# Effects of Exercise on Incretin Hormones

by

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## ABSTRACT

The prevalence of type 2 diabetes (T2D) has been dramatically increasing globally. Exercise is recommended as a first line treatment, along with diet and medications. The mechanisms by which exercise improves glycemic control are not fully understood but are typically attributed to changes in insulin sensitivity, particularly at the level of skeletal muscle. Furthermore, the effects of exercise on other hormones that are involved in the pathogenesis of diabetes, such as incretins, also known as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are much less well understood. Early research has shown that exercise can increase incretins in healthy subjects.

To our knowledge, no studies in patients with T2D have been published at the time this dissertation began. Therefore, a series of studies were conducted to: 1- to systematically review the effect of acute exercise on endogenous incretin hormone concentrations in different populations; 2- to examine the effects of a single bout of moderate exercise, 28 days of metformin and their combination on plasma concentrations of total GLP-1 and GIP before and after meal ingestion; and 3- to examine the effect of two bouts of long duration, moderate intensity aerobic exercise on active and total GLP-1 and GIP.

Results showed that a single bout of aerobic exercise can significantly increase plasma concentrations of GLP-1 only in normal weight healthy participants but not in individuals with obesity. While adding an acute bout of exercise (i.e., 35 minutes) to 28 days of metformin treatment in people with T2D

did not have any effects on incretins, metformin significantly increased total GLP-1 and GIP concentrations. On the other hand, long-duration (i.e., two bouts of 1.5 hours), moderate-intensity aerobic exercise increased next day fasting incretins both in healthy and T2D participants.

In conclusion, there is some evidence to support the role of exercise in increasing GLP-1 concentrations in healthy individuals. On the other hand, the effect of acute exercise on GIP is less consistent. The available literature on the effects of exercise in obesity and T2D is very limited. The two original studies in the current thesis suggest that long duration exercise might be necessary to cause small effect on incretins in T2D.

## PREFACES

The current dissertation is an original work by Saeed Reza Toghi Eshghi. The research projects included in this thesis received research ethics approval from University of Alberta Research Ethics Board, project names: “Exercise, metformin and incretins”, No. 35632, November 27, 2012; and “Exercise and glucose counter-regulation”, No. 36610, April 18, 2013.

Chapter 4 of this dissertation has been published as Eshghi SR, Bell GJ, Boulé NG. Effects of aerobic exercise with or without metformin on plasma incretins in type 2 diabetes. Canadian Journal of Diabetes. 2013;37(6):375-80. I contributed to the design of this sub-study, analyzed the blood samples, analyzed data and wrote the manuscript. Boulé NG and Bell GJ contributed to the design of the original study, collected data, analyzed data and critically revised the manuscript. All authors contributed to the discussion of the article and reviewed the manuscript.

Chapter 5 of this dissertation has been published as Eshghi SR, Fletcher K, Myette-Côté É, Durrer C, Gabr RQ, Little JP, Senior P, Steinback C, Davenport MH, Bell GJ, Brocks DR, Boulé NG. Glycemic and metabolic effects of two long bouts of moderate-intensity exercise in men with normal glucose tolerance or type 2 diabetes. Frontiers in Endocrinology. 201711;8(154):1-12. Boulé NG, Steinback C, Davenport MH, Bell GJ, Senior P, Little JP, Brocks DR, and I contributed to the conception of the study and obtained funding for this project. Boulé NG, Myette-Côté É, and I collected the data. Boulé NG, Myette-Côté É, Fletcher K, Gabr RQ,

Durrer C, and I analyzed the data. Boulé NG and I drafted the manuscript, and all authors critically reviewed the manuscript to provide important intellectual content.

