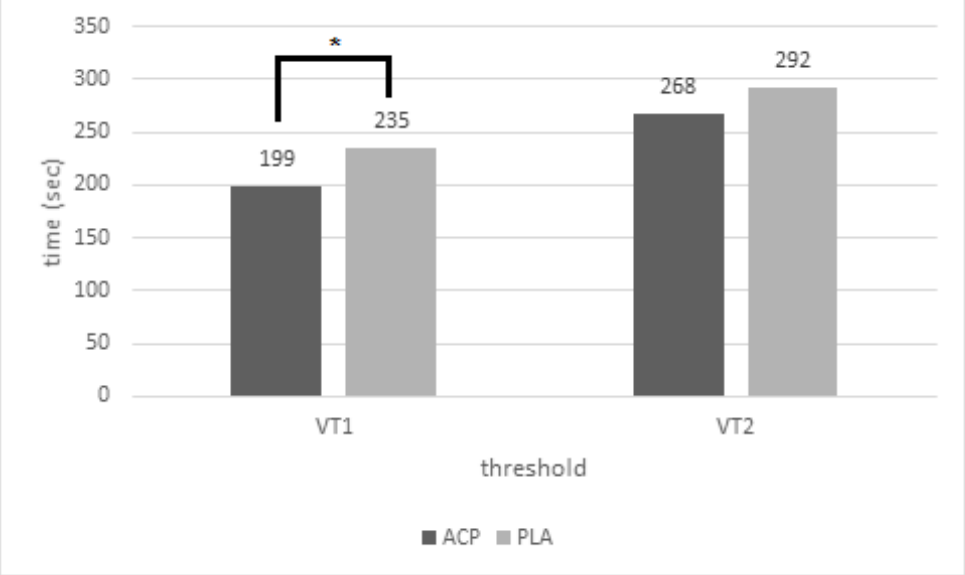
Expressed through mean values

Figure 5.2: Oral glucose tolerance test: insulin levels



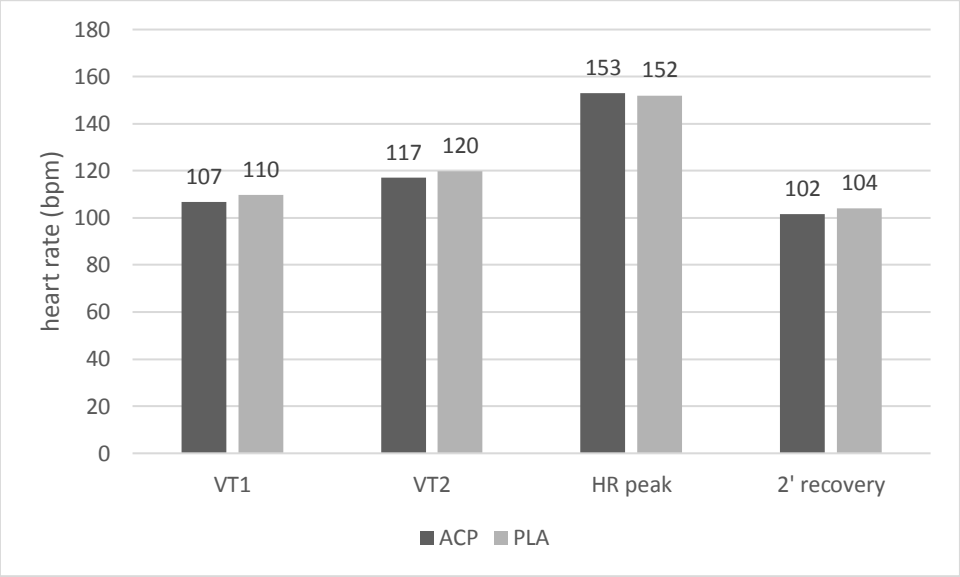
VT1: aerobic threshold, VT2= anaerobic threshold, VO_{2peak}: maximal oxygen uptake; expressed through mean values

Figure 6: $\%VO_{2peak}$ at ventilatory thresholds



VT1: aerobic threshold, VT2= anaerobic threshold; expressed through mean values
*p<0.05 for difference between conditions

Figure 7: Time to ventilatory thresholds



VT1: aerobic threshold, VT2: anaerobic threshold, HR_{peak}: maximal heart rate; expressed through mean values

Figure 8: Heart rate during cardiopulmonary exercise test

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KNOWLEDGE IN ACTION

VOORTGANGSFORMULIER WETENSCHAPPELIJKE STAGE DEEL 2

DATUM	INHOUD OVERLEG	HANDTEKENINGEN
10/10/2017	Mail contact i.v.m. opstarten MP2. De student kan mee helpen in het recruteren van deelnemers.	**Promotor:** Hansen D. Copromotor: Student(e): Martens Hanne Student(e):
2/02/2018	Hoe verder recruteren deelnemers? Wanneer zal studie (*deel 2*) aanvangen?	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
14/02/2018	Mail contact met dhr. Verboven: data *deel 1* van MP2 werden ontvangen.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
14/03/2018	Overlopen data *deel 1* en bespreken statistische verwerking.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
17/04/2018	Nagaan statistiek *deel 1* + overlopen context en introduction.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
3/05/2018	Laatste metingen *deel 2* worden uitgevoerd. Nodige data worden geëxtraheerd uit computerprogramma.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
15/05/2018	Overlopen data *deel 2* en bespreken statistische verwerking.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
16/05/2018	Mail contact met dhr. Verboven. Herwerkte versie van context, introduction, methode en eerste versie van resultaten en discussie worden doorgestuurd.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
21/05/2018	Mail contact met dhr. Hansen: volledig uitgeschreven thesis wordt doorgestuurd.	Promotor: **Copromotor:** Verboven K. Student(e): Student(e):
24/05/2018	Overlopen volledige thesis: laatste bemerkingen worden gemaakt. Invullen formulier ter indienen van de thesis in eerste zittijd.	**Promotor:** Hansen D. Copromotor: Student(e): Student(e):

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Ik/wij verlenen het wereldwijde auteursrecht voor de ingediende eindverhandeling:
Effects of inhibition of adipose tissue lipolysis during exercise on glycaemic control and exercise performance in patients with type 2 diabetes mellitus

Richting: **master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen**
Jaar: **2018**

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