



UHASSELT

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Faculteit Geneeskunde en Levenswetenschappen

master in de revalidatiewetenschappen en de
kinesitherapie

Masterthesis

Optimization of exercise therapy in type 2 diabetes by blocking lipid break down

**Quinten Mertens
Eva Vranken**

Eerste deel van het scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

PROMOTOR :

dr. Kenneth VERBOVEN



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Optimization of Exercise Therapy in Type 2 Diabetes by Blocking Lipid Breakdown.

“Can we optimize glycaemic control by inhibiting lipolysis during exercise in Type 2 diabetes patients?”

HIGHLIGHTS

- No inhibition of adipose tissue lipolysis by lactate infusion during exercise.
- Inhibition of adipose tissue lipolysis by intravenous glucose administration and by nicotinic acid drug 'Acipimox' during exercise.
- Increased whole-body insulin sensitivity during recovery time after lipolysis inhibition for T2DM patients.
- Lower plasma glucose concentrations at two hours post-exercise after lipolysis inhibition in T2DM patients.
- Further research including more subjects and a longer follow-up of the effects of lipolysis inhibition combined with aerobic exercise in patients with T2DM is recommended.

Mertens Quinten
Vranken Eva
Dr. Verboven Kenneth

CONTEXT OF THE MASTER THESIS

The research domain of this master thesis is rehabilitation of metabolic diseases. This domain is characterized by patients with heart and lung conditions. These conditions are heavily studied. But this domain encompasses more than those conditions alone. That is why this master thesis is based on the condition called Type 2 diabetes mellitus (T2DM). T2DM patients are characterized by difficulties maintaining their glycaemic control.

The research on T2DM is important because of its rising prevalence and a lot of things still remaining unknown. One of the topics that needs further investigation is the glycaemic control of T2DM patients during exercise. More specifically, how to control the glycaemic parameters using physical therapy interventions and this possibly in combination with drugs. This topic is relevant to physical therapists in the rehabilitation of T2DM patients, because exercise is known to be, next to pharmacology and diet, one of the key factors in the treatment of T2DM. Therefore, the literature study of this master thesis focused on the following research question: "Can we optimize glycaemic control by inhibiting lipolysis during exercise in T2DM patients?".

This thesis followed the central format.

This master thesis Part 1 was a duo master thesis and is part of the first master year at Hasselt University. Part 2 of this master thesis will be done at the BIOMED-REVAL Rehabilitation research center of Hasselt University. This master thesis is part of a broader research project in the context of optimization of exercise therapy for metabolic diseases coordinated by Prof. Dr. Dominique Hanssen and Dr. Kenneth Verboven.

The literature search was performed and written by two master students of rehabilitation science and physiotherapy, under supervision of their promotor Dr. Kenneth Verboven.

The research question was determined by the two students in consultation with their promotor. The literature search was performed by both students together. Based on the literature study, a new study protocol was written by the students. The research has not started yet.

Both students collaborated equally in the realization of this master thesis Part 1.

PART 1: LITERATURE SEARCH

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1. ABSTRACT

Background: Patients with Type 2 diabetes (T2DM) have difficulties maintaining their glycaemic control, in which the lipid metabolism might intervene. High plasma free fatty acid concentrations are known to inhibit glucose uptake. Therefore, inhibition of lipid breakdown in combination with exercise, one of the cornerstones in T2DM care, may have potential benefits in the management of glycaemic control for T2DM patients.

Methods: This literature search was performed using the databases PubMed and Web of Science. The search strategy included “*lipolysis inhibition*” combined with either “*endurance exercise*”, “*aerobic exercise*” or “*exercise*”. “*Type 2 diabetes mellitus*” was added or not to these combinations. Four studies were included in the final literature study.

Results: In healthy and T2DM subjects, lipolysis inhibition had no significant effect on plasma glucose and plasma insulin concentrations during exercise compared to exercise without lipolysis inhibition.

Discussion and conclusion: In T2DM patients, whole-body insulin sensitivity was significantly increased during recovery. Therefore, further investigation about postprandial and long-term effects is recommended for its potential clinical relevance in treatment of T2DM.

Aim of this research: The aim of this literature search was to give an overview of all the available literature on the topic related to adipose tissue lipolysis inhibition during exercise in T2DM patients.

Operationalization: This master thesis Part 1 is part of a broader research project led by Prof. Dr. Dominique Hanssen and Dr. Kenneth Verboven in which optimization of exercise therapy in rehabilitation of T2DM is the main theme.

Most important key words: T2DM, lipolysis inhibition, exercise

2. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease affecting many people worldwide and its prevalence is rapidly increasing. The past three decades, the number of people diagnosed with diabetes mellitus has doubled, resulting in a prevalence of approximately 260 million people worldwide (Chen, Magliano, & Zimmet, 2011). In Belgium the prevalence in 2013 was approximately 515 thousand and is estimated to be 604 thousand by 2035 (Guariguata et al., 2014).

This trend might be attributed to factors such as aging and an increase in sedentary behaviour and obesity prevalence, the latter being a main contributor to the development of T2DM (Geiss et al., 2014). Individuals with obesity are often characterized by insulin resistance, the pre-stage of T2DM. (Kahn, Cooper, & Del Prato, 2014). Obesity-related whole-body insulin resistance implies a state in which several peripheral tissues (including skeletal muscle, adipose tissue and liver) are less or even not responsive to insulin, resulting in reduced uptake of plasma glucose and the development of hyperglycaemia (Kahn et al., 2014).

Unger (2003) has proposed the 'lipid overflow hypothesis' to explain ectopic fat accumulation. This hypothesis suggests that obesity-associated metabolic alterations result from leptin resistance and a failure in the capacity of appropriate adipose tissue expansion and thereby storing lipids in non-adipose tissues where it results in 'lipotoxicity' (Mittendorfer, 2011). Of interest, accumulation of intramuscular fatty acids, as seen in individuals with obesity and T2DM, plays an important role in the pathogenesis of insulin resistance (Shulman, 2000). Recent data also suggest that high concentrations of plasma free fatty acids (FFAs) inhibit glucose uptake by decreasing whole-body glucose oxidation rates and muscle glycogen synthesis (Shulman, 2000). Ultimately, obesity-related insulin resistance will lead to a state of hyperglycaemia and hyperinsulinemia, and accounts for the progression from impaired glucose tolerance to the development of T2DM (Kahn et al., 2014).

Chronic hyperglycaemia is known to be related to renal, visual, neurological and cardiovascular complications (Park, PhD, Lee, & PhD, 2015), indicating the need for further optimization of glycaemic control in T2DM and its related complications (Park et al., 2015). Along with diet and pharmacologic intervention, exercise is considered as one of the cornerstones in the management of diabetes. For individuals with T2DM, current guidelines recommend aerobic exercise training for minimum 150 min/week (30 min, 5 days/week) at moderate intensity (40-60% VO_2max) or 60 min/week of vigorous intensity (20 min on 3 days) (Colberg et al., 2010). A meta-analysis showed a strong dose-response relationship between exercise intensity and changes in cardiorespiratory fitness and glycated haemoglobin levels, respectively (Colberg et al., 2010).

Furthermore, endurance-type exercise should be combined with resistance exercise at least 2-3 times a week at a moderate (50% 1 Repetition Maximum [RM]) or vigorous (75-80% 1RM) intensity targeting the major muscle groups (Colberg et al., 2010).

Despite these recommendations for physical activity or exercise, long-term compliance is a major concern in patients with T2DM. Recent studies have shown that up to one third of adults with diabetes are completely sedentary and only one out of three exercises regularly (Thomas, Alder, & Leese, 2004).

FFAs are a major link between obesity and insulin resistance/hyperinsulinemia. Interestingly, it has been shown that lowering of chronically elevated plasma FFA concentration would improve whole-body insulin resistance/hyperinsulinemia and glucose tolerance in obese nondiabetic and diabetic individuals (Santomauro et al., 1999). The release of adipose tissue-derived FFAs can be inhibited with a lipid lowering drug, Acipimox, which specifically and temporarily inhibits adipose tissue lipolysis (Santomauro et al., 1999). It is known that acute inhibition of lipolysis also improves insulin action in patients with non-insulin-dependent diabetes mellitus, by increasing both insulin-stimulated glucose oxidation and nonoxidative glucose disposal (Vaag et al., 1991).

Thus, elevated FFA levels may be an important target for the (co-)treatment of insulin resistance T2DM.

However, it remains elusive whether lipolytic inhibition (acutely or chronically) is a suitable alternative or adjuvant therapy to exercise therapy in order to improve metabolic health and thus glycaemic control in patients with T2DM (Santomauro et al., 1999; Vaag et al., 1991). Therefore, the aim of this literature search is to gain insight into literature investigating exercise combined with lipolytic inhibition and the effect on metabolic parameters.