## Contents

# *LIST OF ABBREVIATIONS*

The following is the list of commonly used abbreviations in this dissertation.

| | |
|---|---|
| ADA | American diabetes association |
| CDA | Canadian Diabetes Association |
| CD26 | Cluster of differentiation 26 |
| CI | Confidence interval |
| DPP-4 | Dipeptidyl peptidase-4 |
| ELISA | Enzyme linked immunosorbent assay |
| FBG | Fasting blood glucose |
| GIP | Gastric inhibitory polypeptide/ glucose-dependent Insulinotropic peptide |
| GLP-1 | Glucagon-like peptide-1 |
| GLUT4 | Glucose transporter 4 |
| IL-6 | Interleukin 6 |
| MET | Metabolic equivalent |
| NEFA | Non esterified fatty acid |
| OGTT | Oral glucose tolerance test |
| RER | Respiratory exchange ratio |
| RMR | Resting metabolic rate |
| PPG | Postprandial glucose |
| SD | Standard deviation |
| SEM | Standard error of mean |

| | |
|---|---|
| SI | International system of units |
| SMD | Standardized mean difference |
| T2D | Type 2 diabetes |
| $VO_{2max}$ | Maximum oxygen consumption |
| $VO_{2peak}$ | Peak oxygen uptake |
| VT | Ventilatory threshold |

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# Chapter 1

## 1.1 INTRODUCTION

Type 2 diabetes (T2D) is a progressive and heterogeneous condition characterized by hyperglycemia due to a wide range of deficits in insulin action, secretion or both (1). The global prevalence of diabetes mellitus is rising dramatically. About 387 million people were reported to have diabetes in 2014 and the number is estimated to increase to 592 million by 2035 (2). In 2014, diabetes caused 4.9 million deaths, which means that, on average, every seven seconds a person died from this disorder (3). Although developing countries are experiencing the largest increase, Canada has faced a 230% increase in the prevalence between 1998 and 2009. About 3.7 million Canadians will be diagnosed with diabetes by 2019 (4).

Physical activity is considered a first-line therapy for T2D (5). Improved glycemic control, improved insulin sensitivity, increased cardiorespiratory fitness, and weight loss maintenance are among the positive effects of exercise in this population (6-9). The mechanisms by which exercise improves glycemic control are not fully understood but are typically attributed to changes in insulin sensitivity, particularly at the level of skeletal muscle (10). The effects of exercise on other hormones that are involved in the pathogenesis of diabetes, such as incretins, are much less well understood.

Incretin hormones, also known as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are secreted from the gastrointestinal tract into the portal circulation in response to nutrients. They have been shown to reduce blood glucose by increasing insulin secretion. They could also lead to a decreased rate of gastric emptying in response to

nutrient ingestion. Incretins are degraded shortly after their secretion by an enzyme called dipeptidyl peptidase 4 (DPP-4) (11). Despite the controversy regarding the changes in incretin hormones (increased, unchanged, decreased) during the progression to/of T2D, (12-14) the use of incretin-based therapies is increasingly popular (15, 16). The two main categories involve GLP-1 agonists and DPP-4 inhibitors that increase secretion of GLP-1 and prevent degradation of GLP-1, respectively.

Although there is significant evidence regarding the positive effects of exercise on muscle, liver, and adipose tissue, little is known regarding its effects on gut hormones such as incretins. Early research has shown that exercise can increase incretins in healthy subjects (17, 18) and that the increased incretins could be mediated by secretion of interleukin-6 (IL-6) from skeletal muscle (19). However, to our knowledge, no studies in patients with type 2 diabetes have been published at the time this dissertation began.

Postprandial hyperglycemia is thought to be more strongly associated with peripheral (muscle) insulin resistance, whereas fasting hyperglycemia is associated with increased hepatic insulin resistance (20, 21). As for incretins, exercise would therefore be expected to cause more pronounced reductions in postprandial hyperglycemia in people with T2D (22). Thus, if exercise could increase incretin concentrations (either by increasing their secretion or prolonging their half-life) this could consequently increase the incretin effect and, as a result, further improve glucose control in individuals with T2D.

## 1.2 SUMMARY AND RATIONAL

Despite the increasing popularity of incretin therapies, very limited research has been done on the effects of exercise on endogenous forms of incretin hormones. Research in this area could pave the way toward a more comprehensive understanding of how exercise affects glucose metabolism. Ultimately, this could lead to improved exercise prescriptions.

