2016•2017

FACULTY OF MEDICINE AND LIFE SCIENCES

Master of Biomedical Sciences

Master's thesis

Impact of lipolysis inhibition on exercise physiology in type 2 diabetes mellitus

Supervisor :
Prof. dr. Dominique HANSEN

Boudewijn Lemmens

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences

Transnational University Limburg is a unique collaboration of two universities in two countries: the University of Hasselt and Maastricht University.

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I. Foreword

I would like to thank all members of the REVAL group for their kindness and readiness to help, especially drs. Kenneth Verboven for his guidance throughout the internship.

The members of REGO deserve special thanks as well. Even though it was not required, they went out of their way to provide advice and help, especially with patient recruitment.

Special thanks to Dominique Hansen for the opportunity to work on a grant proposal and his infectious enthusiasm for science.

II. Abstract

Introduction: Endurance-type exercise, combined with adipose tissue lipolytic inhibition using acipimox, has been shown to augment intramuscular lipid use and the increase in whole-body insulin sensitivity in type 2 diabetes mellitus (T2DM) patients after a single bout of exercise but the effects of long term intervention remain to be studied. However, little is known about the exercise physiology during such an inhibition. The goal of this project is to investigate the impact of adipose tissue lipolytic inhibition on exercise physiology in T2DM patients.

Methods: 10 overweight male T2DM patients (age 57±8 years, BMI 31.8±2.8 kg/m², HbA1c 7.1±0.96%,) participated in a double-blind placebo-controlled randomized cross-over study. After 250 mg nicotinic acid derivative acipimox or placebo subjects underwent a 12-minute indirect calorimetry measurement, followed by a maximal cardiopulmonary exercise test. Heart rate was monitored continuously and blood pressure was measured during rest and at exercise cessation.

Results: RER was lowered following acipimox administration at rest and during exercise at 40-60% of VO2Peak ($p < 0.05$). At 6 minutes of exercise lactate concentration was increased in the acipimox group (2.9±0.9 vs. 3.2±0.9, $p = 0.041$), corresponding with a leftward shift of the respiratory compensation point (RCP). Heart rate, oxygen pulse and blood pressure were not altered after acipimox administration ($p < 0.05$)

Discussion & conclusions: Inhibiting adipose tissue lipolysis during a maximal cardiopulmonary exercise test alters substrate metabolism during moderate-intensity exercise. Moderate, not high intensity exercise may be optimal for an acipimox co-intervention.

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III. List of abbreviations

ACP	Acipimox-treated group
ADA	American Diabetes Association
BMI	Body mass index
DM	Diabetes mellitus
EE	Energy expenditure
FFA	Free fatty acids
FPG	Fasting plasma glucose
GWAS	Genome-wide association studies
HbA1c	Blood glycated haemoglobin
HDL	High density lipoprotein
HR	Heart rate
HSL	Hormone sensitive lipase
IGT	Impaired glucose tolerance
IMTG	Intramyocellular triglycerides
IR	Insulin resistance
NGT	Normal glucose tolerance
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
OGTT	Oral glucose tolerance test
PKA	Protein kinase A
PLA	Placebo-treated group
RCP	Respiratory compensation point

RER	Respiratory exchange ratio
RM-ANOVA	Analysis of variance for repeated measurements
SD	Standard deviation
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
TG	Triglycerides
VLDL	Very low density lipoprotein

1. Introduction

An increasing number of people worldwide suffer from diabetes mellitus (DM), of whom more than 95% have type 2 DM (T2DM). It is estimated that this number will increase to more than half a billion by 2030. An estimated 300 million more individuals will have features indicating increased risk of developing T2DM, including impaired glucose tolerance (IGT), fasting hyperglycaemia and euglycemic insulin resistance (IR) (1). The majority of T2DM cases results from westernized lifestyles; decreased exercise and high-fat diets, leading to obesity. Obesity, especially in combination with reduced physical activity, is the major determinant of the development of T2DM (2).

T2DM is, in part, an inherited condition. Offspring of diabetic parents is more likely to suffer from T2DM (3). Furthermore, some ethnic minorities are at greater risk of developing T2DM compared their age- and sex-matched Caucasian counterparts (4). Genome-wide association studies (GWAS) have revealed that certain single nucleotide polymorphisms (SNPs) related to beta-cell function predispose to the development of T2DM, as well as SNPs associated with insulin signalling, glucose metabolism and the development of obesity (5-7).

The progression from normal glucose tolerance (NGT) to T2DM is characterized by progressive increases in fasting and post-prandial plasma glucose concentration, which develops over the course of several years. T2DM is diagnosed by measuring plasma glucose levels, preferably after an overnight fast (between 10 and 16 hours without caloric intake) or the oral glucose tolerance test (OGTT), in combination with other symptoms of diabetes including polyuria, polydipsia, ketonuria and rapid weight loss. The American Diabetes Association (ADA) recommends the following criteria: HbA1c ≥6.5%, fasting plasma glucose (FPG, ≥126 mg/dl (7.0 mmol/l)). 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT or plasma glucose ≥200 mg/dl (11.1 mmol/l) IGT is an intermediate state of hyperglycaemia in which glucose levels do not meet criteria for diabetes, but are too high to be considered normal. For IGT the criteria are FPG <7.0 mmol/l (<126 mg/dl) and 2-h plasma glucose 7.8–11.0 mmol/l (140–198 mg/dl) The American Diabetes Association recognizes another intermediate category of glucose tolerance termed impaired fasting glucose (IFG), which is defined by fasting glucose concentrations ≥ 6.1 but less than 7.0 mmol/l (110 to 126 mg/dl) (8).