3. METHOD

3.1 Research question

The aim of this literature search was to give an overview of all the available literature on the topic related to adipose tissue lipolysis inhibition during exercise in patients with T2DM. The research question of this literature review was: “Can we optimize glycaemic control by inhibiting lipolysis during exercise in T2DM patients?”. Studies with non-diabetic patients were included because of the limited availability of studies on adipose tissue lipolysis inhibition in T2DM patients.

3.2 Literature search

Literature search was performed using the databases of PubMed and Web of Science.

The predetermined PICO of the literature search was:

- Population: Humans, non-diabetics, patients with Type 2 diabetes mellitus
- Intervention: Lipolysis inhibition during endurance exercise
- Comparison: No lipolysis inhibition during endurance exercise
- Outcome: Glycaemic control parameters including plasma glucose concentration and plasma insulin concentration primary and substrate utilization and whole-body insulin sensitivity secondary.

The main term “lipolysis inhibition” was combined with either “endurance exercise”, “aerobic exercise” and “exercise”. “Type 2 diabetes mellitus” was added or not to these combinations. The filter “species: humans” was added to the search build on PubMed. The six different combinations were entered in the web data bases PubMed and Web of Science, of which the resulting number of hits (January 2018) are shown in Table 1. The literature search was repeated in May 2018. There were no new studies found. The results are shown in Table 2.

3.3 Selection criteria

The inclusion criteria of this literature review are:

- Language: English or Dutch
- Non-diabetic patients
- Type 2 diabetic patients
- Humans
- Lipolysis inhibition during endurance exercise
- Monitoring glycaemic control parameters (including plasma glucose concentration, plasma insulin concentration and plasma FFA concentration primary and substrate utilization, whole body insulin sensitivity and lactate concentration secondary)

The exclusion criteria of this literature review are:

- Interventions other than combined exercise and lipolysis inhibition.
- Studies that included specific pathological populations (for example: hypertension, postmenopausal, burned, pregnant, surgical) except for Type 2 diabetes mellitus.
- Involvement of animals.
- The use of medication that does not only inhibit the lipolysis.
- Irrelevant outcome measures.

These exclusion criteria were applied during initial screening for relevance, based on title and abstract of every study.

3.4 Quality assessment

All included studies were experimental studies. No specifications of study design were made by the authors. Consequently, the ExDes checklist was used to assess the quality. An overview of the criteria used to assess the quality is shown in Table 3.

3.5 Data-extraction

The data extraction was performed for all included studies for the following parameters: general subject characteristics (age [yr], weight [kg], baseline VO_2max [$\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$], type of population and the number of subjects), intervention characteristics (method for lipolytic inhibition), exercise characteristics (modalities and protocol details) and relevant outcomes/results with respect to glycaemic control (primary: plasma glucose, plasma insulin and plasma FFA; secondary: substrate utilization, whole body insulin sensitivity and lactate). An overview of the data extraction can be found in Table 4, 5, 6, 7, 8, 9, 10 and 11.

4. RESULTS

4.1 Results study selection

The full study selection is displayed in Figure 1. During the literature search, 98 studies were found on PubMed and 88 studies on Web of Science. One hundred and one duplications were removed. Sixteen of the 85 studies were removed because these were reviews. Sixty-nine studies were maintained following selection based on inclusion criteria and removal based on design (review). These studies were assessed by title and abstract. Sixty-two studies were excluded (no involvement of exercise nor lipolysis inhibition: 13; specific pathological population: 10; animal studies: 23; medication: 14; irrelevant outcomes: 2). Articles meeting selection criteria were: Boyd, Giamber, Mager, and Lebovitz (1974); Coyle, Jeukendrup, Wagenmakers, and Saris (1997); Guler, Walter, Morell, and Froesch (1982); Hales, Luzio, and Siddle (1978); Trudeau et al. (1999); van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005). The full text of three of the last seven studies were inaccessible. The authors of these studies were contacted on LinkedIn and ResearchGate. No response was given. The impact of these studies would be limited because of their date of publication. A total of 4 studies which met the selection criteria were used in this literature study.

4.2 Results quality assessment

The quality assessment was done by two independent researchers using the ExDes Checklist. The results of the quality assessment are shown in Table 3.

All four studies showed similar results in the total score of the quality assessment. The studies van Loon, Manders, et al. (2005), van Loon, Thomason-Hughes, et al. (2005), Trudeau et al. (1999) and Coyle et al. (1997) scored 82%, 80%, 78% and 82% respectively. The arbitrary cut-off was placed at 75% (90 points). All studies were maintained after quality assessment. There are differences between studies in some components of the checklist.

All studies have a score of 0 on "Qualitative Observations". This is because the studies are all quantitative studies rather than qualitative studies. Trudeau et al. (1999) scores the lowest on section "Quantitative Data" because the study does not have condensed table(s) containing most important data. On the section "Possible Experimental Errors" Coyle et al. (1997) scores maximum in contrast to the other studies. The other studies do not discuss the effect errors might have on the data presented. The same studies did not mention application and recommendations for further use.

4.3 Results data-extraction

4.3.1 Subjects

All data with respect to the subjects' characteristics can be found in Table 4. The population studied in three studies were men with good physical fitness (Coyle et al., 1997; Trudeau et al., 1999; van Loon, Thomason-Hughes, et al., 2005). In the other study the subjects were overweight patients diagnosed with T2DM (van Loon, Manders, et al., 2005). The number of subjects included ranged from 6 subjects (Coyle et al., 1997) to 10 subjects (van Loon, Thomason-Hughes, et al., 2005).

The average age (yr) of the subjects was similar in three of the four studies, 23, 22 and 26 in van Loon, Thomason-Hughes, et al. (2005), Coyle et al. (1997) and Trudeau et al. (1999), respectively. In the study of van Loon, Manders, et al. (2005) which included subjects who were overweight and diagnosed with T2DM, the average age was 60.

The average weight (kg) of the subjects was 74, 69, 74 and 91 in the studies of van Loon, Thomason-Hughes, et al. (2005), Coyle et al. (1997), Trudeau et al. (1999) and van Loon, Manders, et al. (2005), respectively.

The average BMI could not be calculated. Coyle et al. (1997) and Trudeau et al. (1999) did not measure the height of the subjects.

The average VO₂max (ml/kg.min) was similar in the same three studies including healthy young men, 62, 72 and 60 in van Loon, Thomason-Hughes, et al. (2005), Coyle et al. (1997) and Trudeau et al. (1999), respectively. In the study of van Loon, Manders, et al. (2005), which included overweight subjects diagnosed with T2DM, the average VO₂max was lower, namely 32.

4.3.2 Method of lipolysis inhibition.

The following data can be found in Table 5.

The aim of all studies was to inhibit adipose tissue lipolysis (directly/indirectly) during exercise to investigate the effect(s) on glycaemic control. The method used to inhibit lipolysis varied among studies.

In the study of Coyle et al. (1997), intravenous glucose feeding (1.4g/kg bodyweight) 60 and 10 minutes before exercise was used to inhibit lipolysis. Plasma FFA concentration in the fasting trial were maintained at pre-exercise concentration during the exercise bout, however in the glucose ingestion trials they were significantly lower at all points in time compared to the fasting trial. Intravenous glucose feeding is an effective method to inhibit lipolysis during exercise, based on these findings.

In the study of Trudeau et al. (1999) adipose tissue lipolysis was partially inhibited by lactate infusion (16 mM) at a perfusion rate of 2.5 µl/min locally in abdominal subcutaneous adipose tissue using the micro-dialysis technique. After lactate infusion, to inhibit adipose tissue lipolysis, dialysate glycerol increased significantly during exercise compared to pre-exercise concentration, and decreased significantly during recovery compared to exercise concentration. No significant difference between the lactate probe and the control probe was found. Plasma FFA and glycerol levels increased significantly during exercise with highest levels after exercise. Lactate infusion was not an effective method to inhibit lipolysis during exercise, based on these findings.

In two studies (van Loon, Manders, et al., 2005; van Loon, Thomason-Hughes, et al., 2005), adipose tissue lipolysis was partially inhibited by Acipimox (250 mg).

In the study of van Loon, Manders, et al. (2005), Acipimox was administered 120 minutes before and 30 minutes into the exercise bout, plasma FFA were significantly lower for subjects in the Acipimox group compared to the placebo group. In the study of van Loon, Thomason-Hughes, et al. (2005), Acipimox was administered 90minutes before and 75 minutes into the exercise bout, plasma FFA were significantly lower at all time points for subjects in the Acipimox group compared to the placebo group.

4.3.3 Exercise modalities

The following data can be found in Table 6.