## 1.3 PURPOSE OF THE DISSERTATION

### 1.3.1 Overall purpose

The overall purpose of this thesis was to investigate the effects of exercise on the plasma concentration of incretin hormones (i.e., GLP-1 and GIP) (Figure 1-1). This dissertation consists of three studies. The first study systematically reviewed the available literature on the effects of acute aerobic exercise on endogenous incretin hormones on people with different glucose profiles (i.e. healthy normal weight, healthy obese, and T2D) while the second and third studies investigated the effects of exercise on healthy people and individuals with T2D.

### 1.3.2 Overall hypothesis

The overall hypothesis was that aerobic exercise increases plasma concentrations of incretins, both immediately post-exercise and subsequent to meal consumption.

### 1.3.2 General purpose of each study

***Study 1 (Chapter 3)***

**Purpose:** The purpose of this study was to systematically review the effect of acute exercise on endogenous incretin hormone concentrations in different populations (e.g., healthy, obese, and T2D). When appropriate, a meta-analysis was also conducted to statistically pool results from different studies, examine overall effects, and the variability among studies.

**Hypothesis:** Exercise would increase concentration of incretin hormones in healthy, obese, and T2D individuals.

**Context:** Recently, several studies have been published on the effect of exercise on incretins. The results have been inconsistent, perhaps due to methodological differences. The proposed systematic review was important since it could provide detailed analyses of the studies to date and how **Study 2** and **Study 3** from this thesis fit within this context.

***Study 2 (Chapter 4)***

**Purpose:** The objectives of this study were to examine the effects of a single bout of moderate exercise, 28 days of metformin and their combination on plasma concentrations of total GLP-1 and GIP before and after meal ingestion.

**Hypothesis:** It was hypothesized that both exercise and metformin would increase GLP-1 and GIP, both pre- and postprandially, in people with T2D.

**Context**: Along with exercise, metformin is also considered a first line therapy for T2D. These were post hoc analyses from a previously conducted study in our lab (23).

***Study 3 (Chapter 5)***

**Purpose:** The objective of this study was to examine the effect of two bouts of long duration, moderate intensity aerobic exercise on active and total GLP-1 and GIP. In addition to the blood samples collected immediately before and after the bouts of exercise, samples were collected on the day after the fasting state and following an oral glucose challenge in people with T2D and in healthy controls.

**Hypothesis:** It was hypothesized that two bouts of long duration moderate intensity exercise would increase plasma incretin hormones concentration in people with T2D and in healthy control participants both post-exercise and following a next day oral glucose challenge.

**Context:** This study built on **study 2** by measuring active GLP-1, the addition of healthy controls and longer duration exercise, which had previously been shown to increase incretins (17, 18). The incretin analysis represents one part of a larger study that also included measures of insulin and glucagon.

