2013•2014
FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

Is lipolysis inhibition during exercise instrumental to augmentation in glycemic control in type 2 diabetes: a pilot study

Promotor :
Prof. dr. Dominique HANSEN

Niels Hermans , Anton Walbers
Proefschrift ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

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ACKNOWLEDGEMENTS

We want to thank Prof. Dominique Hansen for his support, guidance and dedication, both during the experimental visits and afterwards during the processing and analysing of results. We also want to thank Dra. An Stevens and Drs. Kenneth Verboven for their support during the experimental visits.

RESEARCH CONTEXT

This pilot study belongs to a two-part master thesis spread over two years. The first part was a research study, which was performed last year. This second part is a cross-over allocation, double blinded controlled trial (RCT) performed at REVAL-Rehabilitation research centre of Hasselt University. This research belongs to a complete new area in the rehabilitation of type 2 diabetes mellitus (T2DM).

In the past years, Prof. Dominique Hansen has been part of pioneering research dedicated to exercise therapy in obesity, type 2 diabetes mellitus (T2DM) and coronary artery disease. In a recent study by Van Loon et al. (2005), after Acipimox (lipolysis inhibitor) administration and exercise, they found a possible relation between muscle intramyocellular triglyceride (IMTG) content/metabolism and insulin resistance. Due to these findings, Prof. D. Hansen, in collaboration with Prof. Luc van Loon, wanted to assess 24-hour glycemic control after exercise combined with intake of Acipimox in T2DM patients. In the present pilot study, this was assessed with nine T2DM patients, who were subjected to an acute endurance exercise bout, in combination with Acipimox or placebo intake.

To understand the purpose of this study, it is important to elucidate the substrate use in healthy subjects and T2DM patients. Substrate utilisation depends mainly on two factors: exercise intensity and duration [1]. Under normal conditions, the skeletal muscle has the ability to use carbohydrates and plasma non-esterified fatty acids (NEFA). At rest approximately 60% fatty acids and 40% carbohydrates are used as an energy source. When initiating exercise an increase in the utilisation of both substrates is noted. At an exercise intensity of 50% of maximal oxygen uptake (VO_{2max}) the peak fat oxidation rate is often achieved. With increasing exercise intensity the favoured substrate source changes from fatty acids to carbohydrates [2, 3]. It is also known that untrained subjects are more likely to use relatively more carbohydrates as exercise fuel (especially during high-intensity exercise) in comparison to trained counterparts [4]. From start till approximately 40 minutes of mild exercise (at ±50% VO_{2max}), the main energy sources are plasma glucose and plasma NEFA. After 40 minutes till 4 hours of exercise a shift to approximately 70% of metabolized NEFA occurs [1].

Since T2DM is a metabolic disease in which both carbohydrate and fat mechanisms are involved, it is clear that fuel use abnormalities are present [5]. An experiment, performed by Boon et al. (2007) with ten T2DM patients and ten healthy age-matched controls, compared substrate utilization and plasma metabolite concentrations during rest, exercise (at 50% maximal cycling power output (W_{max})) and post-exercise recovery. They found significantly higher total fat oxidation rates at rest and during recovery in the diabetes group. This difference was due to a significantly higher NEFA oxidation rate in the diabetes group ($p<0.05$). As compensation to these higher fat oxidation rates, a lower total glucose oxidation was found. Furthermore, during exercise there was a significant decrease in plasma glucose concentrations in the T2DM group, as glucose utilisation exceeds its rate of appearance during exercise conditions. In addition, they found a significant increase in IMTG use during exercise, compared to resting conditions, in both the diabetes group and the control group, with no differences between groups [6].

It is also important to understand the influence of increased muscle IMTG content on insulin sensitivity. In recent studies [7, 8], after increasing NEFA concentrations using intralipid-infusion, a significant decrease in intramyocellular glucose was noted. This means that blood NEFA levels influence insulin-stimulated glucose muscle uptake in a far earlier stage then suggested by Randle et al [9]. After further research, it is clear that an increased accumulation of muscle IMTG and other fatty acid metabolites results in a disturbed insulin signalling cascade. This disturbance inhibits GLUT-4 translocation, resulting in a decreased insulin-stimulated glucose uptake and subsequent insulin resistance [9]. However, some details remain unclear and further studies are still needed.

Finally, the function of Acipimox needs to be clarified. Acipimox (5-methyl-pyrazine carboxylic acid 4-oxide) is used in the treatment of hyperlipidaemia, to decrease plasma NEFA levels and inhibit adipose tissue lipolysis. Acipimox (a nicotinic acid analogue) is able to reduce blood NEFA levels at rest up to 52% and 72% (250mg) after one hour in T2DM patients and nondiabetic subjects, respectively [10]. In healthy subjects, an acute lowering in blood NEFA levels to 80-85% of baseline levels was found. A suppression of NEFA up to 65% respectively 60% was detected after 1 week of Acipimox use [11, 12]. In the long term, a 27% (250mg x4/d) decrease in blood NEFA concentration was found after 28 days of Acipimox intake in chronic heart failure patients [13].

In response to lowering in blood NEFA levels by Acipimox intake in T2DM patients, a decrease in total fat oxidation at rest [13], and thus an increase in whole-body glucose oxidation, can be achieved [14, 15]. Moreover, several studies [14, 16] have shown that after Acipimox intake, the increase in total glucose oxidation is associated with a decreased plasma glucose level, increased glucose tolerance and increased insulin-stimulated glucose uptake in muscles. This suggests an improvement in insulin action due to Acipimox intake. Lim et al. [15] reported no effect on muscle glycogen utilization rate. Furthermore, no data has been found regarding Acipimox utilization and IMTG oxidation.

In this pilot study, a central format was used and author guidelines were followed, according to Diabetologia. The research question was chosen by our promoter. The research protocol was mostly based on an existing protocol from Prof. D. Hansen. Niels Hermans revised and converted it to the central format. Both, Anton Walbers en Niels Hermans contributed to the experimental visits in November and December 2013. Blood samples were analysed at University of Maastricht, where after Anton Walbers finished the abstract, statistical analysis and results section. Niels Hermans completed the introduction, methods and discussion section.

Is lipolysis inhibition during exercise instrumental to augmentation in glycemic control in type 2 diabetes: a pilot study.