The exercise conditions were similar for all studies. Cycling was the aerobic exercise intervention used in every study. During the exercise, cycling, was performed at 50% VO_2max (determined during an incremental exhaustive exercise test) for a total duration ranging between 40 to 60 min. (Coyle et al., 1997; van Loon, Manders, et al., 2005) and 120 min. (Trudeau et al., 1999; van Loon, Thomason-Hughes, et al., 2005). In the studies conducted by van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005), a recovery period of 120 minutes was included. In the study of Trudeau et al. (1999) this recovery period lasted 30 minutes. No recovery period was included in the study of Coyle et al. (1997).

Dietary conditions differ among the studies. Trudeau et al. (1999) and Coyle et al. (1997) both prescribed an overnight fasting period. Trudeau et al. (1999) added that the subjects had to abstain from caffeine containing beverages and medication, 24 h before their presence in the laboratory. van Loon, Thomason-Hughes, et al. (2005) prescribed a standardized meal (72% carbohydrate, 11% fat, 17% protein) to the healthy subjects on the evening before each trial and van Loon, Manders, et al. (2005) prescribed a standardized meal (53% carbohydrate, 31% fat, 11% protein) to the subjects with T2DM on the evening before each trial.

4.3.4 Primary outcomes

The main outcome measures of interest in these studies were the (1) plasma glucose concentration and (2) plasma insulin concentration, pre, post and during an exercise bout (Table 7).

4.3.4.1 Plasma glucose

In the study of Coyle et al. (1997), lipolysis inhibition was done by intravenous glucose feeding. This resulted in a significantly ($p < 0.05$) higher plasma glucose concentration before exercise in the glucose ingestion trial compared to the fasting trial. During exercise and recovery, plasma glucose concentration of the glucose ingestion trial was not significantly ($p > 0.05$) different compared to the fasting trial.

In the study of Trudeau et al. (1999), adipose tissue lipolysis was partially inhibited by local lactate infusion in the subcutaneous adipose tissue. Plasma glucose concentration during exercise was significantly ($p < 0.05$) decreased compared to pre-exercise values. The effect was retained during recovery.

In the study of van Loon, Thomason-Hughes, et al. (2005), lipolysis inhibition was done by Acipimox administration pre- and during exercise. Before exercise no significant ($p > 0.05$) change was found in the plasma glucose concentration. During exercise a significant ($p < 0.05$) decline was observed with no significant differences between the placebo group and the Acipimox group at any point in time. During recovery there was no significant decline.

In the study of van Loon, Manders, et al. (2005), lipolysis inhibition was done by Acipimox administration pre- and during exercise. Plasma glucose concentration was not significantly declined before exercise. During exercise, the plasma glucose concentration tended to decline more in the Acipimox group in comparison to the placebo group, but the difference was not significant ($p=0.08$). At the end of the recovery period (120 minutes after cessation of exercise) the plasma glucose was significantly ($p<0.05$) lower in the Acipimox group compared to the control group.

4.3.4.2 Plasma insulin

In the study of Coyle et al. (1997), lipolysis inhibition was done by intravenous glucose feeding. During pre-exercise, the plasma insulin concentration was significantly ($p<0.05$) raised in the glucose ingestion trial compared to the fasting trial. During exercise significantly ($p<0.05$) higher plasma insulin concentrations were found in the glucose trial compared to the fasting trial.

In the study of Trudeau et al. (1999), adipose tissue lipolysis was partially inhibited by lactate infusion. During exercise, plasma insulin concentration was significantly ($p<0.05$) decreased compared to pre-exercise values. The effect was not retained during recovery.

In the study of van Loon, Thomason-Hughes, et al. (2005), lipolysis inhibition was established by Acipimox administration pre- and during exercise. Plasma insulin was not measured in this study.

In the study of van Loon, Manders, et al. (2005), lipolysis inhibition was established by Acipimox administration pre- and during exercise. Plasma insulin levels were similar at pre-exercise. During exercise, plasma insulin levels decreased significantly ($p<0.05$), but with no significant difference between the Acipimox group and the placebo group. During recovery, plasma insulin levels were significantly ($p<0.05$) lower in the Acipimox group compared to the placebo group.

4.3.5 Secondary outcomes

The secondary outcome measures of interest in these studies were the (1) substrate utilization, (2) whole body insulin sensitivity and (3) lactate concentration, pre, post and during exercise. All data can be found in Tables 9, 10 and 11.

4.3.5.1 Substrate utilization

There are different methods to calculate substrate utilization. Coyle et al. (1997) used stoichiometric equations to calculate carbohydrate and fat oxidation rates. van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005) used the non-protein respiratory quotient (Formula 1) to calculate fat and carbohydrate oxidation rates.

The overall findings in both studies, van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005), are similar for healthy and T2DM subjects. For both healthy and T2DM subjects, the Acipimox group had a significantly higher carbohydrate and a significantly lower plasma FFA use during rest.

Same results were found during recovery for T2DM patients. During exercise, both studies detected a significantly lower plasma FFA use in the Acipimox group.

Coyle et al. (1997) found that plasma FFA oxidation and oxidation of intramuscular triglyceride (IMTG) was significantly reduced during exercise in the glucose ingestion trial compared to the fasting trial. A significantly higher carbohydrate oxidation in the glucose ingestion trial compared to the fasting trial was also established during exercise. Substrate utilisation was not monitored during rest and during recovery. Trudeau et al. (1999) did not monitor substrate utilization at any point.

4.3.5.2 Whole body insulin sensitivity

In the study of van Loon, Manders, et al. (2005), changes in whole body insulin sensitivity, a ratio between plasma glucose rate of disappearance (calculated using the single-pool non-steady state Steele equations) (Formula 2) and the plasma glucose and insulin concentration, were calculated for patients with T2DM. During exercise, insulin sensitivity increased progressively with no significant differences between groups. During recovery, insulin sensitivity increased significantly in the Acipimox group only, resulting in a significant ($p < 0.01$) difference between trials. The other studies did not measure whole body insulin sensitivity (Coyle et al., 1997; Trudeau et al., 1999; van Loon, Thomason-Hughes, et al., 2005).

4.3.5.3 Lactate concentration

Lactate was measured in two studies van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005), and showed a similar trend in both studies. Lactate concentration increased significantly ($p < 0.05$) during exercise above pre-exercise concentration and declined significantly ($p < 0.05$) during recovery to near baseline values. However, for healthy subjects, the Acipimox group showed significantly higher plasma lactate concentrations during recovery while for T2DM patients there were no differences. Trudeau et al. (1999) and Coyle et al. (1997) did not measure lactate levels.

5. DISCUSSION

5.1 Reflection of study quality

Scores on the ExDes checklist ranged between 94 (Trudeau et al., 1999) and 98 (Coyle et al., 1997; van Loon, Thomason-Hughes, et al., 2005) on a total of 120 points. Higher scores indicated better quality. So we can thus conclude that all studies had similar level of quality. All studies had an acceptable level of quality based on the arbitrary cut off score of 75% (90 points). All four studies scored zero points on 'qualitative observations' because there was no clear description of qualitative observations of results or procedures given. Because interventions in all studies were described very detailed and procedures were followed strictly, these qualitative observations were not essential. The study of Trudeau et al. (1999) did not include any tables with quantitative data. Therefore, it scored only four points on 'quantitative data'. In the studies of van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005); and Coyle et al. (1997) there were no suggestions for further research or suggestions for improvement of research. Although the results suggest a potential role of exercise and glycemic control in the management of T2DM, no recommendations for implication in practical settings were present in any of the four studies.

All four studies included a reference for comparison. In the studies of van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005), subjects in the control group received a placebo. Therefore, subjects were blinded for the experimental trial (lipolysis inhibition) in these two studies. The study of Coyle et al. (1997) used the contralateral arm as comparison, where the study of Trudeau et al. (1999) used a microdialysis control probe contralateral at the umbilicus. Blinding of therapist or statisticians is not described in any of the studies.

5.2 Reflection of findings correlated to the research question

Coyle et al. (1997) found that the plasma glucose was significantly higher during pre-exercise in the glucose trials. This is a consequence provoked by the glucose ingestion to inhibit the lipolysis. The high plasma glucose levels before and during exercise evoked the significantly ($p < 0.05$) higher plasma insulin concentration during pre-exercise, exercise and recovery in the glucose trials. Because insulin is known to be a potent inhibitor of lipolysis (Campbell, Carlson, Hill, & Nurjhan, 1992), plasma FFAs concentration in the glucose trial were significantly ($p < 0.05$) lowered compared to the control group. The similar plasma glucose concentration can also be explained by the exercise intervention. During the aerobic exercise (50% VO_2max) the subjects used plasma glucose as a fuel, where normally plasma FFA would be used. This is supported by the finding of raised carbohydrate oxidation during exercise. Plasma FFA concentrations were significantly lower since the FFA oxidation and the IMTG oxidation were reduced. Based on the above, the conclusion is that Coyle et al. (1997) successfully inhibited the lipolysis with glucose ingestion, by significantly ($p < 0.05$) increasing plasma insulin concentration, but this had no beneficial effects on glycaemic control.