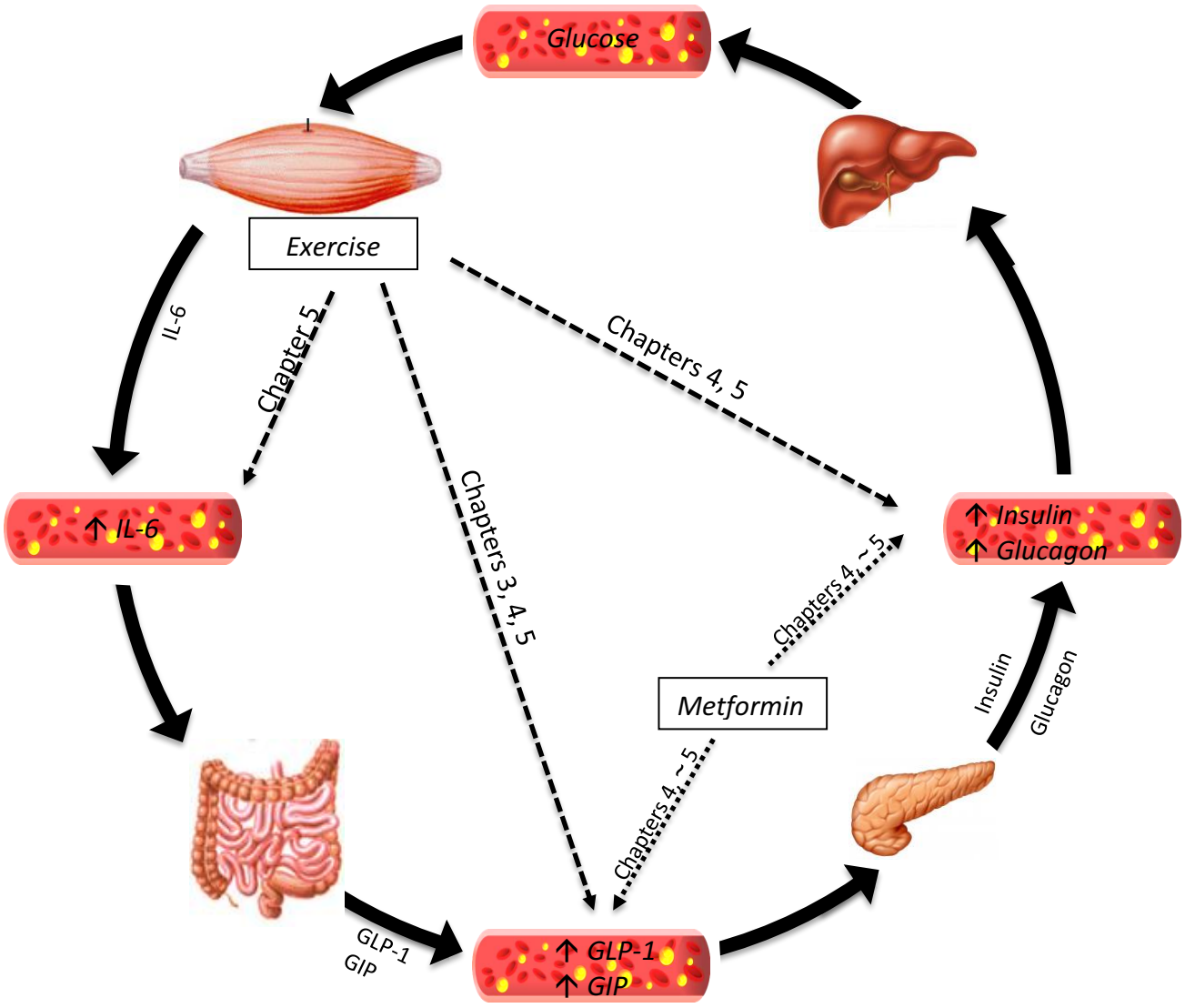



**Figure 1-1 Summary of dissertation hypothesis**

Legend: GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide; IL-6, Interleukin 6

## 1.4 LIMITATIONS, DELIMITATIONS AND SIGNIFICANCE

This dissertation focused on the effects of exercise on endogenous incretin hormones, GLP-1 and GIP, in individuals with T2D who were not on exogenous insulin and had a relatively well-controlled glycemia. Exercise might also affect pharmacokinetics or pharmacodynamics of exogenous incretins or DPP-IV inhibitors, but this was not examined in the present thesis.

Furthermore, we included individuals with different glucose profiles in the first study (i.e. healthy normal weight, healthy obese, prediabetes) and healthy participants in the third study. Studies on animal models or cells could provide a more in-depth understanding of the effects of exercise on incretin secretion or sensitivity but were also beyond the scope of this thesis. All three studies considered a non-exercise rest condition as the control condition to investigate the effects of exercise intervention on incretin hormones.

A limitation of study 1 was that we could not include any eligible studies with T2D participants. In **study 2**, there was no DPP-4 inhibitor added to the previously collected plasma samples. Hence, we were able to only measure total GLP-1. Other limitations of **studies 2 and 3** include a small sample size and the fact that only the acute effect of exercise (not regular training) was considered.

These studies represent important first steps towards exploring the effects of exercise on these hormones in humans. It is anticipated that these studies will broaden the understanding of the effects of exercise on glucose regulation and perhaps contribute to the development of more optimal exercise interventions for T2D. If exercise can increase postprandial incretin concentrations, this would improve our understanding of how exercise

can affect glucose control. They also provide more information about this area and further define the direction for future studies.