In accordance to author guidelines of “Diabetologia”:
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ABSTRACT

Aims/hypothesis:

In this pilot study, it was hypothesized that oral Acipimox administration during exercise would lead to a greater improvement in 24-hour glycemic control in type 2 diabetes mellitus (T2DM) patients.

Methods:

Nine T2DM patients underwent three trials in a cross-over double blinded study. ACI trial existed of intake of Acipimox (nicotinic acid analogue) and exercise, PLA trial existed of placebo intake and exercise, and a control trial (CON) with only placebo intake without exercise. The exercise consisted of cycling during 60 minutes at 45% of baseline peak power output (W_{max}) in fasting condition. Afterwards, patients were followed for 24 hours in which three standardized meals were provided. Blood samples were taken every 30 minutes during exercise and follow-up, to determine blood glucose, blood non-esterified fatty acid (NEFA), lactate and triglyceride content. After a 24-hour follow-up an oral glucose tolerance test (OGTT) was performed.

Results:

NEFA content was significant lower after Acipimox intake during exercise and the first three hours (p<0.001as opposed to other trials), thus verifying a blunted lipolysis during exercise. However, no significant differences were detected in blood glucose levels between trials, both during exercise, as well as during the 24-hour follow-up period and OGTT. Moreover, a significant correlation was found between glucose control during OGTT and age (p<0.05) and between delta glucose control during OGTT and total lean tissue mass (p<0.05). Furthermore, a correlation was found between delta FFA and age (p<0.05).

Conclusions/interpretation:

The intake of Acipimox during exercise does not lead to a greater improvement in 24-hour glycemic control in T2DM.

Keywords: Acipimox, Exercise, T2DM, Glycemic control

Abbreviations: T2DM: type 2 diabetes mellitus, ACI: Acipimox trial, PLA: placebo trial, CON: control trial, NEFA: non-esterified fatty acids (= FFA: free fatty acids), OGTT: oral glucose tolerance test, IMTG: intramyocellular triglyceride, HbA1c: blood glycosylated haemoglobin, TG: triglyceride

INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic disease that affects mostly adults due to the lack in physical exercise and/or excessive adipose tissue mass [17]. In the last decade, there has been an alarming increase in T2DM prevalence, rising from 171 million in 2000 up to an estimated staggering 366 million in 2030 [18]. Most recent figures in Belgium demonstrate a prevalence of T2DM in adults of 83.3/1.000, which will increase up to 100/1000 subjects in 2030 [19]. Risk factors for the development of T2DM are genetic predisposition (up to 40% by having first-degree relatives with T2DM), ethnicity (Asian, Hispanic and Africans have a higher risk for developing T2DM compared to Caucasians) [20] and lifestyle (low physical activity, obesity, and carbohydrate rich diet) [17].

Because of the excessive rise in prevalence of T2DM, there is a greater need for substantiated evidence-based and effective treatment. In the care of T2DM patients, exercise intervention is considered a cornerstone. Structured long-term exercise training in T2DM patients leads to a ~0.7% reduction in blood glycosylated haemoglobin (HbA1c) content [21]. Considering the significant relation between blood HbA1c content and risk of cardiovascular complications and premature death, such a decline in blood HbA1c content would translate into a reduction in risk of micro- and macro vascular disease, and premature death [22, 23]. Moreover, participation in exercise training intervention leads to an improvement in exercise capacity, decrease in adipose tissue mass and increase in lean tissue mass, improvement in blood lipid profile, lowering in blood inflammatory markers and blood pressure and an improvement in quality of life [24-27]. Various official international instances therefore confirm the importance of exercise intervention in the care of T2DM [28-30]. According to clinical guidelines, patients with T2DM should exercise 3-5 days/week, at a low-to-moderate intensity (at 40-70% of maximal oxygen uptake), achieving a minimal exercise duration of 150 min/week, in combination with resistance exercises (5-10 exercises/session, three series/exercise, 10-15 repetitions/series).

In recent decades, it has been pursued to further optimize the clinical benefits of exercise intervention in T2DM [31]. It has been shown that a greater improvement in glycemic control is achieved in T2DM patients when prolonging exercise intervention [31], adding resistance exercises on top of endurance exercises [24], and exercising more frequently [32]. Despite the promising improvement in the clinical effectiveness of exercise intervention in T2DM patients, room for further improvement remains present.

In this regard, the manipulation of substrate selection (carbohydrate versus blood NEFA) during exercise in T2DM patients could be relevant. More specifically, the suppression of lipolysis during exercise has been studied in T2DM patients. By oral administration of Acipimox during acute endurance exercise, lowered blood free fatty acid levels have been observed in T2DM patients [33]. A more recent study replicated these findings and additionally discovered that intramuscular lipid oxidation and glycogen use is substantially increased when administrating Acipimox during acute endurance exercise in T2DM patients [34].

This finding might be clinically relevant to T2DM patients. It is hypothesized that acute exercise combined with oral Acipimox intake up regulates intramuscular fatty acid turnover and hereby prevents accumulation of intramuscular fat and/or improves fat oxidation capacity [34]. Moreover, the intake of Acipimox during

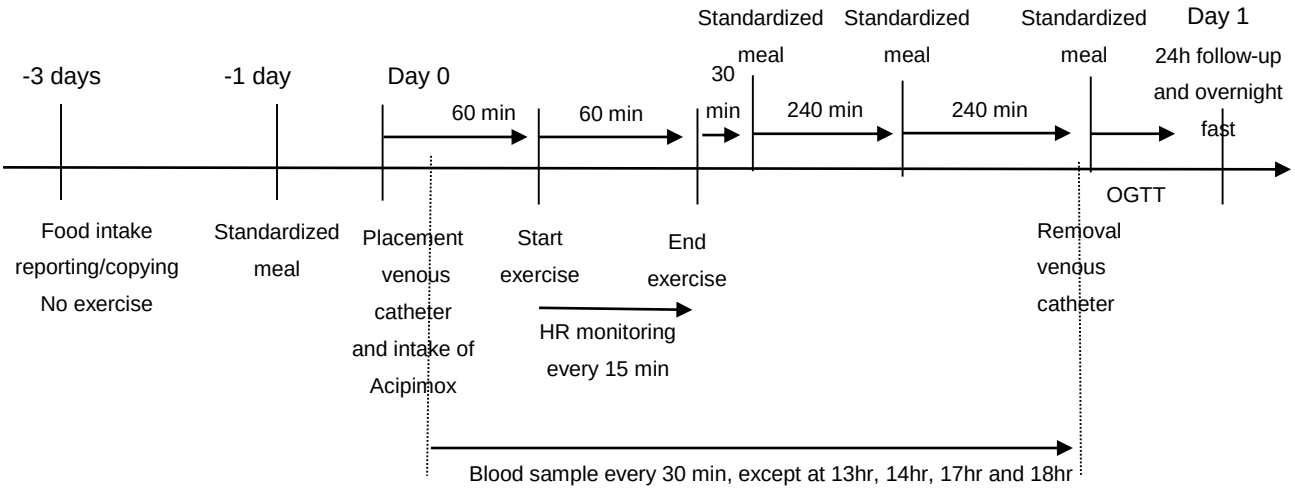
exercise would lead to a significant increase in muscle glycogen use. This would translate into a greater improvement in insulin sensitivity during and after exercise. Whether such pharmacologic support during exercise would lead to significant greater improvements in 24-hour glycemic control in T2DM patients remains however unknown. Therefore we hypothesized that oral Acipimox administration during exercise would lead to a greater improvement in 24-hour glycemic control.