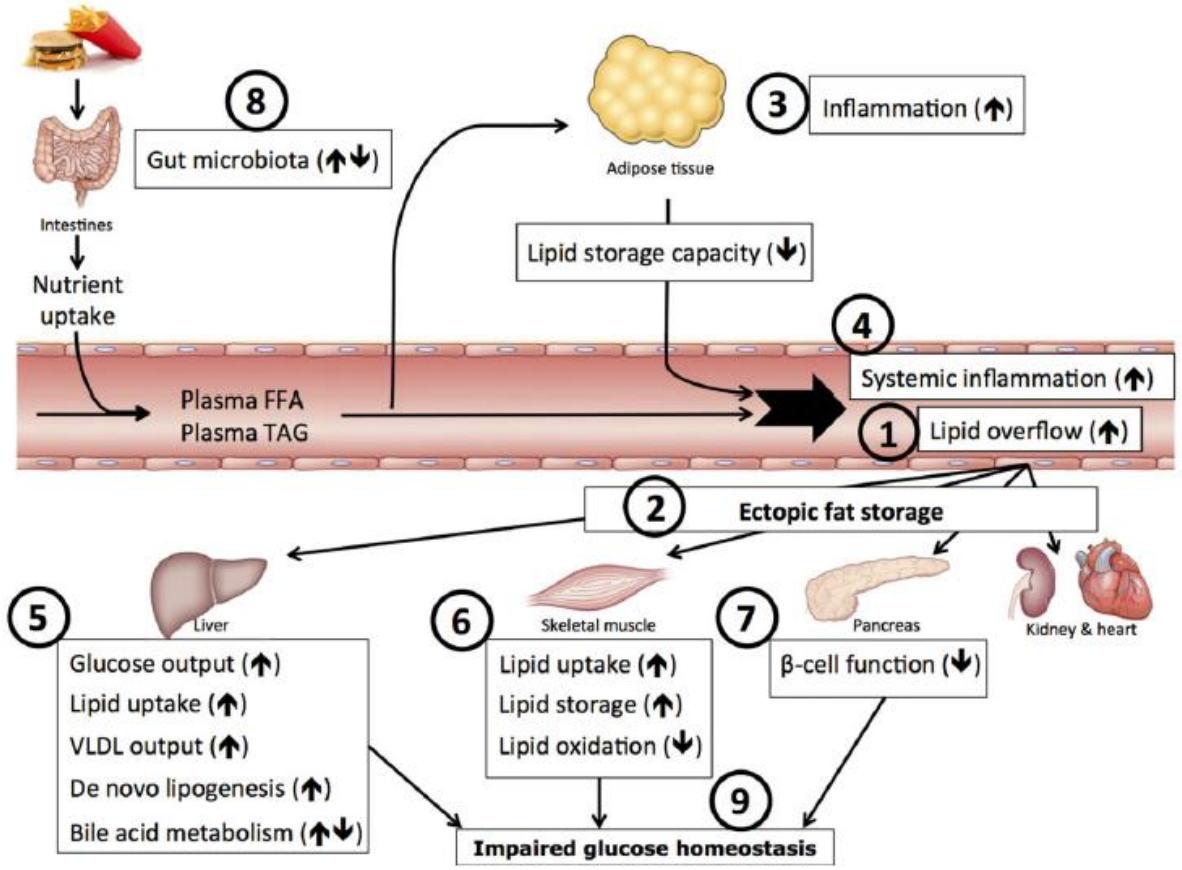

Figure 1:Inter-organ crosstalk in fatty acid metabolism and insulin resistance. (1) An impaired adipose tissue lipid metabolism, as observed in obesity and characterized by elevated plasma FFA levels, is associated with a decreased lipid storage capacity, which contributes to lipid overflow in the circulation., resulting in (2) excessive fat storage in peripheral tissues such as skeletal muscle, liver, pancreas, kidney and heart (ectopic fat storage). (3) Furthermore, adipose tissue dysfunction is characterized by an altered expression and secretion of adipokines, inducing a state of chronic low-grade inflammation. (4) This inflammatory state may on the one hand affect local adipose tissue lipid metabolism and on the other hand contribute to systemic inflammation, which together may affect lipid handling in peripheral tissues such as liver and skeletal muscle and promote insulin resistance through interference with insulin signalling. (5) An increased lipid supply to the liver may result in a higher glucose production, an increased hepatic TG content and VLDL-TAG output and a reduced insulin clearance by the liver. The increased insulin concentration stimulates de novo lipogenesis and, together with an increased VLDL-TG output, results in hypertriglyceridemia, ultimately leading to insulin resistance and glucose intolerance. (6) In skeletal muscle, besides an increased fatty acid supply and uptake, an impaired muscle lipid turnover may contribute to accumulation of TAG and bioactive lipid metabolites (DAG, LCFA-CoA and ceramides), which may interfere with insulin signalling. (7) Hyperglycaemia may, together with the formation of hyperlipidaemia-related toxic metabolites and lipid accumulation in the pancreas, result in decreased glucose-stimulated insulin secretion by the pancreatic β-cells. (8) Finally, alterations in gut microbiota composition and function may affect adipose tissue, liver and skeletal muscle lipid and glucose metabolism, possibly through effects on SCFA production and bile acid metabolism. (9) Ectopic fat storage is associated with impaired function of the liver, skeletal muscle and pancreas, leading to derangements in whole-body glucose homeostasis and, consequently, type 2 diabetes (Adapted from Stinkens et al. 2015 (9)).

After a meal, very low density lipoprotein (VLDL)- and chylomicron triglycerides (TG) are hydrolysed by lipoprotein lipase (LPL). The free fatty acids (FFA) released during this process are taken up by adipose tissue, or spill over into the plasma FFA pool (10). Fatty acid spillover is more pronounced in more overweight T2DM patients compared to their less overweight counterparts (11). Excess FFAs are stored within peripheral tissue peripheral tissues such as skeletal muscle, liver and heart, leading to impaired glucose homeostasis (Figure 1).

FFAs stored within skeletal muscle fibres are located adjacent to muscle mitochondria and function as a readily available substrate use (12). Accumulation of these intramyocellular triglycerides (IMTG) is associated with IR, T2DM and obesity (13-16).

However, IMTG pools are also elevated in endurance-trained subjects and, in sedentary subjects, increase in response to endurance training intervention. Indeed, endurance trained athletes have higher levels of IMTG while being highly insulin sensitive (17, 18). It follows that IMTG accumulation alone is not responsible for IR (12, 19, 20). The difference between endurance athletes and untrained men is the oxidation rate (21, 22). An increased IMTG pool allows for increased contribution of IMTG as a fuel source during exercise, much like the increased muscle glycogen storage in endurance trained athletes (23).