Trudeau et al. (1999) attempted to inhibit adipose tissue lipolysis by lactate infusion in abdominal subcutaneous adipose tissue and described a significant decrease of plasma glucose concentration

during exercise. The significant decrease of plasma glucose is probably due to the exercise intervention. During exercise the body uses the glucose for the glycolysis. The plasma insulin concentration significantly decreased during exercise. The significant decrease in plasma insulin concentration can be explained by the significant decrease of plasma glucose levels, which translates to less need for insulin. Because of the low plasma glucose levels, the subjects needed an alternative fuel. Because dialysate glycerol levels in the control and lactate probe were similar, they did not succeed to inhibit adipose tissue lipolysis. Therefore, it is not surprising that plasma FFAs concentration significantly increased during exercise. A possible explanation for this failing of lipolysis inhibition might be that the amount of lactate infused in this study (16 mM) was too low.

van Loon, Thomason-Hughes, et al. (2005) found a significant decrease in plasma glucose during exercise in healthy subjects. This is the effect of the exercise intervention, in which the subjects used glucose for the glycolysis. This is supported by the results of the substrate utilization since there was a significantly higher carbohydrate oxidation rate. The significantly lower plasma FFA levels in the intervention group is due to the lipolysis inhibitor and confirms the fact that it successfully suppresses the lipolysis. This statement is confirmed by the results of the substrate utilization. There was a significantly lower plasma FFA use. The higher carbohydrate and IMTG use and lower plasma FFA use during rest shows that the lipolysis inhibitor also works without the exercise intervention, but the effects are larger when combined with aerobic exercise. Significantly increased lactate concentration was due to the exercise intervention and the use of glycogen due to the lipolysis inhibition.

van Loon, Manders, et al. (2005) described a decline in plasma glucose concentration in both groups. This is due to the exercise intervention. During recovery, the plasma glucose concentration was significantly lower compared to control. This result suggests that the intervention group used more plasma glucose during recovery, compared to the control trial. This theory is confirmed by the results of the substrate utilization measurement which shows an increased muscle glycogen and IMTG use during exercise. This causes significantly higher lactate levels during exercise and recovery. In combination with the decrease in plasma insulin during exercise with significant difference during recovery, we can state that the intervention group is able to control the blood glucose levels with less insulin. This statement is empowered by the finding of the insulin sensitivity. During recovery there was a significant increase in the intervention group. The plasma FFA concentration was significantly lower at all points in time during the experiment in the intervention group. This means that the lipolysis was inhibited successfully.

5.3 Reflection of the strengths and weaknesses of the literature search

The weaknesses of the literature study are the following:

- Because of the small amount of human studies that investigated the inhibition of lipolysis during exercise, only a very small number of studies (4) were included in this systematic review.
- The population of interest (T2DM) was incorporated in only one study (van Loon, Manders, et al., 2005), while the other three studies incorporated healthy, trained men.
- As T2DM occurs predominantly in the elderly population, there was a larger age difference between the subjects in the studies including of healthy men (Coyle et al., 1997; Trudeau et al.,

1999; van Loon, Thomason-Hughes, et al., 2005) and the subjects with T2DM that were included in the other study (van Loon, Manders, et al., 2005).

- Secondary outcomes varied over the four studies making the comparison between interventions difficult. As shown in Table 10 only van Loon, Manders, et al. (2005) calculated the whole-body insulin sensitivity, while the other studies did not. Substrate utilisation was not measured in the study of Trudeau et al. (1999) and only during exercise in the study of Coyle et al. (1997). The two studies van Loon, Manders, et al. (2005); van Loon, Thomason-Hughes, et al. (2005) did measure lactate accumulation, while Coyle et al. (1997) and Trudeau et al. (1999) did not.
- All four studies had a small sample sizes ranging from six to ten subjects. How subjects were recruited is not described in any of the included studies.
- All four studies investigated the acute effects of lipolysis inhibition during exercise but none of these studies had a long term follow-up. Thus, it is not possible to draw long term conclusions for glycaemic control.

The strengths of this literature study are the following:

- In all studies the physical activity and diet was controlled for all subjects several days before the trials. Subjects had to write down their food intake before the first trial and were asked to repeat this food intake pattern in the following trials. They also all started the trials after an overnight fast.
- All exercise modalities were similar over the included studies. (%VO₂max, duration, rest, exercise and recovery) Only Coyle et al. (1997) did not incorporate measurements during recovery.
- The response on glycaemic control was in all four studies the main outcome of interest. Concentration plasma glucose, plasma insulin and plasma FFA were measured in all four studies, which makes it possible to make a solid comparison.

5.4. Recommendations for future studies

Because moderate to vigorous aerobic exercise is known to increase the peripheral insulin sensitivity and glycaemic control (Park et al., 2015), further research including more subjects and a longer follow-up of the effects of lipolysis inhibition combined with aerobic exercise in patients with T2DM is recommended. Additional recommendations are: a bigger sample size to increase the power of the evidence, homogeneous population (including men and women) and a period of follow-up (e.g. whole day).

6. CONCLUSION

In conclusion, glucose ingestion and administration of a nicotinic acid analogue both effectively suppressed the mobilization of plasma FFAs during exercise. However, no significant differences in glycaemic control were found between the intervention and control group during exercise for both methods of lipolysis inhibition. In contrast, during the recovery time, administration of a nicotinic acid analogue had a beneficial influence on insulin sensitivity for patients with T2DM. This finding may provide useful information for further research concerning the management of T2DM.

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8. APPENDICES PART 1

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VOORTGANGSFORMULIER WETENSCHAPPELIJKE STAGE DEEL 1

DATUM	INHOUD OVERLEG	HANDTEKENINGEN
26/10/2017	Algemene afspraken & ondertekenen contract	Promotor: Copromotor: Student(e): Student(e):
13/11/2017	Brainstormen onderzoeksvraag en zoekstrategie	Promotor: Copromotor: Student(e): Student(e):
19/12/2017	Zoekstrategie	Promotor: Copromotor: Student(e): Student(e):
16/11/2018	Zoekstrategie verfijnen	Promotor: Copromotor: Student(e): Student(e):
28/2/2018	Voortgang overleg.	Promotor: Copromotor: Student(e): Student(e):
10/4/2018	Voortgang overleg	Promotor: Copromotor: Student(e): Student(e):
8/5/2018	voortgang overleg	Promotor: Copromotor: Student(e): Student(e):
4/6/2018	voortgang overleg	Promotor: Copromotor: Student(e): Student(e):
		Promotor: Copromotor: Student(e): Student(e):
		Promotor: Copromotor: Student(e): Student(e):

Naam & Voornaam STUDENT: Mertens Quinten.....
Naam & Voornaam (CO)PROMOTOR & PROMOTOR: dr. Verboven Kenneth.....
TITEL masterproef (Nederlandstalig of Engels): Optimization of Exercise Therapy in Type 2 Diabetes by Blocking Lipid Breakdown.....

LITERATUURSTUDIE	Gestelde deadline	Behaald op	Reflectie
De belangrijkste concepten en conceptuele kaders van het onderzoekdomein uitdiepen en verwerken	04/11/2017	03/11/2018	
De belangrijkste informatie opzoeken als inleiding op de onderzoeksvraag van de literatuurstudie	04/11/2017	04/11/2018	
De opzoekbare onderzoeksvraag identificeren en helder formuleren in functie van de literatuurstudie	13/11/2017	13/11/2017	
De zoekstrategie op systematische wijze uitvoeren in relevante databanken	19/12/2017	12/01/2018	
De kwaliteitsbeoordeling van de artikels diepgaand uitvoeren	16/01/2018	15/02/2018	
De data-extractie grondig uitvoeren	28/02/2018	18/03/2018	
De bevindingen integreren tot een synthese	10/04/2018	22/04/2018	

ONDERZOEKSPROTOCOL

	Gestelde deadline	Behaald op	Reflectie
De onderzoeksvraag in functie van het onderzoeksprotocol identificeren	24/04/2018	23/04/2018	
Het onderzoeksdesign bepalen en/of kritisch reflecteren over bestaande onderzoeksdesign	08/05/2018	07/05/2018	
De methodesectie (participanten, interventie, uitkomstmaten, data-analyse) uitwerken	04/06/2018	03/06/2018	

ACADEMISCHE SCHRIFVEN

	Gestelde deadline	Behaald op	Reflectie
Het abstract tot he point schrijven	04/06/2018	04/06/2018	
De inleiding van de literatuurstudie logisch opbouwen	20/05/2018	19/05/2018	
De methodesectie van de literatuurstudie transparant weergegeven	20/05/2018	20/05/2018	
De resultatensectie afstemmen op de onderzoeksvragen	25/05/2018	25/05/2018	
In de discussiesectie de bekomen resultaten in een wetenschappelijke tekst integreren en synthetiseren	25/05/2018	26/05/2018	