METHODS

Study design

In this pilot study, subjects were randomly assigned, by envelope, to an acute exercise bout with Acipimox administration (ACI), followed one week later by an acute exercise bout with placebo intake (PLA), followed one week later by oral placebo administration without exercise (CON), or to the opposite follow order (=three experimental visits/subject). Both participants and researchers were blinded. At entry of study, following measurements had been executed (screening): fasting blood sample for assessment of glycemic control, OGTT, maximal cardiopulmonary exercise test, and body composition assessment. Oral blood-glucose and/or lipid-lowering medication intake remained constant during the study. During the three experimental visits, serial blood samples were taken to analyse following blood concentrations: NEFA, triglycerides (TG), glucose and lactate levels. During the visits food intake was standardized as well. The chronology of measurements during the experimental visits is displayed in figure 1.

Figure 1 Measurements during experimental visits (no exercise was implemented in the control situation)



Participants

Nine male T2DM patients (fasting blood glucose level >125mg/dl, and/or HbA1c >6.5%) were included. Based on previous studies, fifteen subjects will be a sufficient sample to detect clinically relevant and statistically significant changes [34]. However, because of the pilot study design, we only investigated nine subjects. Patients were recruited by endocrinologists of the Jessa Hospital Hasselt through advertisements. In addition, informed consent was obtained from the patients and the study was approved by the Ethics Committee of the Jessa Hospital and the University of Hasselt. Patients with following characteristics were excluded: exogenous insulin therapy, history of coronary events/revascularization, presence of chronic pulmonary or renal disease, gastric complaints/disease, and/or orthopaedic disease that interferes with exercise, involvement in exercise training and/or caloric restriction program for at least one year. All

subjects maintained normal physical activity and dietary patterns, and refrained from exhaustive physical exercise three days prior to each experimental day. In addition, participants recorded dietary intake over three days prior to the first experimental visit and copied their diet prior to the subsequent experimental visits. During the laboratory stay, all participants received the same standardized meals.

Intervention and outcomes

Pre-study screening

Fasting blood sample and OGTT

Participants arrive at the hospital by car or public transport and report at the laboratory at 08:00 hours following an overnight fast. After 20 min of supine rest, a venous blood sample was collected. Thereafter, an OGTT was performed (oral intake of 75 gr glucose, dissolved in 250 ml water). Blood samples were immediately centrifuged at 1.000 *g* and 4°C for 5 min, after which plasma was frozen in liquid nitrogen and stored at −80°C until analysis. Blood samples were analysed for glucose (Beckman Synchron LX 20 Analyzer, Beckman Coulter, Fullerton, CA, USA, HbA1c (Hi-Auto A1c Analyzer, Menarini Diagnostics, Florence**,** Italy), and total plasma TG (Beckman Synchron LX 20 Analyzer, Beckman Coulter). NEFA levels were quantified by an enzymatic method (Wako Chemicals, Neuss, Germany).

Maximal cardiopulmonary exercise test

Peak oxygen uptake capacity ($VO_{2\ peak}$) and maximal workload capacity (W_{max}) were assessed during an exhaustive incremental exercise test on a cycle ergometer (Ebike basic, Ergoline, Germany) using a 1-min work stage protocol. VO_2 measurements were performed continuously (Oxycon Pro, Jaeger, Germany) to assess VO_{2peak}. In addition, expiratory volume, carbon dioxide output, and respiratory gas exchange ratio (RER) was monitored continuously. Cardiac function was monitored using a 12-lead electrocardiogram with heart rate recorded continuously.

Body composition

Body mass was measured using a calibrated analogue weight scale. Segmental and whole body fat mass and fat-free mass were determined using whole body dual X-ray absorptiometry (DEXA Delphi QDR, Bedfort, USA).

Measurements during experimental visits

Blood samples

Ahead of, during, and after exercise serial venous blood samples were collected and treated the same way as during the pre-study screening. They were analysed for blood glucose, blood NEFA and plasma TG (all same method as pre-study), lactate (Wako Chemicals, Neuss, Germany) and insulin (Advia Centaur Immunoassay System, Bayer Diagnostics, Tarrytown, NY, USA).

Oral Acipimox and placebo administration

In one experimental trial, subjects were orally administered one capsule of 250mg Acipimox (Nedios, Altana Pharma bv, Hoofddorp, NL). Potential side-effects of Acipimox intake are: flushing, skin rashes, gastrointestinal complaints and headaches. In the other experimental trials, subjects were administered one capsule of 250mg of placebo.

Exercise

All participants performed an acute endurance exercise bout in fasting condition on bike (Bike Exc 500i, Technogym, Italy) for a total duration of 60 minutes, at exactly 45% W_{max}. The exercise intensity was monitored by continuous heart rate monitoring (Polar, Oy, Finland).

Data analysis

Data have been analysed with the SPSS 22.0 statistical software programme. Statistical significance was set at $p<0.05$. First descriptive statistics have been performed to define means and standard deviation. A boxplot was used to determine data-distribution and outliers. When assessing normality, the Shapiro-Wilk Test was performed due to small sample size (n=9). Because data were not normally distributed, further data analysis consisted of a Friedman test and post hoc analysis with a Bonferroni correction. In addition possible correlations were checked by a Spearman Test. Blood sample data of two subjects were missing, one in de ACI group and one in the PLA group. Due to an automatic correction in the SPSS software programme, these missing data had no influence on the outcome measures.