Contrary to endurance athletes, IMTG pools are less utilized as a substrate source in sedentary, obese or T2DM patients (22). Elevated plasma FFA levels in these patients inhibit the mobilisation and oxidation of IMTG stores (24-26). As the IMTG are neither mobilised nor used both the IMTG and its metabolites accumulate. FA metabolites fatty acyl-CoA, ceramides and diacylglycerol have been shown to cause defects in insulin signalling (figure 2) (12, 27). The defects in insulin signalling cause a decline in insulin sensitivity which gives rise to a more pronounced insulin resistant state, shifting substrate use towards glucose and thus further impairing FFA oxidation, stimulating further storage of TG (12).

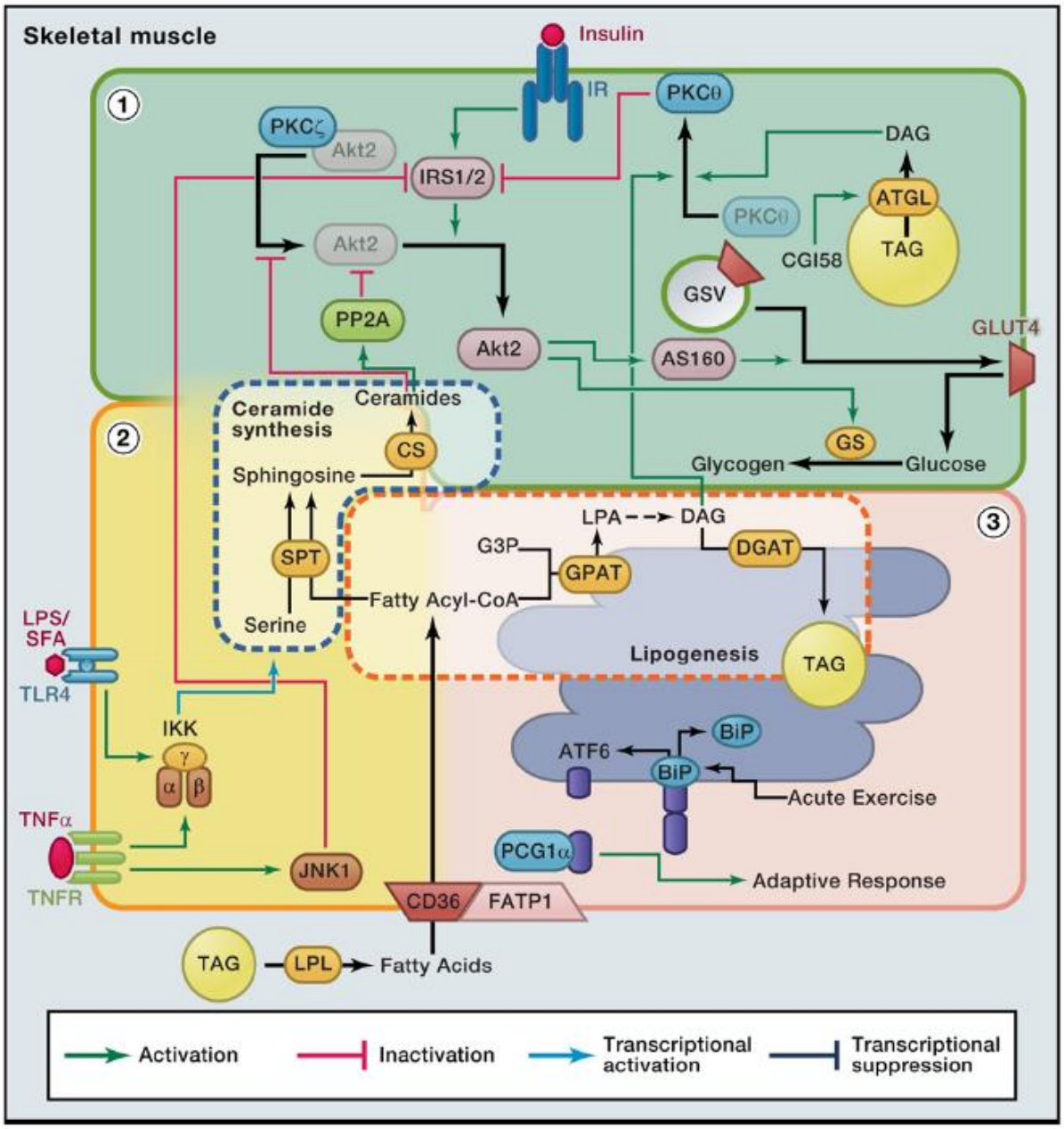

Figure 2: Insulin activates the insulin receptor (IR) tyrosine kinase, which subsequently tyrosine phosphorylates IRS1. Through a series of intermediary steps, this leads to activation of Akt2. Akt2 activation, via AS160 and Rab-GTPase (not shown), promotes the translocation of GLUT4-containing storage vesicles (GSVs) to the plasma membrane, permitting the entry of glucose into the cell, and promotes glycogen synthesis via glycogen synthase (GS). This central signalling pathway is connected to multiple other cellular pathways that are designated by numbers 1–3.

(1) The green shaded areas represent mechanisms for lipid induced insulin resistance, notably diacylglycerol (DAG)-mediated activation of PKCq and subsequent impairment of insulin signalling, as well as ceramide-mediated increases in PP2A and increased sequestration of Akt2 by PKCz. Impaired Akt2 activation limits translocation of GSVs to the plasma membrane, resulting in impaired glucose uptake. Impaired Akt2 activity also decreases insulin-mediated glycogen synthesis.

(2) The yellow areas depict several intracellular inflammatory pathways—notably, the activation of IKK, which may impact ceramide synthesis, and the activation of JNK1, which may impair insulin signalling via serine phosphorylation of IRS1.
(3) The pink area depicts activation of the unfolded protein response (UPR), which under some instances (such as acute extreme exercise) may lead to activation of ATF6 and a PGC1a-mediated adaptive response. The endoplasmic reticulum membranes also contain key lipogenic enzymes and give rise to lipid droplets. Proteins that regulate the release from these droplets (e.g., ATGL and PNPLA3) may modulate the concentration of key lipid intermediates in discrete cell compartments.
CS, ceramide synthase; G3P, glycerol 3-phosphate; IRS, insulin receptor substrate; LPA, lysophosphatidic acid; SPT, serine palmitoyl transferase; TAG, triacylglycerol (from Varman et al. 2012 (27)).