Het onderzoeksprotocol deskundig technisch uitschrijven	04/06/2018	04/06/2018	
Referenties correct en volledig weergeven	11/06/2018	11/06/2018	

ZELFSTUREND EN WETENSCHAPPELIJK DENKEN EN HANDELEN	Aanvangsfase	Tussentijdse fase	Eindfase
Een realistische planning opmaken, deadlines stellen en opvolgen	Goed	Goed	Zeer goed
Initiatief en verantwoordelijkheid opnemen ten aanzien van de realisatie van de wetenschappelijke stage	Zeer Goed	Goed	Zeer goed
Kritisch wetenschappelijk denken	Goed	Voldoende	Goed
De contacten met de promotor voorbereiden en efficiënt benutten	Voldoende	Goed	Zeer goed
De richtlijnen van de wetenschappelijke stage autonoom opvolgen en toepassen	Goed	Goed	Goed
De communicatie met de medestudent helder en transparant voeren	Zeer Goed	Goed	Zeer goed
De communicatie met de promotor/copromotor helder en transparant voeren	Zeer Goed	Goed	Zeer goed
Andere verdiensten:			

Naam & Voornaam STUDENT: Eva Vranken

Naam & Voornaam (CO)PROMOTOR & PROMOTOR: Kenneth Verboven

TITEL masterproef (Nederlandstalig of Engels): Optimization of exercise therapy in type 2 diabetes by blocking lipid breakdown

LITERATUURSTUDIE	Gestelde deadline	Behaald op	Reflectie
De belangrijkste concepten en conceptuele kaders van het onderzoekdomein uitdiepen en verwerken	4/11/2017	03/11/2017	
De belangrijkste informatie opzoeken als inleiding op de onderzoeksvraag van de literatuurstudie	4/11/2017	04/11/2017	
De opzoekbare onderzoeksvraag identificeren en helder formuleren in functie van de literatuurstudie	13/11/2017	13/11/2017	
De zoekstrategie op systematische wijze uitvoeren in relevante databanken	19/12/2017	05/01/2018	
De kwaliteitsbeoordeling van de artikels diepgaand uitvoeren	16/01/2018	15/02/2018	
De data-extractie grondig uitvoeren	28/02/2018	18/03/2018	
De bevindingen integreren tot een synthese	10/04/2018	22/04/2018	

ONDERZOEKSPROTOCOL	Gestelde deadline	Behaald op	Reflectie
De onderzoeksvraag in functie van het onderzoeksprotocol identificeren	24/04/2018	24/04/2018	
Het onderzoeksdesign bepalen en/of kritisch reflecteren over bestaande onderzoeksdesign	08/05/2018	08/05/2018	
De methodesectie (participanten, interventie, uitkomstmaten, data-analyse) uitwerken	04/06/2018	04/06/2018	

ACADEMISCHE SCHRIFVEN	Gestelde deadline	Behaald op	Reflectie
Het abstract tot he point schrijven	04/06/2018	04/06/2018	
De inleiding van de literatuurstudie logisch opbouwen	20/05/2018	19/05/2018	
De methodesectie van de literatuurstudie transparant weergegeven	20/05/2018	20/05/2018	
De resultatensectie afstemmen op de onderzoeksvragen	25/05/2018	25/05/2018	
In de discussiesectie de bekomen resultaten in een wetenschappelijke tekst integreren en synthetiseren	25/05/2018	26/05/2018	

Het onderzoeksprotocol deskundig technisch uitschrijven	04/06/2018	04/06/2018	
Referenties correct en volledig weergeven	11/06/2018	11/06/2018	

ZELFSTUREND EN WETENSCHAPPELIJK DENKEN EN HANDELEN	Aanvangsfase	Tussentijdse fase	Eindfase
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Initiatief en verantwoordelijkheid opnemen ten aanzien van de realisatie van de wetenschappelijke stage	Zeer goed	Goed	Zeer goed
Kritisch wetenschappelijk denken	Goed	Goed	Goed
De contacten met de promotor voorbereiden en efficiënt benutten	Zeer goed	Goed	Goed
De richtlijnen van de wetenschappelijke stage autonoom opvolgen en toepassen	Goed	Goed	Zeer goed
De communicatie met de medestudent helder en transparant voeren	Zeer goed	Goed	Zeer goed
De communicatie met de promotor/copromotor helder en transparant voeren	Goed	Goed	Zeer goed
Andere verdiensten:			

9. APPENDICES PART 2

Table 1

Hits per combination per database January 2018.

Combinations	PubMed	Web of Science
<i>Lipolysis inhibition - Endurance exercise -Type 2 diabetes mellitus</i>	0 hits	0 hits
<i>Lipolysis inhibition - Endurance exercise</i>	12 hits	15 hits
<i>Lipolysis inhibition - Aerobic exercise - Type 2 diabetes mellitus</i>	2 hits	0 hits
<i>Lipolysis inhibition - Aerobic exercise</i>	41 hits	2 hits
<i>Lipolysis inhibition - Exercise - Type 2 diabetes mellitus</i>	2 hits	2 hits
<i>Lipolysis inhibition - Exercise</i>	41 hits	69 hits

Table 2

Hits per combination per database May 2018.

Combinations	PubMed	Web of Science
<i>Lipolysis inhibition - Endurance exercise -Type 2 diabetes mellitus</i>	0 hits	0 hits
<i>Lipolysis inhibition - Endurance exercise</i>	12 hits	15 hits
<i>Lipolysis inhibition - Aerobic exercise - Type 2 diabetes mellitus</i>	2 hits	0 hits
<i>Lipolysis inhibition - Aerobic exercise</i>	41 hits	2 hits
<i>Lipolysis inhibition - Exercise - Type 2 diabetes mellitus</i>	2 hits	2 hits
<i>Lipolysis inhibition - Exercise</i>	41 hits	69 hits

Table 3**Results Quality Assessment ExDes**

	van Loon, Thomason- Hughes, et al. (2005)	van Loon, Manders, et al. (2005)	Trudeau et al. (1999)	Coyle et al. (1997)
A. Statement of problem (4)	4	4	4	4
B. Hypothesis (8)	8	8	8	8
C. Variables (20)	20	20	20	20
D. Experimental control (4)	4	4	4	4
E. Materials (6)	4	4	4	4
F. Procedure: including diagrams (12)	12	10	10	10
G. Qualitative Observations (8)	0	0	0	0
H. Quantitative Data (12)	10	10	4	10
I. Graphs (10)	10	10	10	10
J. Statistic Divisions B&C (6)	6	6	6	6
K. Analysis and interpretation of data (8)	8	8	8	8
L. Possible Experimental Errors (6)	4	4	4	6
M. Conclusion (8)	8	8	8	8
N. Application and Recommendations for further use (8)	0	0	4	0
Total (120)	98	96	94	98

Table 4**General Subject Characteristics**

	Amount	Type	Age (yr)	Weight (kg)	VO ₂ max (ml/kg.min)
van Loon, Manders, et al. (2005)	10	Overweight & diagnosed with T2DM	60 ± 2	91 ± 3	32 ± 2
van Loon, Thomason-Hughes, et al. (2005)	10	Active men	23 ± 1	74 ± 3	62 ± 3
Trudeau et al. (1999)	8	Men in good condition	26 ± 2	74 ± 2	60 ± 3
Coyle et al. (1997)	6	Endurance trained men	22 ± 2	69 ± 2	72 ± 2

Table 5
Lipolysis Inhibition

	Method	Pre-exercise	During exercise	Recovery
Coyle et al. (1997)	Indirect Intravenous glucose feeding (1.4g/kg bodyweight)	Significantly ($p<0.05$) lower plasma FFA in the glucose ingestion trials compared to the fasting trial.	Significantly ($p<0.05$) lower plasma FFA in the glucose ingestion trials compared to the fasting trial.	Significantly ($p<0.05$) lower plasma FFA in the glucose ingestion trials compared to the fasting trial.
Trudeau et al. (1999)	Local lactate infusion (16mM, perfusion rate: 2.5 μ l/min) in abdominal subcutaneous adipose tissue using the micro-dialysis technique	Significantly ($p<0.05$) lower plasma FFA, plasma glycerol and dialysate glycerol compared to exercise.	Significantly ($p<0.05$) increased plasma FFA, plasma glycerol and dialysate glycerol compared to pre-exercise. No significant difference in dialysate glycerol between the probes.	Significantly ($p<0.05$) increased plasma FFA and plasma glycerol compared to pre-exercise with highest levels after exercise. Dialysate glycerol decreased significantly compared to exercise levels. No significant difference between the probes.
van Loon, Thomason-Hughes, et al. (2005)	Acipimox (250mg) administration pre- and during exercise	Significantly ($p<0.05$) lower plasma FFA in the Acipimox group compared to placebo group.	Significantly ($p<0.05$) lower plasma FFA in the Acipimox group compared to placebo group.	Significantly ($p<0.05$) lower plasma FFA in the Acipimox group compared to placebo group.
van Loon, Manders, et al. (2005)	Acipimox (250mg) administration pre- and during exercise	Significantly ($p<0.05$) lower plasma FFA in the Acipimox group compared to placebo group.	Significantly ($p<0.05$) lower plasma FFA in the Acipimox group compared to placebo group.	Significantly ($p<0.05$) lower plasma FFA in the Acipimox group compared to placebo group.