RESULTS

Patient characteristics

Patient characteristics and medication intake are summarized in table 1.

Amount of patients		N=9
General Features		
	Age, y	67.2 ± 6.6
	Body Weight, kg	88.1 ± 13.3
	Height, cm	171.6 ± 4.9
	BMI, kg/m²	29.9 ± 3.6
Blood Parameter		
	HbA1c, %	6.9 ± 0,6
Body Composition		
	Total adipose tissue, kg	29.8 ± 6.4
	Percentage adipose tissue, %	33.6 ± 3.7
	Total lean mass, kg	55.3 ± 7.3
	Percentage lean Mass, %	63.0 ± 3,7
	Total BMC, kg	3.0 ± 0.6
Exercise Capacity		
	Peak VO_2max, W	1947.4 ± 623.6
	Peak heart rate, Bpm	138.7 ± 15.9
	Peak RER	1.16 ± 0.16
Medication		
	Metformin, n	9
	Sulfonylurea, n	1
	Statin, n	5
	Fibrate, n	1
	ACE-inhibitor, n	4
	Agiontensin Receptor Blocker, n	1
	Vildagliptin, n	2
	Acetylsalicylic acid, n	7
	Liraglutide, n	1
	Allopurinol, n	1
	Hydrochlorothiazide, n	1
	Amlodipine, n	1
	Meglitinide, n	2
	Clopidogrel, n	1
	Sitagliptin, n	1
	Benzodiazepine, n	1
	tiotropium bromide, n	1
	Terazosin, n	1

-Abbreviations: BMI = body mass index, HbA1c = glycosylated haemoglobin , BMC = bone mineral content, ACE = angiotensin converting enzyme, RER = Respiratory gas exchange ratio, Bpm = beats per min
-Data are expressed as mean ± SD. P<0.05

Table 1 Patient characteristics.

Impact of exercise

Blood NEFA concentrations

Mean blood NEFA concentrations are shown in figure 2. At the onset of the trial, blood NEFA levels were significant different between the PLA trial and the CON trial (p<0.05). Hereafter plasma NEFA concentrations in PLA group increased (t=6; p<0.05) during exercise and continued to rise for half an hour after finishing the exercise bout. Hereafter the blood NEFA concentrations sharply declined to below baseline levels even two hours after a first meal (t=11; p<0.05). In the CON group similar but smaller variations were observed (t=6; p<0.05, t=11; not significant). In the ACI group plasma NEFA values dropped immediately after Acipimox intake and remained below baseline levels during and after exercise (p<0.05)**.** Three hours after exercise, the blood NEFA levels slightly increased but remained below baseline levels in the ACI trial. At the end of all three trials, blood NEFA values remained lower, but did not significantly differ from baseline values. Between PLA and CON trials, a statistical difference was detected at t=1, t=14 and t=15 (p<0.05). Before, during and after exercise (t=3-9), the ACI trial was significant different from the PLA trial (p<0.05) and the CON trial (p<0.05). Only at t=6, a significant difference was seen between the PLA trial and the CON trial (p<0.05).

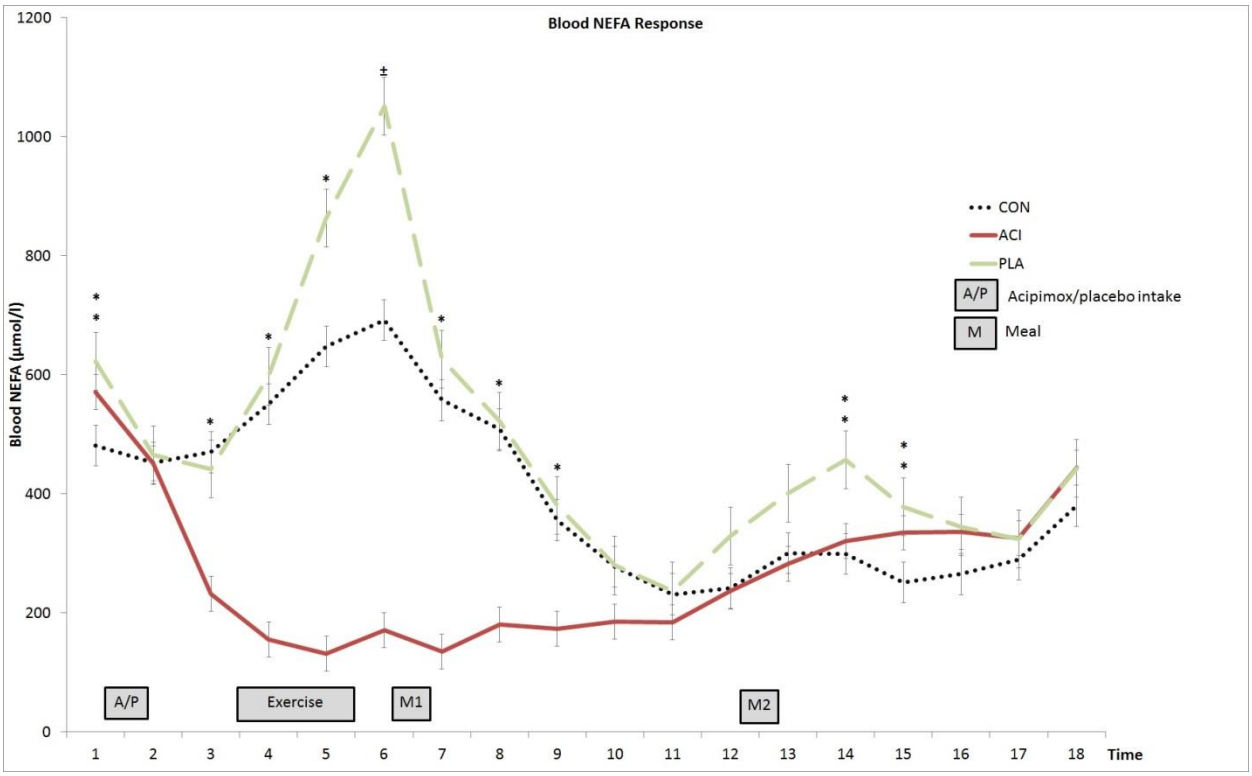


Figure 2 Blood NEFA concentrations. * p<0.05 difference between ACI trial and CON or PLA trial ‡ p<0.05 difference between PLA and CON trial. ± p<0.05 difference between all three trials.