Skeletal muscle lipids are a potential substrate source for energy needs in muscle during exercise in healthy subjects. Exercise has proven to be a viable strategy to increase IMTG use (18, 19, 22, 28). Fat oxidation is increased especially in moderate-intensity exercise (55% of W_{max}), but decreases during high intensity exercise (75% of W_{max}) (28). Furthermore, exercising in an overnight fasted state has been shown to further increase IMTG breakdown (19, 29). However, IMTG breakdown has been shown to be reduced in the presence of high plasma FFA levels (19). Lowering plasma FFA levels by inhibition of adipose tissue lipolysis using acipimox during fasted moderate-intensity exercise has been shown to increase IMTG use and short term insulin sensitivity in T2DM patients (25).

Acipimox is a nicotinic acid/niacin derivate, resulting from the oxidation of the N4 of 5-Me-pyrazine-2-carboxylic acid (figure 3) (30). Niacin has been in use as a antidyslipidemic drug since the 1950s, lowering low density lipoprotein (LDL) and raising high density lipoprotein (HDL)**.** The anti-lipolytic effect of nicotinic acid and its derivates result from the activation of the nicotinic acid receptor HM74A (also known as GPR109)**,** inhibiting adenylyl cyclase (31). This in turn reduces activation of protein kinase A (PKA**).** PKA is known to result in translocating hormone sensitive lipase (HSL) from cytosol to the lipid droplets in adipocytes (32). Acipimox has proven to be more potent than nicotinic acid as an inhibitor of lipolysis with longer-lasting activity (33).

The major unwanted side-effect of nicotinic acid when given at pharmacologically active doses is flushing affecting the upper body and face. The effect consists of cutaneous vasodilation accompanied by a burning sensation (34). While the flushing itself is harmless, it interferes with double-blind study designs.

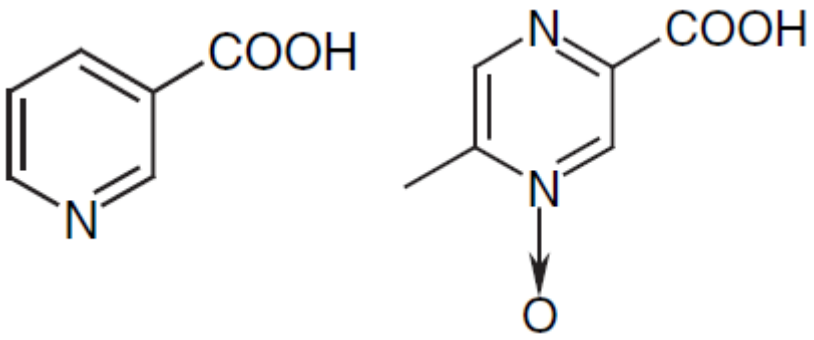

Figure 3: Structure of nicotinic acid receptor ligands niacin (left) and acipimox (right)

In fasting conditions, absorption of the drug is rapid with peak plasma levels being reached within 2 hours after administration. Mean plasma level decrease is fast for the first 8 to 10 hours after oral administration, with a slower phase thereafter (35).

Reducing plasma FFA levels using acipimox has been shown to acutely improve insulin sensitivity in nondiabetic subjects predisposed to T2DM through family history (36), diabetic subjects, (37) and both healthy normal and T2DM in a fasting state (38).

Longer term acipimox administration did not affect glucose tolerance in nondiabetic subjects (39). However, in subjects with a family history of T2DM insulin sensitivity was improved (40). In T2DM patients, long term treatment has been shown to both improve insulin sensitivity (41, 42), and decrease insulin sensitivity through increased levels of plasma FFA in the mornings (43).

Endurance exercise at 50% of VO_{2Max} after acipimox administration in overweight men showed increased IMTG oxidation during exercise, but equal IMTG stores before and after exercise, suggesting increased turnover (44). Lipolytic inhibition through acipimox during moderate-intensity exercise in an overnight fasted state has been shown to increase both glycogen and IMTG use in fit, healthy men and in type 2 diabetic patients (24, 25).

Under physiological circumstances the heart is able to adapt to altered availability of energy sources. This adaptive potential is impaired in insulin resistant states, causing the cardiomyocytes to be dependent on FFA oxidation (45). The effects of reducing FFA availability are not well known. It has been shown that acute acipimox administration in T2DM patients resulted in reduced systolic heart function (46). However, stores of myocardial lipids (MYCL) can serve as an energy source during the temporary reduction in FFA availability.

The effects of acipimox administration during steady-state, moderate-intensity exercise are relatively well known. However, the effects during a maximal cardiopulmonary exercise test

have not yet been described. The goal of this study is to further elucidate the effects of inhibition of adipose tissue lipolysis on acute exercise physiology in male T2DM subjects

2. Methods

2.1 Subjects

Ten male T2DM patients were selected to participate in this study. Inclusion criteria were: blood glycated haemoglobin (HbA1c) >6.5% (48 mmol/mol), age 40-70 years, BMI 25-37.5 kg/m², sedentary lifestyle (<2h sports related activity/week) and Caucasian ethnicity. Exclusion criteria were exogenous insulin therapy or self-reported coronary artery, pulmonary, renal or gastric disease or orthopaedic symptoms that would interfere with exercise. This study was approved by the local medical ethical committee (Jessa Hospital and Hasselt University, Hasselt, Belgium), and the study was performed conform to the standards set by the latest revision (2013) of the Declaration of Helsinki. After thorough explanation about the nature and risks of the experimental procedures, all subjects gave their written informed consent before participating in the study.

2.2 Study design

Each subject was examined twice, one week apart. Subjects arrived at the lab after an overnight fast (>11 h). At the first visit, a blood sample was taken for further analysis**.** Each patient received either a single capsule containing either 250 mg acipimox (ACP) (Nedios, Altana Pharma, Hoofddorp, The Netherlands). or a placebo (PLA) capsule. Subjects were instructed to cease glucose-lowering medication on test days. Subjects recorded their dietary intake the day prior to test days and copied their evening meal to the next visit.