Table 6*Exercise Modalities*

	Modality	Intensity	Preexercise	Exercise	Recovery	Diet
van Loon, Manders, et al. (2005)	Cycling	50%VO ₂ max	90min.	60min.	120min.	Standardized meal, overnight fast and 24h abstain from medication
van Loon, Thomason-Hughes, et al. (2005)	Cycling	50%VO ₂ max	90min.	120min.	120min.	Standardized meal and overnight fast
Trudeau et al. (1999)	Cycling	50%VO ₂ max	30min.	120min.	30min.	Overnight fast, 24h abstain from caffeine containing beverages and medication
Coyle et al. (1997)	Cycling	50%VO ₂ max	60min.	60min.	-	Overnight fast

Table 7*Data Extraction: Plasma Glucose Concentration*

	Pre-exercise	During exercise	Recovery
Coyle et al. (1997)	Significant (p<0.05) higher plasma glucose concentration in the glucose ingestion trial compared to the fasting trial.	No significant (p>0.05) difference in plasma glucose concentration in the glucose ingestion trial compared to the fasting trial.	-
Trudeau et al. (1999)	Significant (p<0.05) higher plasma glucose concentration compared with pre-exercise and recovery values.	Significant (p<0.05) decrease in plasma glucose concentration compared with pre-exercise values.	Significant (p<0.05) decrease in plasma glucose concentration compared with pre-exercise values retained.
van Loon, Thomason-Hughes, et al. (2005)	No significant (p>0.05) decline.	Significant (p<0.05) decline with no significant differences between the placebo group and the Acipimox group.	No significant (p>0.05) decline.
van Loon, Manders, et al. (2005)	No significant (p>0.05) decline.	Tended to decline more in the Acipimox group in comparison to the placebo group, but not significant (p>0.05).	Significantly (p<0.05) lower in the Acipimox group compared to the control group.

Table 8*Data Extraction: Plasma Insulin Concentration*

	Pre-exercise	During exercise	Recovery
Coyle et al. (1997)	Significantly ($p < 0.05$) raised plasma insulin concentrations in the glucose ingestion trial compared to the fasting trial.	Significantly ($p < 0.05$) higher plasma insulin concentrations in the glucose trial compared to the fasting trial.	-
Trudeau et al. (1999)	Significant ($p < 0.05$) higher plasma insulin concentration compared with exercise and recovery values.	Significant ($p < 0.05$) decrease in plasma insulin compared to pre-exercise values.	Significant ($p < 0.05$) decrease in plasma insulin compared to pre-exercise values retained.
van Loon, Thomason-Hughes, et al. (2005)	-	-	-
van Loon, Manders, et al. (2005)	Not significant ($p > 0.05$) higher compared to exercise values.	Significant decrease ($p > 0.05$) compared to pre-exercise values and no significant ($p > 0.05$) difference between the Acipimox group and the placebo group.	Significantly ($p < 0.05$) lower in the Acipimox group compared to the placebo group.

Table 9*Data Extraction: Substrate utilization*

	Pre-exercise	During exercise	Recovery
Coyle et al. (1997)	-	Plasma FFA oxidation and FFA oxidation from intramuscular triglyceride (IMTG) was significantly ($p < 0.05$) reduced in the glucose ingestion trial compared to the fasting trial.	-
Trudeau et al. (1999)	-	-	-
van Loon, Thomason-Hughes, et al. (2005)	Acipimox group had a significant ($p < 0.05$) higher carbohydrate and a significant ($p < 0.05$) lower plasma FFA use.	Significant ($p < 0.05$) lower plasma FFA use in the Acipimox group.	-
van Loon, Manders, et al. (2005)	Acipimox group had a significant ($p < 0.05$) higher carbohydrate and a significant ($p < 0.05$) lower plasma FFA use.	Significant ($p < 0.05$) lower plasma FFA use in the Acipimox group.	Acipimox group had a significant ($p < 0.05$) higher carbohydrate and a significant ($p < 0.05$) lower plasma FFA use.

Table 10*Data Extraction: Whole body insulin sensitivity*

	Pre-exercise	During exercise	Recovery
Coyle et al. (1997)	-	-	-
Trudeau et al. (1999)	-	-	-
van Loon, Thomason-Hughes, et al. (2005)	-	-	-
van Loon, Manders, et al. (2005)	-	Insulin sensitivity increased progressively with no significant ($p > 0.05$) differences between groups.	Insulin sensitivity increased significantly ($p < 0.05$) in the Acipimox group only.

Table 11*Data Extraction: Lactate concentration*

	Pre-exercise	During exercise	Recovery
Coyle et al. (1997)	-	-	-
Trudeau et al. (1999)	-	-	-
van Loon, Thomason-Hughes, et al. (2005)	Significantly ($p < 0.05$) lower compared to pre-exercise concentration.	Significantly ($p < 0.05$) increased above pre-exercise concentration.	Decline significantly ($p < 0.05$) compared to exercise values. Acipimox group significant higher ($p < 0.05$) plasma lactate concentration.
van Loon, Manders, et al. (2005)	Significantly ($p < 0.05$) lower compared to pre-exercise concentration.	Significantly ($p < 0.05$) increased above pre-exercise concentration.	Decline significantly ($p < 0.05$) compared to exercise values. No significant difference between groups.

Formula 1*Non-protein respiratory quotient*

$$\text{Fat oxidation rate} = 1.695 V_{O_2} - 1.701 V_{CO_2}$$

$$\text{Carbohydrate oxidation rate} = 4.585 V_{O_2} - 3.226 V_{CO_2}$$

Formula 2*Steele Equation*

$$R_a = \frac{F - V \left[\frac{C_2 + C_1}{2} \right] \cdot \left[\frac{(E_2 - E_1)}{(t_2 - t_1)} \right]}{(E_2 + E_1)/2}$$