Blood triglyceride concentrations

Mean blood TG concentrations are shown in figure 3. At baseline, no significant difference was found between the three trials. In the ACI trial, during and 30 minutes after exercise (t=6; $p<0.05$) blood TG levels dropped significantly. Hereafter plasma TG values rose to above baseline levels ($p<0.05$). In comparison, similar but higher values were seen in the CON and PLA trial alike. Throughout the complete PLA trial, blood TG concentrations rose longer and higher in comparison to the ACI trial (non-significant). Between trials, only at t=8 a significant difference was detected between CON and ACI trial ($p<0.05$).

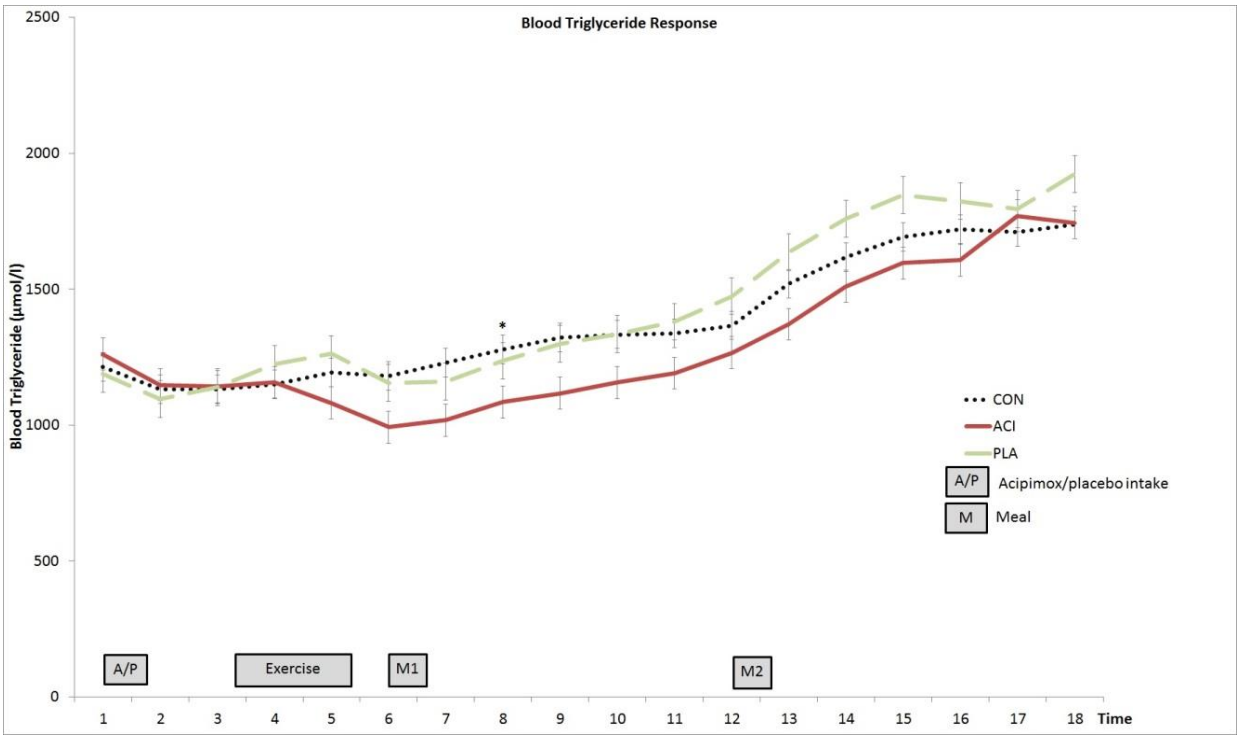


Figure 3 Blood triglyceride concentrations.* $p<0.05$ for difference between ACI and CON trial.

Blood lactate concentrations

Mean blood lactate concentrations are shown in figure 4. At the start, no difference was measured between all three trials. After initiating and during exercise, lactate values more than doubled in ACI and PLA trials alike (t=4 p<0.05). After exercise, blood lactate values decreased again to almost pre-exercise level (non-significant). Throughout the complete ACI and PLA trials, no significant differences were measured. While in the CON trial no exercise was initiated, lactate values remained around baseline levels before the first meal. A significant difference (p<0.05) was seen between the CON and ACI trail and between CON and PLA trial at t=4 and t=5 (p<0.05). Herafter, at t=6, only a significant diference between the CON and the PLA trial was found (p<0.05). After meal, there were no more signifcant differences between trials.