# Chapter 2

# LITERATURE REVIEW

## 2.1 INTRODUCTION

The discovery of incretin hormones (i.e., GLP-1 and GIP) has opened a new window for management of T2D. Despite the availability of new incretin therapies which function as DPP-4 inhibitors or GLP-1 agonist, very little was known about the effectiveness of exercise on endogenous forms of incretin hormones in individuals with T2D. This was important since exercise is considered a first line intervention for prevention and management of type 2 diabetes (24-26). Preliminary research has shown that exercise can increase incretins in healthy subjects (17, 18). However, only a limited number of studies in patients with type 2 diabetes (including some who may have impaired incretin responses) have been published. This literature review focuses on glucose homeostasis in healthy and T2D populations and the effects of exercise on glucose control, incretin hormones, and DPP-4.

## 2.2 GLUCOSE HOMEOSTASIS IN HEALTHY AND T2D

In the postabsorptive state (10-12 hour overnight fast) only 25% of glucose uptake occurs by insulin dependent tissues (mainly muscle) (27). Another 25% of glucose uptake occurs in the splanchnic area (liver and gastrointestinal tissue) (28). The remaining 50% is taken by the brain. In contrast to insulin dependent tissue glucose uptake, the two later processes are both insulin independent (29, 30).

The liver produces approximately 85% of endogenous glucose and the remaining amount is derived from the kidney (31, 32). Following glucose ingestion, the changing balance between glucose production and uptake results in increased amounts of plasma glucose. This in turn stimulates an elevation in insulin secretion. Both hyperglycemia and hyperinsulinemia trigger an increase in glucose uptake by the liver, kidney, and peripheral tissues. They also suppress endogenous glucose production (28-30, 33-37).

In scenarios where there is no hyperinsulinemia, hyperglycemia has been shown to stimulate muscle glucose uptake and also to supress the endogenous glucose production in a dose dependent manner (38, 39). The majority (about 80-85%) of glucose taken by peripheral tissue is disposed in muscle (28-30, 33-37), and only a fraction of that (about 4-5%) is metabolized by adipocytes (40). Even slight increases in insulin concentrations would lead to a potent antilipolytic effect, which in turn decreases plasma free fatty acid concentrations (41). Consequently, reductions in plasma concentrations of free fatty acids result in elevated muscle glucose uptake (42) and contribute to the inhibition of endogenous glucose production (39, 43). Thus, changes in plasma fatty acids due to increased plasma insulin or glucose concentrations play an important role in the maintenance of normal glucose homeostasis (44).

### 2.2.1 Insulin secretion

Glucose and other stimuli can cause two phases of insulin secretion. The first phase is proportional to the magnitude of stimuli and causes a sharp arterial rise of insulin (45). This early rise is followed by a rather lower more constant second-phase insulin release. The early phase, though, is significantly diminished in people with T2D (45, 46). Studies have shown that

insulin responses that include first-phase response are more effective in suppressing hepatic glucose release and also decreasing the glycemic responses to feeding than ones only including second-phase response (45, 46).

### 2.2.2 Insulin resistance

The presence of normal glucose homeostasis is dependent on two primary factors: normal insulin secretion by pancreatic beta-cells and normal sensitivity of tissues to the independent influences of hyperinsulinemia and hyperglycemia to increase glucose uptake.

Insulin resistance is present in a majority of patients with T2D and usually occurs before incidence of hyperglycemia. Impaired insulin action in several tissues (e.g., skeletal muscle, liver and adipose tissue) leads to elevations in the secretion of insulin from the pancreas to counterbalance the lower insulin action. The resulting hyperinsulinemia helps to maintain glucose concentrations near the normal range. In progressing to T2D, insulin secretion is impaired in absolute terms or in relation to the degree of insulin resistance, which leads to hyperglycemia. Between 2% to 14% (on average 5%) of individuals with impaired glucose tolerance progress to T2D each year (47).