2.3 Anthropometry

At the first visit, after PLA or ACP administration weight and height were assessed in a standardised manner Waist circumference was measured at the midpoint between the top of the iliac crest and the lower margin of the last palpable rib (47). Waist circumference was measured at the top of the iliac crest.

2.4 Indirect calorimetry

Energy expenditure (EE) and respiratory exchange ratio (RER) were measured by indirect calorimetry (Cortex 3000, CORTEX Biophysik GmbH, Germany) over a period of 12 minutes, starting 45 minutes after acipimox or placebo intake.

2.5 Maximal cardiopulmonary exercise test

Subjects performed a maximal cardiopulmonary exercise test on a cycle ergometer (Cortex 3000, CORTEX Biophysik GmbH, Germany) to assess peak oxygen uptake capacity (VO2peak) and maximal workload capacity (Wpeak), using a 1-min work stage protocol (starting workload of 40 W, incremental workload of 20 W). The VCO2 (l/min)), VO2 (ml/min) VE (l/min), tidal volume and respiratory rate were continuously measured on a breath by breath basis with (Cortex 3000, CORTEX Biophysik GmbH, Germany) equipped with an oxygen and carbon dioxide analyser. Volume, ambient air and gas (5.17% CO2, 17.35% O2) calibration was performed before every measurement. Heart rate (HR) was monitored continuously using a 12-lead electrocardiogram. Blood pressure was measured before the start of the test and directly after stopping. 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. Borg Rating of Perceived Exertion Scale for dyspnea and leg fatigue was obtained before exercise and directly after cessation. (48)

2.6 Blood chemistry

During exercise, lactate (Accutrend Plus system, Roche, Basel, Switzerland) concentrations were assessed every two minutes and two minutes post cessation. Venous blood samples (9 ml) were collected in sodium heparin and silicon coated tubes and centrifuged after 15 minutes at 2000 rpm for 10 minutes and after 30 minutes at 3000xg for 10 minutes respectively. Aliquots of plasma and serum were stored at -80 °C until analysis. A venous blood sample (4 ml) was collected in an EDTA tube for blood HbA_{1c} concentration determination (Hi-Auto A1c Analyzer, Menarini Diagnostics, Florence, Italy).

2.7 Calculations

From respiratory measurements during indirect calorimetry total fat oxidation and carbohydrate oxidation rates, as well as energy expenditure were calculated using the nonprotein respiratory quotient (49). Gross exercise efficiency was calculated by $\frac{Mechanical\ power\ (\ kcal/min}{Metabolic\ power\ (kcal/\mathrm{min})} \times 100\%$ (50).

2.8 Statistics

Data are presented as mean±SD. Significance was set at 0.05. Normal distribution was tested with the Shapiro-Wilk test. For indirect calorimetry, Borg ratings and blood pressure comparisons between ACP and placebo groups were performed using the paired T-test or

Wilcoxon signed-rank test. Comparisons between groups at different exercise intensity was performed using two-way analysis of variance for repeated measurements (RM-ANOVA). The paired t-test with Bonferroni correction for multiple comparison was used for post hoc testing. All data were analysed in IBM SPSS Statistics v24.0 (SPSS Inc., Chicago, IL, USA)

3. Results

3. 1 Subject characteristics

Ten male T2DM patients (age 57±8 years, 8.6±4.0 years since diagnosis) with well controlled diabetes (HbA_{1c} 7.2±0.97% (55.1±10.6 mmol/mol)) were screened and selected for this study. Subjects were overweight or obese (BMI 31.8±2.8 kg/m²). All subjects were treated with oral glucose lowering medication metformin (n=10). One subject has been excluded in heart rate and oxygen pulse analyses due to increased heart rate caused by atrial fibrillation (table 1).

Table 1: Subject characteristics

Variable	
Age (years)	57±8
Weight (kg)	104±11
Height (cm)	181±5
BMI (kg/m²)	31.8±2.8
Waist/Hip ratio	1.09±0.03
HbA1c (%)	7.1±0.96
HbA1c (mmol/mol)	54.1±10.5
Years since diagnosis	8.6±4.0
Metformin (n)	10
Statins (n)	6
Sulfonylurea (n)	3
Salicylate (n)	2
ACE inhibitor (n)	1
Calcium antagonist (n)	1
Fibrates (n)	1
Meglitinides (n)	1

Data are presented as mean±SD. HbA1c, blood glycated hemoglobin

3.2 Indirect calorimetry

VO_2 and VCO_2 (l/min) were not different between PLA and ACP (0.40±0.07 vs. 0.41±0.06, p = 0.456 and 0.33±0.05 vs. 0.35±0.05 l/min, p = 0.111, respectively). RER tended to be lower in PLA compared to ACP (0.81±0.04 vs. 0.84±0.04, p = 0.052). Carbohydrate oxidation was increased in ACP (0.75±0.28 vs. 1.00±0.28 kcal/min, p = 0.036), whereas fat oxidation was

not significantly different (1.25±0.42 vs. 1.08±0.39 kcal/min, p = 0.147). Energy expenditure was equal between PLA and ACP (2.00±0.33 vs. 2.08±0.32 kcal/min, p = 0.349) (Table 2).

Table 2: Substrate use during indirect calorimetry.

	Placebo	Acipimox	p-value
VO_2 (l/min)	0.40±0.07	0.41±0.06	0.456
VCO_2 (l/min)	0.33±0.05	0.35±0.05	0.111
RER	0.81±0.04	0.84±0.04	0.052
Carbohydrate ox. (kcal/min)	0.75±0.28	1.00±0.28	**0.036**
Carbohydrate ox. (% EE)	38.5±15.2	49.03±15.2	0.059
Fat ox. (kcal/min)	1.25±0.42	1.08±0.39	0.147
Fat ox. (% EE)	61.5±15.2	50.97±15.2	0.059
Energy expenditure (kcal/min)	2.00±0.33	2.08±0.32	0.349

Data are shown as mean±SD. RER, respiratory exchange ratio; EE, energy expenditure; ox. oxidation.