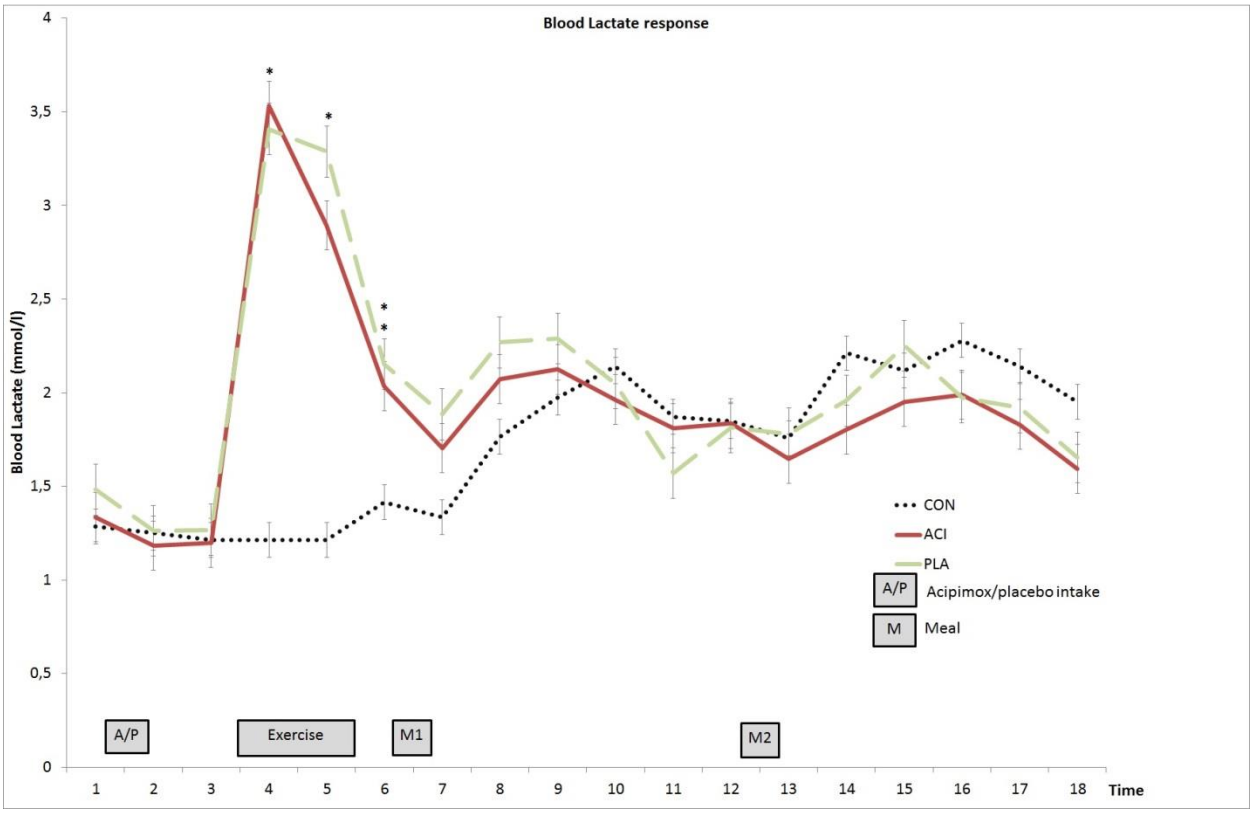


Figure 4 Blood lactate concentrations. * p<0.05 difference between CON trial and ACI or PLA trial. ** p<0.05 difference between PLA and CON trial.

Blood glucose concentrations

Mean blood glucose concentrations are shown in figure 5. No significant differences were found throughout the complete length of all three trials. After the first meal of the day, blood glucose levels increased over an one hour period to ±12 mmol/l (t=9; p<0.05) and subsequently decreased to baseline levels. After consuming a second meal, a more profound increase in blood glucose was seen in the CON (not significant) and PLA trial. During the OGTT no difference was found between the three groups. At the end of the OGTT, a non-significant lower blood glucose concentration was found in the PLA trial, compared to the CON trial and the ACI trial.

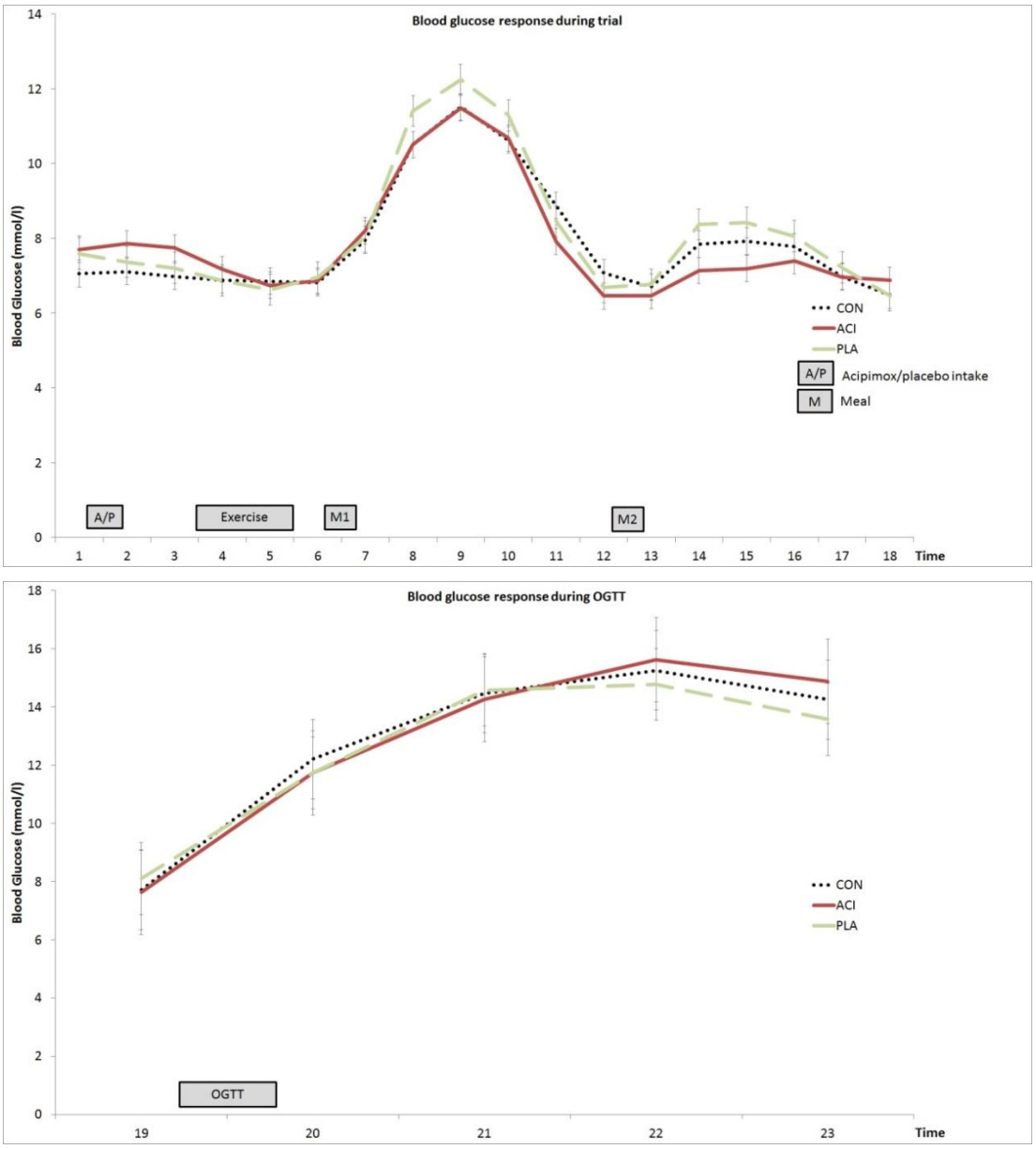


Figure 5 Blood glucose concentrations during the trial and OGTT.