### 2.2.3 Hepatic glucose control

The brain utilizes glucose at a relative rate of 1-1.2 $mg \cdot kg^{-1} \cdot min^{-1}$(27). Brain glucose uptake occurs independently from insulin (27, 48). In order to fulfill this essential need, the liver of a healthy individual produces about 1.8-2.0 $mg \cdot kg^{-1} \cdot min^{-1}$ glucose in an overnight fasted state (28, 29, 33, 41, 49). As mentioned earlier, brain glucose uptake accounts for 50-60% of

postabsorptive glucose disposal. This glucose uptake rate is a constant in both absorptive and postabsorptive phases even in individuals with T2D (50).

Following ingestion of macronutrients, insulin secretion inhibits the secretion of glucagon and enters the portal vein. This high insulin: glucagon ratio then inhibits hepatic glucose production. Should the liver not receive this signal, uptake of glucose from the gastrointestinal tract along with continued hepatic glucose production would lead to significant hyperglycemia. As mentioned earlier, under normal circumstances, following glucose ingestion significant hyperglycemia and hyperinsulinemia occurs (51).

In T2D the basal hepatic glucose production is increased by about 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$. This is significantly correlated with the severity of fasting hyperglycemia (28, 29, 33, 41, 49, 52-54). Consequently, in T2D the elevated hepatic glucose production is the major cause of higher fasting plasma glucose concentrations (55-59).

In individuals at the early stages of T2D, plasma insulin concentrations are 2-4 fold greater in comparison to nondiabetic subjects in the postabsorptive state (29). Hyperinsulinemia has been shown as a potent inhibitor of hepatic glucose production (28, 29, 33, 39). Therefore, in the presence of postabsorptive hyperglycemia, hepatic insulin resistance could explain the excessive glucose production by the liver. On the other hand, hyperglycemia itself is known to supress hepatic glucose production (60-62). Consequently, the liver is also resistant to the inhibitory effect of glucose on hepatic glucose production (60, 63, 64). The severity of hepatic insulin resistance has been shown to depend on the severity of diabetes state (41).

### 2.2.4 Peripheral (Muscle) glucose uptake

Muscles are a major site for glucose uptake in human beings (28-30, 38) and account for approximately 80% of glucose uptake in euglycemic hyperinsulinemic conditions (28-30). Following an oral glucose load, muscle takes up approximately one-third of the glucose (51). In contrast to healthy individuals, those with T2D show a blunted ability of insulin to stimulate muscle glucose uptake (28).

As illustrated in Firgure 2-1, in order for blood glucose to get phosphorylated in muscle it first needs to enter the interstitial space. The determining factors for this to happen are the amount of blood flow to skeletal muscle, capillary recruitment and endothelial permeability to glucose. The next step is to pass the membrane barrier, which depends on the number of glucose transporters and their activity. Once in the intramyocellular space, glucose is phosphorylated. Muscle glucose uptake is regulated by an alteration in one or more of these factors. Among all the above-mentioned factors, the membrane transport plays the major role in glucose uptake in basal conditions (65-68).

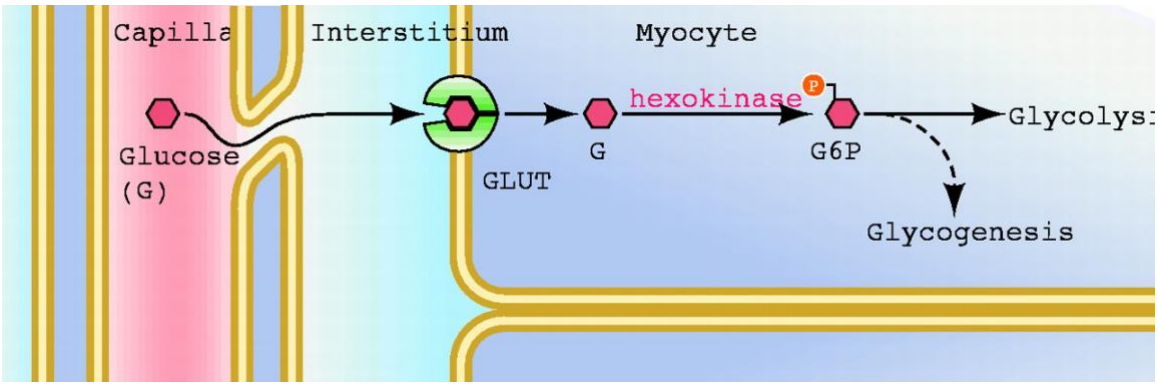



**Figure 2-1 Muscle glucose uptake**

Adopted from Skeletal muscle glucose uptake during exercise: how is it regulated? (69)