3.3 Maximal cardiopulmonary exercise test

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated for exercise intensity in the case of VO_2, VCO_2, VE, $VEqO_2$ and $VEqCO_2$ (χ^2 (20) = 67.77, p < 0.001, χ^2 (20) = 52.79, p < 0.001, χ^2 (20) = 72.89, p < 0.001, χ^2 (20) = 55.37, p < 0.001, χ^2 (20) = 77.57, p < 0.001, respectively), therefore the Greenhouse-Geisser correction was used. A significant main effect of treatment on RER was found (F (1, 9) = 12.50, p = 0.006), but not for other parameters (p > 0.05). In all cases, there was a significant main effect of exercise intensity (p < 0.05) (table 3, 4. Figure 4).

Table 3: Two-way RM ANOVA of exercise parameters

	Treatment		Intensity	
	F (1, 9)	p–value	F (6, 54)	p-value
VO_2, l/min	0.027	0.0874	628.66	< **0.001**
VCO_2, l/min	2.14	0.178	550.54	< **0.001**
RER	12.5	0.006	192.73	< **0.001**
VE, l/min	0.66	0.438	145.34	< **0.001**
$VEqO_2$	3.47	0.096	48.14	< **0.001**
$VEqCO_2$	0.368	0.559	6.130	**0.025**

Table 4: parameters during exercise

Exercise Intensity, %VO_{2peak}	VO_2, l/min			VCO_2, l/min			RER		
	placebo	acipimox	p-value	placebo	acipimox	p-value	placebo	acipimox	p-value
40	1.05±0.08	1.03±0.13	0.534	0.81±0.09	0.85±0.13	0.229	0.77±0.04	0.82±0.07	**0.030**
50	1.26±0.10	1.25±0.18	0.803	1.04±0.15	1.09±0.19	0.117	0.83±0.09	0.88±0.08	**0.019**
60	1.49±0.14	1.46±0.21	0.443	1.37±0.24	1.40±0.24	0.486	0.91±0.10	0.96±0.08	**0.007**
70	1.71±0.13	1.71±0.22	0.932	1.71±0.26	1.78±0.30	0.193	1.00±0.10	1.04±0.10	0.083
80	1.95±0.15	1.96±0.28	0.946	2.08±0.27	2.13±0.33	0.376	1.07±0.09	1.09±0.10	0.287
90	2.19±0.18	2.20±0.32	0.875	2.47±0.30	2.57±0.35	0.225	1.13±0.08	1.17±0.08	0.058
100	2.40±0.20	2.39±0.34	0.898	2.85±0.37	2.91±0.40	0.458	1.19±0.09	1.22±0.09	0.120
	VE, l/min			$VEqO_2$			$VEqCO_2$		
	placebo	acipimox	p-value	placebo	acipimox	p-value	placebo	acipimox	p-value
40	25.0±3.3	25.7±3.9	0.357	22.5±3.0	23.7±2.8	0.112	29.2±3.5	28.9±3.4	0.581
50	31.1±4.8	31.5±4.6	0.690	23.6±3.4	24.3±2.7	0.285	28.7±3.5	27.8±3.2	0.320
60	39.6±7.2	39.9±6.5	0.849	25.5±3.7	26.3±2.7	0.179	28.0±3.2	27.6±3.1	0.386
70	49.5±8.3	51.1±9.10	0.449	28.0±3.8	28.9±4.4	0.340	28.1±3.3	27.9±3.1	0.426
80	61.6±11.9	62.9±11.3	0.673	30.6±5.3	31.4±5.2	0.561	28.7±3.8	28.7±3.4	0.677
90	74.9±15.1	79.5±12.4	0.250	33.3±6.1	35.4±5.0	0.140	29.5±4.2	30.2±3.2	0.734
100	93.4±20.3	94.3±20.6	0.792	37.9±7.7	38.6±7.3	0.192	31.9±5.3	31.4±4.1	0.693

Data are presented as mean±SD. RER, respiratory exchange ratio

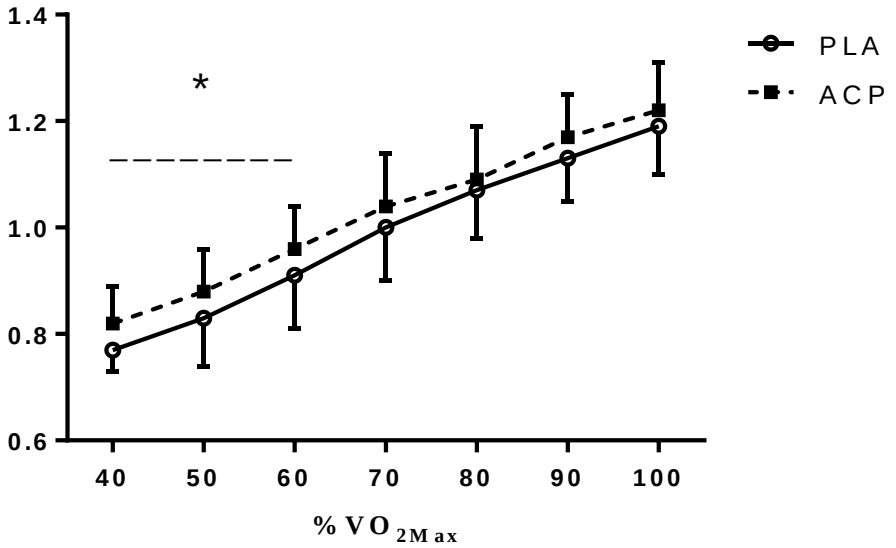

Figure 4: Respiratory exchange ratio during exercise. *, significant at $p < 0.05$

3.4 Ventilatory thresholds

During exercise, at the first ventilatory threshold. VO_2 tended to be lower in ACP (PLA 1.42 ±0.24 vs. ACP 1.37±0.13, p = 0.051). The respiratory compensation point (RCP) was reached earlier in ACP (PLA 424±56 vs. ACP 382±59 s, p = 0.009), corresponding with a lower VE (PLA 69.5±13.2 vs. ACP 61.9±14.7 l/min, p = 0.026) and VO_2 (PLA 2.1±0.24 vs. 1.93±0.25 l/min, p = 0.026) (table 5).