Correlations

We found a significant correlation between glucose control during OGTT and age (p<0.05, figure 6) and between delta glucose control during OGTT (the difference in blood glucose content between PLA and ACI trial) and total lean tissue mass (p<0.05, figure 7). Furthermore, a correlation was found between delta FFA (the difference in blood NEFA content between PLA and ACI trial) and age (p<0.05, figure 8).

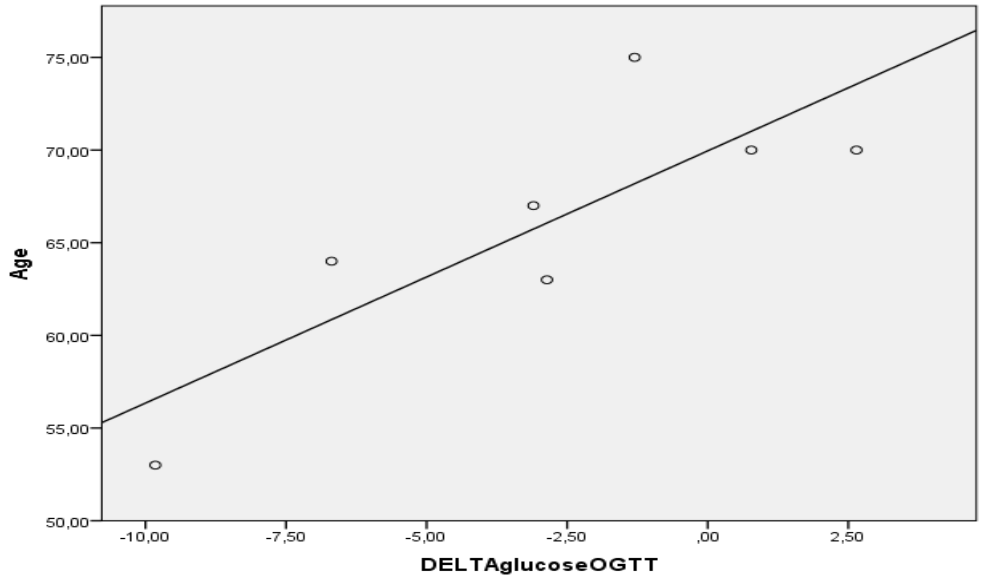


Figure 6 Correlation scatterplot between age and Δ glucose during OGTT.

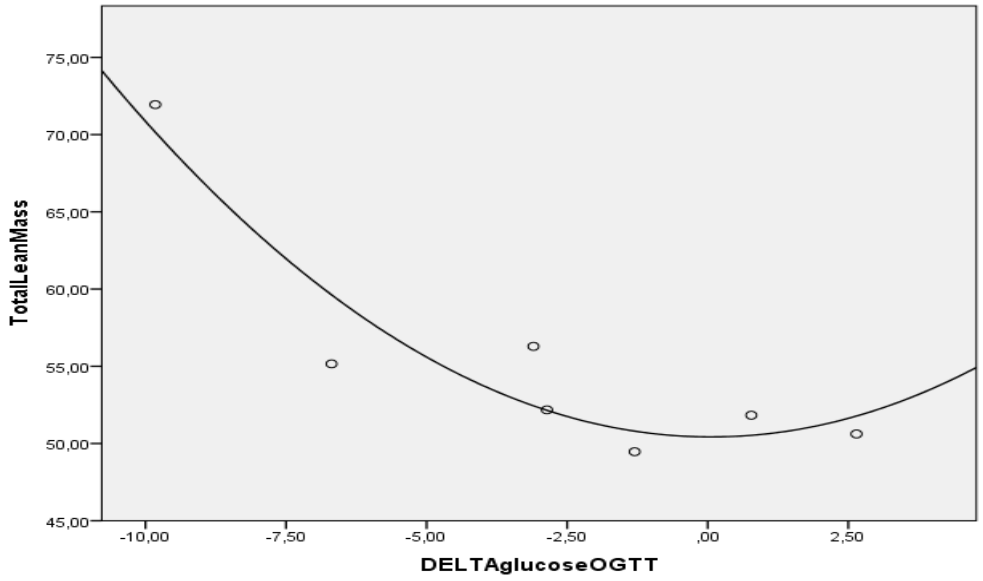


Figure 7 Correlation scatterplot between total lean mass and Δ glucose during OGTT.

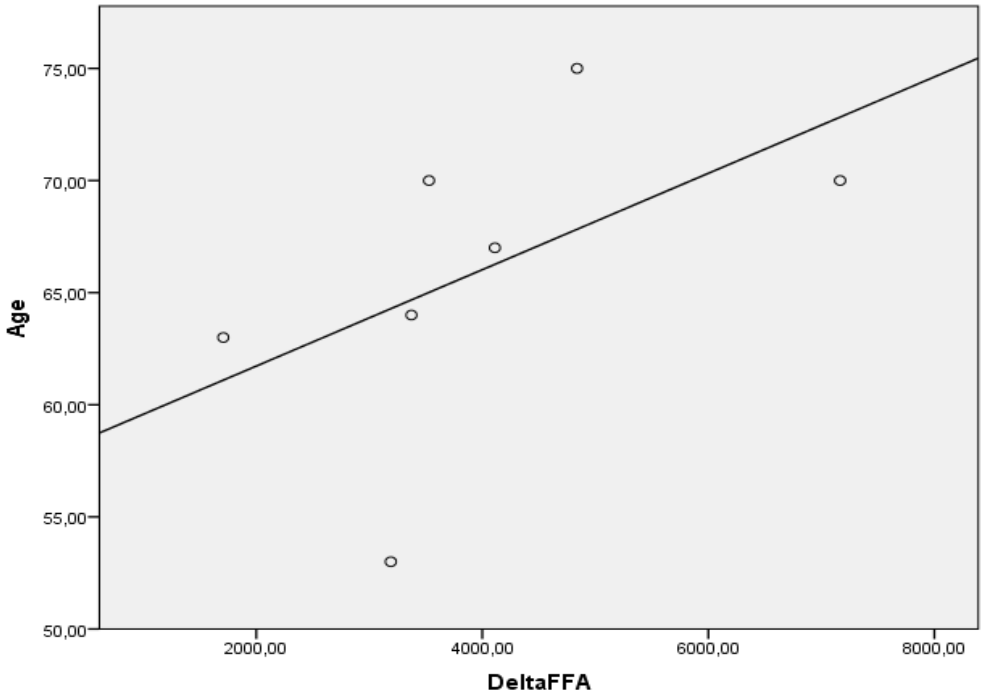


Figure 8 Correlation scatterplot between age and ΔFFA.