It has also been reported that muscle contraction can stimulate distinct, but parallel, glucose uptake pathways compared to insulin. Although both exercise and insulin increase translocation of glucose transporter 4 (GLUT-4) into the cell membrane, the origins of the GLUT-4 is from different intracellular pools (70, 71).

In the absence of increased blood flow to a working muscle, the muscle interstitial glucose would fall sharply and the glucose transport gradient would be insufficient to maintain glucose uptake. A close correlation of muscle blood flow to glucose uptake has been reported in working muscles (72). As mentioned earlier, exercise also increases glucose transport by stimulating plasma membrane translocation of GLUT-4 (73, 74). Although exercise increases muscle glucose uptake in an insulin independent manner, it could also increase the insulin sensitivity that in return stimulates further muscle glucose uptake.

## 2.3 THE EFFECT OF EXERCISE ON GLUCOSE CONTROL

### 2.3.1 Effects of exercise in healthy people

Under normal circumstances, there is fairly constant ratio between glucose uptake and glucose production to make sure plasma glucose concentrations stay stable. This is required for optimal function of systems like the nervous system. To maintain homeostasis and prevent hypoglycemia during early phases of exercise, skeletal muscles breakdown their own glycogen and triglycerides stores. Following a decrease in insulin concentrations, hepatic glucose production increases in the presence of unchanged glucagon concentrations. In subsequent stages, glucagon and catecholamine are increased. As a result, plasma concentrations of glucose remain fairly constant during exercise in healthy individuals (75). A major drop in blood

glucose will only be observed if exercise lasts for several hours (76). This constant ratio between muscle glucose uptake and hepatic glucose production remains as long as there is enough glycogen stored in the liver. In contrast, if exercise becomes more intense, blood glucose can even increase as a result of hepatic glucose production exceeding muscle glucose uptake (77).

The magnitude of increase in muscle glucose uptake during exercise is affected by both intensity and duration of exercise. In the postabsorptive state, exercise intensity is probably the main considering factor that increases muscle glucose uptake (78, 79). This is thought to be due to combination of greater fiber recruitment (80) and higher metabolic stress on active muscle fibers (81, 82) during exercise at higher intensities.

The positive effects of exercise, such as an increase in insulin sensitivity, have been reported to last up to 48 hours after a single bout of exercise (83). Further continuation of exercise training would lead to additional improvements in insulin sensitivity (83).

Studies also have shown that exercise can increase net hepatic glucose uptake (84), suggesting that increased glucose disposal following exercise is due to improved ability of muscle and liver to uptake more glucose (84).

One of the first studies that investigated the effects of exercise on incretin hormones (note: incretin hormones are described in section 2.4) was the study by O'Connor et al. (18). They showed the concentration of GLP-1 was significantly increased following a marathon race. The increasing effects of exercise on incretins might be due to secretion of interleukin 6 (IL-6) from contracting muscle. Contracting muscle during exercise can increase circulating

concentrations of IL-6 (85, 86). Furthermore, it has been recently suggested that elevated IL-6 concentrations in response to exercise could increase GLP-1 (an incretin hormone) secretion from intestinal L cells and pancreatic alpha cells. This could in turn improve insulin secretion and glycemia (19). To our knowledge, to date this has been the only potential mechanism suggested to be responsible for the effect of exercise on incretins.

### 2.3.2 Effects of exercise in people with type 2 diabetes

In contrast to stable plasma glucose concentrations during exercise in healthy individuals, exercise of moderate to high intensities normally decreases plasma glucose concentrations in people with T2D. This occurs due to insulin-independent activation of glucose transport (87) as well as increased insulin sensitivity (88).