Table 5: Parameters at the anaerobic threshold and the respiratory compensation point

AT	Time, s	WR. W	VE. l/min	VO_2. l/min	VCO_2. l/min
Placebo	222±77	107±26	38.3±10.8	1.42±0.24	1.29±0.28
Acipimox	186.67±35	91±15	35.8±4.9	1.37±0.13	1.22±0.13
p-value	0.23	0.235	0.2	0.051	0.384
RCP					
Placebo	424±56	168±22	69.5±13.2	2.1±0.24	2.33±0.36
Acipimox	382±59	158±18	61.9±14.7	1.93±0.25	2.1±0.32
p-value	**0.009**	0.461	**0.026**	**0.028**	0.053

AT	RER	$VEqO_2$	$VEqVCO_2$	% VO_2Peak
Placebo	0.9±0.05	25.5±3.7	28.1±3.2	59±12.9
Acipimox	0.89±0.08	25.0±3.1	28.1±2.7	56.3±7.4
p-value	0.586	0.202	0.42	0.35
RCP				
Placebo	1.10±0.07	32.1±4.9	29.0±3.8	81.6±5.4
Acipimox	1.09±0.09	31.0±5.5	28.4±3.6	80.2±8.2
p-value	0.528	0.282	0.292	0.48

3.4 Lactate

Lactate was not different between groups after 2, 4 and 8 minutes of exercise ($p > 0.05$). After 6 minutes of exercise lactate values were higher in ACP compared to PLA (PLA 2.9±0.9 vs. ACP 3.2 ±0.9 mmol/l). No differences were detected during the recovery phase ($p = 0.553$) (table 6).

Table 6: Lactate concentrations during CPET

Time (minutes)	Placebo	Acipimox	p-value
2	1.9±0.6	2.2±0.5	0.201
4	2.3±0.6	2.5±0.3	0.325
6	2.9±0.9	3.2±0.9	**0.041**
8	3.6±1.0	4.1±1.3	0.283
Recovery	6.1±2.0	5.8±2.2	0.553

Data are shown as mean±SD, mmol/l

3.5 Borg ratings

Before exercise, Borg rating were not different between groups for both dyspnea (PLA 9.3±2.6 vs. ACP 7.8±1.5, $p = 0.415$) and physical exertion (PLA 8.8±2.1 vs. ACP 10.0±1.4, $p = 0.403$). After exercise, ratings were significantly higher compared to pre-exercise conditions ($p < 0.05$), but not different between PLA and ACP (dyspnea PLA 16.3±1.9 vs. ACP 17.3±1.7, $p = 0.480$, physical exertion PLA 15.0±2.8 vs. 16.5±1.9, $p = 0.597$) (table 7).

Table 7: Borg rating of perceived exertion and dyspnea before and after exercise

Placebo		Acipimox		p-value
Pre-exercise				
Dyspnea	9.3±2.6	Dyspnea	7.8±1.5	0.415
Physical exertion	8.8±2.1	Physical exertion	10.0±1.4	0.403
Post-exercise				
Dyspnea	16.3±1.9	Dyspnea	17.3±1.7	0.480

Physical exertion	15.0±2.8	Physical exertion	16.5±1.9	0.597

Data are shown as mean±SD.

3.6 Exercise efficiency

There was a significant main effect of exercise intensity ($F(6, 54) = 9.07$, $p < 0.001$), but not of treatment ($F(1, 9) = 0.473$, $p = 0.509$). Post hoc testing revealed no significant differences between PLA and ACP at any exercise intensity ($p > 0.05$), or between different levels of exercise intensity ($p > 0.05$) (table 8).

Table 8: Gross exercise efficiency during exercise

Exercise intensity	Placebo	Acipimox	p-value
40%	20.0±3.0	18.9±4.5	0.481
50%	20.8±2.8	20.7±3.5	0.955
60%	21.2±2.4	22.0±1.8	0.172
70%	23.1±2.7	22.4±1.8	0.287
80%	22.9±1.5	22.5±1.3	0.192
90%	22.9±1.9	23.4±1.8	0.463
100%	23.8±1.9	23.2±0.8	0.292

Data are shown as mean±SD, gross efficiency %.

3.7 Heart rate

During rest, heartbeat was equal between both groups (72±12 vs. 74±10 beats/min, $p = 0.496$). Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated for exercise intensity, $\chi^2(20) = 39.54$, $p = 0.011$, and therefore the Greenhouse-Geisser correction was used. There was a significant main effect of exercise intensity ($F(6, 48) = 175.75$, $p < 0.001$), but not of treatment ($F(1, 8) = 0.18$, $p = 0.682$). Post hoc testing revealed no significant differences at any exercise intensity ($p > 0.05$) (table 9).

Table 9: Heart Rate during rest and exercise

Exercise intensity	Placebo	Acipimox	p-value
Rest	72±12	74±10	0.496
40%	92±11	92±10	0.861
50%	97±14	99±11	0.430
60%	109±17	109±14	0.692
70%	121±20	119±15	0.460
80%	129±21	132±16	0.512
90%	142±21	145±17	0.282
100%	149±18	151±17	0.678

Data are shown as mean±SD, beats/min

3.8 Oxygen pulse

During exercise, there was a significant main effect of exercise intensity ($F(6, 48) = 59.52$, $p < 0.001$), but not of treatment ($F(1, 8) = 0.001$, $p = 0.972$) on oxygen pulse. Post hoc testing indicated no significant differences between placebo and acipimox treatment at any level of exercise intensity ($p > 0.05$) (table 10).

Table 10: Oxygen pulse at rest and during exercise

Exercise intensity	Placebo	Acipimox	p-value
Rest	5.7±1.4	5.7±1.3	0.924
40%	11.7±1.6	11.4±1.6	0.586
50%	13.2±1.7	13±1.9	0.655
60%	13.9±1.8	13.8±2.2	0.828
70%	14.5±2.2	14.9±2.5	0.340

80%	15.5±2.7	15.4±3	0.789
90%	15.6±2.4	15.7±2.9	0.881
100%	16.3±1.9	16.4±2.9	0.872

Data are shown as mean±SD, ml O_2/heartbeat

3.9 Blood pressure

Diastolic and systolic blood pressure before exercise were not significantly different between conditions (87.1±7.4 vs. 87.6±8.5 and 143.4±10.6 vs. 139.0±12.1 mmHg, respectively). Diastolic blood pressure was not significantly different post-exercise between PLA and ACP (88.9±5.0 vs. 92.4±14.2 mmHg), but systolic pressure tended to be lower in the ACP group (176.2±12.3 vs. 165.2±22.1 mmHg, p = 0.094) (table 11).