$$R_d = R_a - V \cdot \left(\frac{C_2 - C_1}{t_2 - t_1} \right)$$

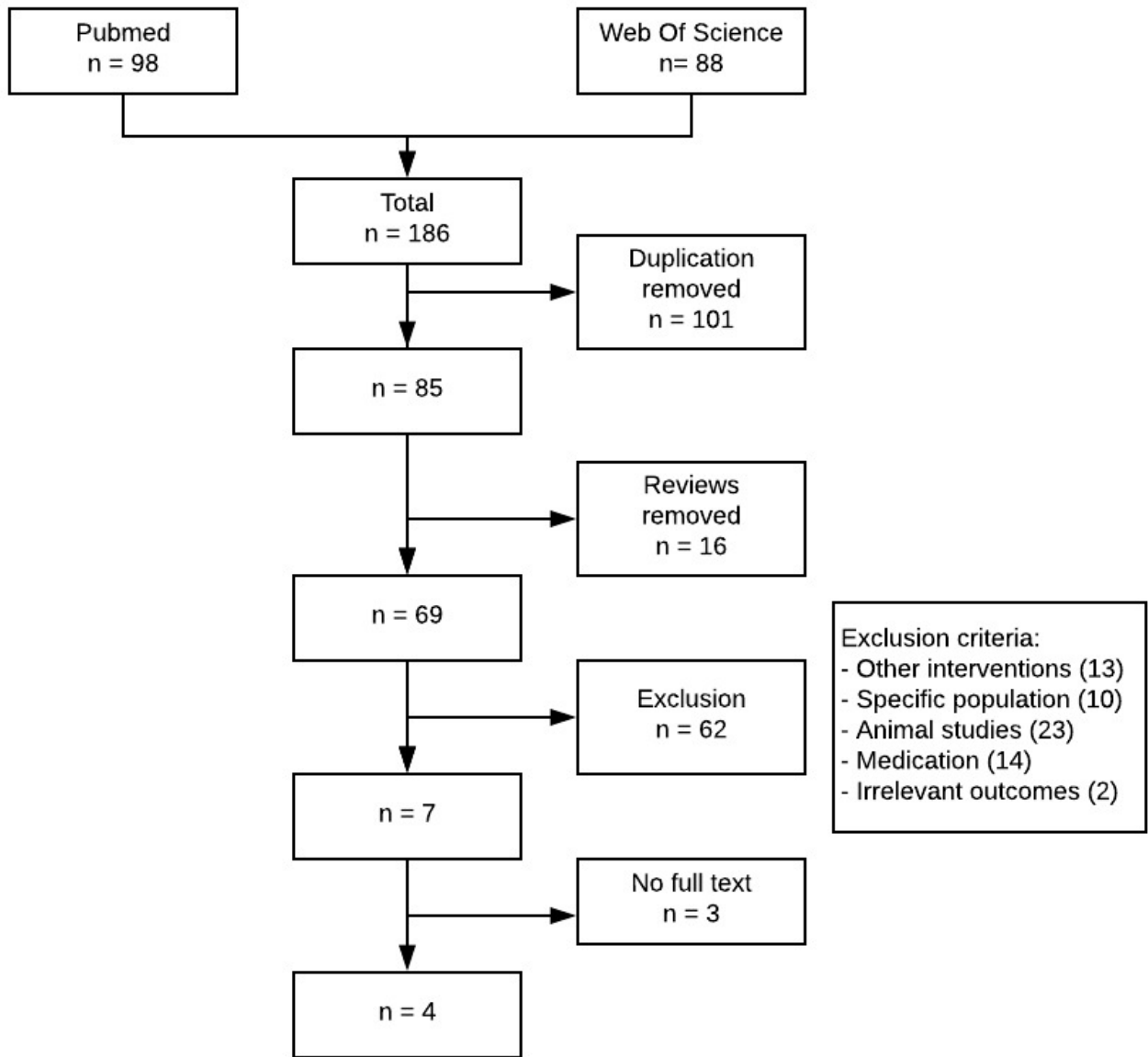


Figure 1
Study selection flowchart

PART 2: RESEARCH PROTOCOL

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1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease affecting many people worldwide and its prevalence is rapidly increasing. The past three decades, the number of people diagnosed with diabetes mellitus has doubled, resulting in a prevalence of approximately 260 million people worldwide (Chen, Magliano, & Zimmet, 2011). In Belgium the prevalence was approximately 515 thousand in 2013 and is estimated to be 604 thousand by 2035 (Guariguata et al., 2014). This unfortunate trend might be attributed to factors such as aging and an increase in sedentary behaviour and obesity prevalence, the latter being a main underlying cause of T2DM (Geiss et al., 2014).

Individuals with obesity are often characterized by insulin resistance, the pre-stage of T2DM (Kahn, Cooper, & Del Prato, 2014). Obesity-related whole-body insulin resistance implies a state in which several peripheral insulin sensitive tissues (including skeletal muscle, adipose tissue and liver) are less or even not responsive to insulin, resulting in reduced uptake of plasma glucose and the development of hyperglycaemia (Kahn et al., 2014).

Postprandial hyperglycaemia is a major concern in patients with T2DM. According to van Dijk, Tummers, Stehouwer, Hartgens, and van Loon (2012). T2DM patients experience a hyperglycaemic state during 32 +/- 4% time of the day. As hyperglycaemia is associated with an increased risk for cardiovascular events (Cavalot et al., 2006), more effective management of postprandial hyperglycaemia is warranted.

Furthermore, FFAs are the link between obesity and insulin resistance/hyperinsulinemia. Recent data also suggest that high concentrations of plasma free fatty acids (FFAs) inhibit glucose uptake by decreasing whole-body glucose oxidation rates and muscle glycogen synthesis (Randle, Garland, Newsholme, & Hales, 1965). The release of adipose tissue-derived FFAs can be inhibited with a lipid lowering drug, Acipimox, which specifically and temporarily inhibits adipose tissue lipolysis (Santomauro et al., 1999). A study of Lim, Hollingsworth, Smith, Thelwall, and Taylor (2011) found that lowering the plasma FFA availability (by Acipimox) in patients with T2DM, increased the whole-body glucose uptake, thereby reducing hyperglycaemic states. The whole-body glucose oxidation rate was increased with 25% by Acipimox administration (Lim et al., 2011). These data indicate that elevated FFAs levels may be an important target for the (co-)treatment of insulin resistance T2DM.

Furthermore, it is known that a single exercise bout lowers the circulating blood glucose due to an increase in whole-body insulin sensitivity up to 48 hours after cessation of exercise in patients with T2DM (van Dijk et al., 2012). Exercise is thus, next to pharmacological intervention and diet, a fundamental factor in the management of T2DM because it can reduce the hyperglycaemic episodes throughout the day (van Dijk et al., 2012).

In previous research, we have observed that aerobic exercise in combination with lowering of the plasma FFA availability (by Acipimox), has a significantly higher increase in insulin sensitivity during the recovery period (60 until 120 minutes after cessation of exercise) than aerobic exercise alone for patients with T2DM (van Loon et al., 2005).

However, it remains elusive whether lipolytic inhibition (acutely or chronically) is a suitable alternative or adjuvant therapy (accompanying exercise therapy) to improve metabolic health in patients with T2DM (Santomauro et al., 1999; Vaag et al., 1991).

Furthermore, so far, no research has been done about the postprandial effects and long term effects of aerobic exercise therapy in combination with lipolysis inhibition (by Acipimox) on the glycaemic control for T2DM patients. To investigate the potential clinical benefits of the combination of these two therapies, the aim of this study is to investigate the acute and postprandial effects of aerobic exercise in combination with lipolysis inhibition by Acipimox. Long-term effects are not included in this study due to the feasibility.

2. STUDY AIM

2.1 Research questions

The aim of the study is to investigate the acute effects of lipolysis inhibition in combination with aerobic exercise therapy on the glycaemic control during a whole day in T2DM patients.

We formulated the following research question:

How does aerobic exercise therapy in combination with lipolysis inhibition (Acipimox) affect the glycaemic control acutely and throughout the postprandial period in patients with T2DM?

2.2 Hypotheses

We formulated the following hypotheses based on the previous research question:

H1: Aerobic exercise therapy in combination with lipolysis inhibition (Acipimox) positively affects the glycaemic control acutely for patients with T2DM.

H2: Aerobic exercise therapy in combination with lipolysis inhibition (Acipimox) positively affects the glycaemic control throughout the whole day (postprandial) for patients with T2DM.

The effect of the intervention on glycaemic control (acutely as well as postprandial) will be tested by measuring plasma glucose concentrations, plasma insulin concentrations and plasma FFA concentrations.

3.METHOD

3.1 Research Design

The research design is described in Figure 1

The research design is a Randomized Controlled Trial (RCT), whereby four groups will be composed. Group one will receive Acipimox in combination with an aerobic exercise bout. Group two will receive a placebo in combination with an aerobic exercise bout. Group three will receive solely the Acipimox with no additional exercise bout. Group four will receive the placebo and no exercise bout. The subjects will be randomly allocated to one of the four groups using closed envelopes. The same subjects will come back 4 times, each time allocated to a different group. The placebo will be a capsule with flour. Presence of Type 2 diabetes is based on medical diagnosis (confirmed by general practitioner [GP]) and will be verified with an oral glucose tolerance test (OGTT) according to the World Health Organization criteria.

3.1.1 Pretesting

The pretesting procedure is described in Figure 2.

One week prior to the experimental trials, the glucose tolerance of the subjects will be measured using an OGTT to determine all subjects' individual glucose tolerance profile. The test starts with a measurement of the blood glucose concentration in a fasting state. The subjects will then take an oral glucose drink (100 g). Every 10 minutes blood glucose levels will be measured for 2 hours. Furthermore, each subject will fill in a self-report questionnaire about their medication use. After the OGTT and the questionnaire, all subjects' height and weight will be measured using the Norbert MPE scale. The subjects' body composition will be measured using a Dual-energy X-ray absorptiometry (DEXA) scan. A DEXA scan measures bone mineral content, bone mineral density, fat-free mass and provides estimates of percent body fat (Clasey et al., 1997). After these measurements the subjects will receive a standardized meal prior to the maximal cardiopulmonary exercise test (CPET). The CPET will be performed on a cycle ergometer in REVAL at the UHasselt. CPET is a non-invasive simultaneous measurement of the cardiovascular and respiratory system during exercise to assess a patient's exercise capacity. During this test heart rate, oxygen uptake and CO₂ emissions will be measured. Intensity will be raised by 25 Watt every minute and the subjects' peak oxygen uptake capacity (VO_{2peak}) will be measured. The absolute intensity of the aerobic intervention will be individually determined based upon their obtained VO_{2peak}. We chose to take the VO_{2peak} as parameter because measuring the VO_{2max} in this population is difficult.

3.1.2 Diet and activity prior to testing

The subjects will be instructed to maintain their usual eating and exercise habit during the experimental period. Additionally, the subjects will fill out a diary of their food intake 2 days prior to the experimental trial. The evening before the trial, subjects will receive a standardized meal (800 kcal, containing 55% carbohydrate, 30% fat and 15% protein) as earlier described by van Loon et al. (2005) followed by an overnight fast.

3.1.3 Protocol

The protocol is described in Figure 3.