DISCUSSION

The present study shows that restricted blood NEFA availability, due to oral Acipimox intake, does not improve the 24-hour glycemic control after endurance exercise in T2DM. However, certain T2DM patients might benefit more from Acipimox intake during exercise, as opposed to others (e.g. younger patients, patients with higher lean body mass, etc.).

Acipimox administration significantly lowered blood NEFA levels during endurance exercise in the ACI group, compared to PLA group (p<0.05). These changes in blood NEFA concentrations were in accordance to previous studies [10-12]. However, this lowering in blood NEFA content during exercise did not lead to an improved 24-hour glycemic control: no significant differences were found in plasma glucose concentrations between ACI and PLA group over the entire 24-hour follow-up period or during the subsequent OGTT. So even though previous studies discovered that by the oral intake of Acipimox, muscle IMTG and glycogen oxidation is augmented during exercise [34], this change in muscle physiology did not translate into an improved 24-hour glycemic control in T2DM. Therefore, it might be speculated that the oral intake of Acipimox during long-term training programs would fail to improve the glycemic control with greater magnitude in T2DM.

The present data could be, at least in part, in conflict with previous studies. Recently, Van Loon et al. [34] demonstrated that NEFA availability plays an important role in the regulation of IMTG use. Following oral Acipimox administration, they found reduced plasma NEFA concentrations (p<0.001). The lower plasma NEFA oxidation rates at rest, during exercise and subsequent recovery were compensated by greater muscle and (lipoprotein) - derived TG as well as endogenous carbohydrate use. This augmentation in IMTG use was hypothesized as an important mechanism to improve skeletal muscle insulin sensitivity in T2DM. This was confirmed by Van Loon et al. [34], demonstrating significantly increased insulin sensitivity in the Acipimox trial, compared to the control trial (p<0.01). However, plasma glucose concentrations only tended to be lower after Acipimox administration (p=0.08). This is in accordance to our study, where plasma glucose concentrations between groups did not differ at all. They even seemed to be lowered in the control group, which may be explained by a rebound effect. Van Loon et al. [34] conducted a short follow up period up to two hours post-exercise, where we did a 24-hour follow-up. Other differences in outcomes between our study and the study of Van Loon et al. [34] could be explained by different patient characteristics: Van Loon et al. [34] conducted the experiment with slightly younger patients (mean age 60 ± 2.1 years vs. 67 ± 6.6 years), who had a higher HbA1c (mean HbA1c 7.3±0.3% vs 6.9±0.6%) and lower BMI (mean BMI 28.4±1.0 kg/m² vs 29.9±3.6 kg/m²). Both studies used almost the same exercise bout characteristics: 60 minutes protocol with a 50% W_{max} (Van Loon et al. [34]), where we used a 45% W_{max} protocol. This is in accordance to clinical guidelines. Furthermore, Van Loon et al. applied different measurement techniques, which could explain differences in outcomes.

However, during the OGTT, correlations were found between age and changes in blood glucose levels (when comparing the PLA vs ACI trial): the younger the T2DM patient, the greater the lowering in blood glucose concentrations due to Acipimox intake. This may indicate a better improvement in glycemic control

after exercise due to Acipimox intake in younger patients. For this reason, Acipimox intake could lead to more effective exercise treatment strategies in younger compared to older T2DM patients. A possible explanation for this correlation could be that liver function decreases over age, which leads to diminished drug metabolizing capacity, which in turn results into less effective treatment with Acipimox. However, this remains speculative and more research is warranted to verify this hypothesis. Moreover, during the OGTT, a correlation between lean body mass and changes in blood glucose levels due to Acipimox intake (comparing the PLA vs. ACI trial) was found: a higher lean body mass predicted a greater lowering in blood glucose content after exercise with Acipimox intake. This could be clarified by the fact that lean body mass is the main system in the uptake of blood glucose. A final correlation was found between age and changes in blood NEFA levels after exercise with Acipimox intake: the older the patient, the greater the lowering in blood NEFA levels after exercise when taking Acipimox. The underlying mechanism for the greater lowering in blood NEFA levels after Acipimox intake in older T2DM patients is speculative. It could be argued that in older individuals, due to higher relative adipose tissue content, lipolysis is more prominent, since the lipolysis process to a large extent takes place in adipose tissue cells [35]. In addition, a possible mechanism for this correlation could be an increased sensitivity of adipose tissue cells for Acipimox in older compared to younger subjects. However, these hypotheses need to be verified in future research.

A lack of an effect of Acipimox intake during exercise on 24-hour glycemic control was not due to methodological flaws. Patients received strictly standardized meals during the experimental visits, in order to exclude contamination or bias of results by nutritional grounds. In addition they also filled out a detailed dietary report, beginning 3 days prior to every experimental visit, making it possible to control dietary intake conscientiously. Moreover, exercise bout characteristics were in accordance to clinical guidelines and lactate data supporting the fact that the intensity of the exercise bout was sufficiently high [31]. Furthermore, it was shown that 250mg of Acipimox was an appropriate dose to effectively lower plasma NEFA concentrations.

The present study is limited by the absence of insulin data. Moreover, only male subjects were included. Finally, the small sample size could be seen as a limitation. However, based on the findings of this pilot study, further research can be optimized.

Future research should be focused on the feasibility of Acipimox in specific T2DM subpopulations (e.g. older patients, patients with higher HbA1c, etc.), whether this could optimize the effect of the drug on T2DM glycemic control. In addition, not only 24-hour follow-up after a single exercise bout, but also long term effects after several training sessions should be investigated. Finally, Acipimox administration in other exercising conditions should be explored. In this regard, more research is warranted.

In conclusion, Acipimox administration combined with physical exercise does not lead to a greater improvement in 24-hour glycemic control, as opposed to physical exercise only, in T2DM patients.

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