Table 11: Blood pressure before and after exercise

Placebo		Acipimox		p-value
Pre-exercise				
Diastolic pressure	87.1±7.4	Diastolic pressure	87.6±8.5	0.754
Systolic pressure	143.4±10.6	Systolic pressure	139.0±12.1	0.342
Post-exercise				
Diastolic pressure	88.9±5.0	Diastolic pressure	92.4±14.2	0.45
Systolic pressure	176.2±12.3	Systolic pressure	165.2±22.1	0.094

Data are shown as mean±SD, mmHg.

4. Discussion

In the present study, we show that by inhibiting adipose tissue lipolysis, using nicotinic acid derivative acipimox, during a maximal cardiopulmonary exercise test in male T2DM patients results in a left shift of respiratory compensation point (RCP) compared to placebo intake. This shift corresponds with higher lactate concentrations at this threshold. At rest and during the incremental protocol until the anaerobic threshold (AT), acipimox significantly altered substrate oxidation with higher glucose oxidation. After the AT, acipimox did not alter substrate use. Acipimox did not alter heart rate (HR), oxygen pulse or blood pressure.

4.1 Acipimox changes substrate oxidation during rest

During the resting period, acipimox increased carbohydrate oxidation, along with a proportionally higher carbohydrate oxidation and lower fat oxidation, as reflected by an increased RER in ACP. These data correspond with the lowered availability of plasma free fatty acids (FFA) as a result of acute acipimox intake and the resulting shift in substrate use (25, 44).

4.2 Moderate-intensity exercise is optimal for acipimox invervention

Acipimox administration alters substrate use during at moderate-intensity exercise as well (25, 44). At high intensity exercise substrate use shifts to glucose, with minimal fat oxidation (51) Above the anaerobic threshold (AT), to lactate use becomes more pronounced as well (28). In ACP, RER is higher at $\%VO_{2Peak}$ between 40 and 60%, suggesting increased carbohydrate oxidation during moderate-intensity exercise. Beyond the anaerobic threshold no differences in RER were observed. Acipimox caused a leftward shift in RCP, corresponding with increased lactate concentrations after 6 minutes of exercise.

During high-intensity exercise, is the primary fuel source (51). Acipimox does not appear to further shift substrate use during high intensities. An elevated carbohydrate oxidation during the moderate-intensity stages of the incremental exercise test causes a depletion of glucose stores and thus shift to lactate use earlier in the test.

In high-intensity interval training (HIIT), brief intervals of high intensity exercise are interspersed with periods of low activity or rest. The goal of HIIT is to accumulate activity at an intensity normally unsustainable for prolonged periods of time (52). is has been shown to acutely improve same-day postprandial glucose control in T2DM patients (52), and HIIT exercise programmes increase IMTG breakdown (20). As acipimox no longer affects substrate metabolism at high-intensity exercise, there may be little added value of combining acipimox with a HIIT exercise intervention.

4.3 Acipimox administration does not affect heart function

Relatively little is known of the effects of acipimox administration on the heart. Wolf et al. have shown that, during rest, acipimox administration results in reduced systolic function (reduced ejection fraction and cardiac index) (46), whereas longer term acipimox administration in non-diabetic patients with chronic heart failure did not change diastolic or systolic function (53, 54). Wolf et al. studied 8 T2DM patients, comparable to the subjects in this study (age 56±11 years, BMI 28±3.5 kg/m², HbA1c 7.3±0.9% vs. age 57±8 years, BMI

31.8±2.8 kg/m², HbA1c 7.1±0.96%, respectively) by ^{1}H-magnetic-resonancespectroscopy and tomography at baseline, 2 and 7 hours with either 250 mg acipimox or placebo administration at baseline and 3 hours. A significant reduction in ejection fraction was found only after 7 hours, or 4 hours after the last acipimox administration. The reduction in systolic function was accompanied by a significant reduction in myocardial lipids (MYCL) (46). The acute impact of acipimox administration on systolic function appears to be of no relevance during exercise one hour post administration.

Both heart rate and oxygen pulse increase as a function of exercise intensity, with no differences between PLA and ACP. Oxygen pulse can be used as an indicator for left-ventricle stroke volume and peripheral oxygen extraction during exercise further suggesting no impact of acipimox on systolic function during exercise one hour post acipimox administration (55). Pre-exercise and post-exercise systolic and diastolic blood pressure show no difference between PLA and ACP, suggesting no compensation for an altered heart rate.

However, it should be noted that other parameters of heart function have not been measured (i.e. ejection fraction, stroke volume). It may be of added value to evaluate heart function in more detail during a stress echocardiogram at different timepoints post acipimox administration.

4.4 Study Limitations

There are some limitations to the present study. First, the study has a small number of participants, leaving it underpowered. Second, the study only included male T2DM patients treated with oral glucose-lowering medication and no exogenous insulin therapy. Furthermore, this subset of participants had relatively well controlled T2DM, which may influence acipimox administration effects (56). Thus, extrapolating the results to female T2DM patients, patients using other diabetes medication or patients with worse diabetes control should be done cautiously.

5. Conclusion

Our results show that adipose tissue lipolytic inhibition during maximal cardiopulmonary exercise testing shifts substrate metabolism towards carbohydrate use at intensities corresponding to moderate-intensity exercise, suggesting that moderate-intensity exercise is optimal for an acipimox-assisted exercise intervention.

5.1 Future recommendations

Future studies should aim to elucidate the effect of acipimox during exercise on insulin sensitivity (using a two—step hyperinsulinemic euglycemic clamp protocol). While one bout of exercise combined with acipimox improves glucose control in fasted T2DM subjects (25), the effects of long-term exercise intervention with acipimox remain unknown. The increased glucoregulatory effects of each bout of exercise may prove further augment glycemic control in T2DM patients (57). However, over time plasma FFA levels may rise during the day, counteracting the effects of acipimox and potentially reducing the efficacy of such an intervention (43). Lastly, as T2DM patients are at great risk of developing cardiovascular diseases, the effects of acipimox during exercise could be further investigated.

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