After an overnight fast, subjects will arrive at the REVAL at 8.00 o'clock, either by car or public transport. A Teflon catheter will be inserted into an antecubital vein of one arm for blood sampling. This catheter will remain inserted throughout the whole trial and the whole day. Blood samples will be taken every 10 minutes during the resting period, exercise bout and recovery, and every 30 minutes throughout the day. The last blood sample will be taken 30 minutes after the last meal. After the catheter insertion the subjects will receive an oral dose of 250 mg Acipimox or placebo (t=0). Followed by a 30-minute resting period. After the resting period (t=30) the two groups including exercise will start the 60-minute aerobic exercise bout at 50% VO₂peak. After 10 minutes into the exercise bout (t=40), the subjects will receive a second oral dose of 250 mg Acipimox or placebo. The exercise bout is followed by a recovery period of 30 minutes (t=90).

The subjects in the two groups including non-exercise, will be asked to bring a book or laptop and refrain from any exertion throughout the whole experimental trial.

After the recovery period (t=120), all subjects will receive a light breakfast containing 500 kcal (65% CHO, 25%fat, 10%prot). After finishing this meal, subjects will be asked to refrain from any exertion until the end of the complete trial, which will be eight hours post-recovery (t=600). The subjects will get another 2 standardized meal. One standardized lunch containing 700 kcal (55% CHO, 30% fat, 15% prot) at t=330 and another standardized meal containing 250 kcal (55% CHO, 30% fat, 15% prot) at t= 570. Medication will be withheld for 24 h prior to, until the end of the experimental trial. The exercise bout is described below.

3.1.4 Measurements

During the experimental trials, blood and breath samples will be collected at regular intervals (10 minutes during the resting period, exercise bout and recovery and every 30 minutes throughout the day). Blood samples (7 ml) will be collected in EDTA-containing tubes and centrifuged at 1,000xg for 10 min at 4°C. Aliquots of plasma will be frozen immediately in liquid nitrogen and stored at -80°C. Plasma glucose, lactate and FFA concentrations will be analyzed with a COBAS semi-automatic analyzer. Plasma insulin concentration will be measured by radioimmunoassay. Expired breath samples will be measured by spirometry with the Cortex metalyzer II.

3.2 Subjects

3.2.1 Inclusion criteria

The following inclusion criteria are used to include subjects:

- Men and women
- Adults with age ranging from 25 to 70 years
- BMI ranges from 20 to 50 kg/m²
- Diagnosed with T2DM (no distinction between types)
- Signed informed written consent

3.2.2 Exclusion criteria

The following exclusion criteria are used to exclude subjects:

- Other metabolic, neurological or musculoskeletal diseases.
- Administration of medication that is due to medical safety not possible to withheld for 24h prior and during the trials (in deliberation with GP).
- Pregnancy.

3.2.3 Recruitment

Recruitment of participants will happen in cooperation with the Jessa hospital in Hasselt. Every patient diagnosed with T2DM for over 5 years, administered at the hospital will be contacted by a standard e-mail or a phone call.

3.3 Medical Ethics

The participants will be informed about the nature and risks of the experimental procedures before signing an informed written consent. The study will have to be approved by the Medical Ethics Committee of Hasselt University.

3.4 experimental trial

3.4.1 Exercise bout

The aerobic exercise bout is a 60-minute cycling exercise at 50% of their individually determined VO_2 peak with a pre-exercise resting period (30 minutes) and recovery period (30 minutes) provided. During both the pre-exercise resting period and recovery period, subjects are told to rest in a seated or supine position. Determination of the individual subjects' VO_2 peak is described earlier. The exercise bout will be performed on an electronically braked cycle ergometer (eBike Basic General Electric GmbH, Bitz, Germany).

3.4.2 Lipolysis inhibition

The lipolysis inhibition will be performed with administration of Acipimox. The groups where no lipolysis inhibition is prescribed, will receive a placebo. Acipimox or placebo administration will be done at $t=0$. Side effects of Acipimox include: flushing, skin rashes, gastrointestinal complaints and headaches. Subjects will be informed in advance of these potential side effects.

3.5 Outcomes

3.5.1 Primary Outcomes

The primary outcomes are the following:

- Plasma glucose concentration will be measured by COBAS semiautomatic analyzer
- Plasma insulin concentration will be measured by radioimmunoassay
- Plasma FFA concentration will be measured by COBAS semiautomatic analyzer

These measurements are performed every thirty minutes to constantly observe the changes in concentrations during the trial.

3.5.2 Secondary Outcomes

The secondary outcomes are the following:

- Lactate concentration measured by COBAS semi-automatic analyzer
- Substrate utilization measured by expired breath samples
- Insulin resistance estimated by the homeostasis model assessment for insulin resistance index (HOMA-IR)
- Age
- VO₂ peak measured by incremental exercise test on a cycle ergometer
- Body weight measured by Norbert MPE scale
- BMI calculated using weight, height and age
- Body composition measured by the DEXA scan
- Medication use based on self-report questionnaire
- Glycemic control profile by an OGTT

3.6 Data analysis

Two-way ANOVA will be used for the data analysis, if our data meet the following criteria: normality of the residuals, equal variances between the groups (homoscedasticity) and independence of the groups and all measurements.

The following tests will be used for controlling model assumptions:

- For testing normality of the data: Shapiro-Wilk
- To check if the variances are equal: Brown-Forsythe

If data are normally distributed and variances are equal between groups, the following test will be used:

- Analysis of variance (F-test)

The data analysis will be performed within the JMP-software.

4. TIME PLANNING

The planning of the recruitment period ranges from July 2018 until December 2018. Application for the medical ethics committee will be done in September 2018 and is expected to be approved in October 2018. The testing period for the clinical trial ranges from October 2018 until February 2019. Statistical analysis will be done in March 2019 and is expected to be finished April 2019. Reporting of the data will be done during April 2019 and May 2019.

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6. APPENDICES PART 3

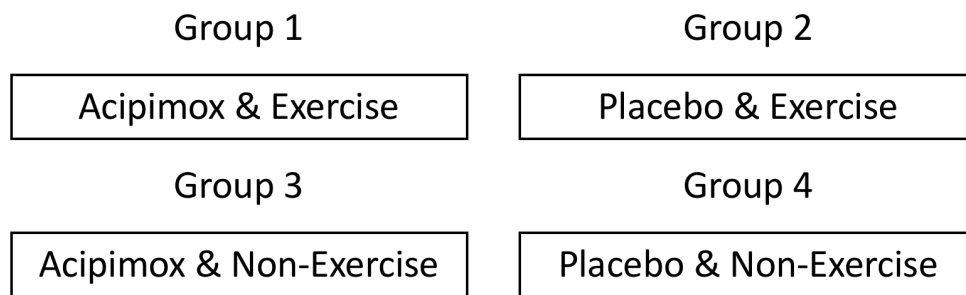


Figure 1
Research groups

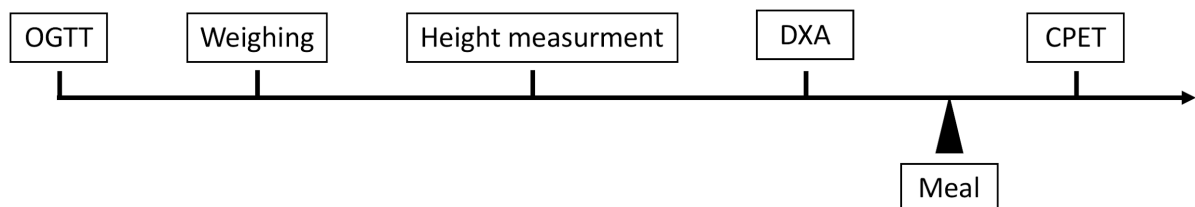


Figure 2
Protocol- pretesting

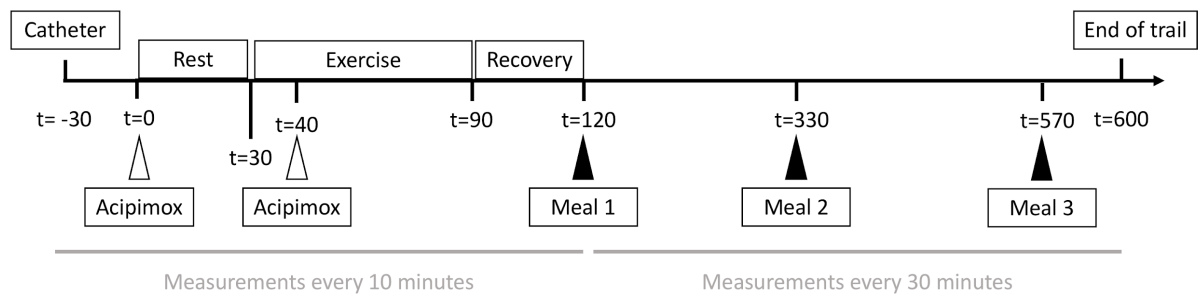


Figure 3
Protocol- testing