The effects of exercise on hyperglycemia in people with T2D might be investigated in two separate states i.e. fasting and postprandial. Although the postprandial hyperglycemia is thought to be more strongly associated with peripheral (i.e., muscle) insulin resistance, fasting hyperglycemia is associated with increased hepatic insulin resistance (20, 21). Therefore, it might be assumed that exercise would more likely affect post-prandial compared to fasting hyperglycemia. This is consistent with a recent meta-analysis by MacLeod et al. (22) which suggested that short-term exercise can significantly reduce the amount of time spent in hyperglycemia (defined as glucose above 10.0 mmol/L) by 129 minutes per day despite not having any significant effects on fasting glucose. These acute benefits of exercise are short-lived and dissipate within 48 to 72 hours of last exercise session (89), showing the importance of regular and persistent physical activity (90).

In a systematic review by Umpierre et al. (91) on T2D participants, regularly performed structured aerobic, resistance, and combined (aerobic + resistance) training were all shown to decrease Glycated Hemoglobin A1c (further explained following this section) by 0.73%, 0.57% and 0.51%, respectively. They also suggested that more than 150 minutes of structured exercise per week reduces A1c more significantly compare to less than 150 minutes (0.89% vs 0.36%).

**Glycated Haemoglobin A1c (A1c)**

Glycated haemoglobin A1c has been considered as a "gold standard" in assessment of glycemic control for patients with diabetes since 1980s (92). It was first shown that a minor component of haemoglobin A is increased in patients with diabetes (93), and then by mid-1970s, A1c was reported to decrease with improvements in glycemic status (94). Over the last 30 years, A1c has been broadly used to evaluate the average glycemia over the previous 12-16 weeks.

A1c is quite stable in relation to acute perturbations like exercise, diet, and stress. This makes A1c a great candidate for clinical test to investigate conditions of T2D (95). Furthermore, many observational and interventional studies have shown how lowering A1c is correlated with a decrease in the risk and progression of diabetes complications. For instance, the UK Prospective Diabetes Study (UKPDS) has shown that reduction in A1c of patients with T2D was accompanied by significant reductions in in the risk of all diabetes-related end points, particularly microvascular complications (96). Based on this study, every 1% reduction in A1c was associated with a 37% and 14% reduction in the risk of all-diabetes related end points and microvascular complications, respectively. This study and the other similar ones are the basis

for Canadian Diabetes Association's (CDA) current recommended treatment goal that aims for an HbA1c of less than 7% (97).

**2.4 INCRETIN HORMONES**

Incretin hormones are secreted from the gastroinstinal tract into the portal circulation. The incretin effect was proposed after observing higher amounts of insulin secretion following oral glucose administration compared to glucose given intravenously. This was despite similar blood glucose profiles resulting from both techniques (98, 99). Incretins are responsible for about 70% of total insulin secretion in response to ingested glucose (100).

Nauck et al. (101) investigated the effect of ingesting different amounts of glucose (i.e., 25, 50 and 100 grams) on insulin secretion and glucose excursion. Despite the increasing amount of glucose ingested, the glucose excursions stayed relatively the same. On the other hand, insulin was raised in relation to increasing amounts of ingested glucose. Thus, another way of describing the incretin effect might be to say that it keeps glucose excursion at a certain level regardless of the amount of glucose consumed by healthy individuals.

The two main incretin hormones accounting for the incretin effect are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP, formerly known as gastric inhibitory polypeptide). GIP is secreted from the K-cells primarily located in the duodenum, but these cells can be found throughout the small intestine (102-104). GLP-1 gets secreted from L-cells. Although L-cells are found throughout the small intestine, their density is higher in distal ileum and also in the large intestine (102, 103, 105). A population of cells in which both GLP-1 and GIP are co-localized has also been noted (102). Both L- and K-cells are

open-type endocrine cells that can be influenced by direct contact of nutrients found in the intestinal lumen.

Besides having positive effects on glucose control, incretins are degraded shortly after secretion by an enzyme called dipeptidyl peptidase 4 (DPP-4) (11). About half of the endogenous incretins are degraded before they enter the systemic circulation. In addition to deactivation by DPP-4, both GLP-1 and GIP exit the circulation via the kidney (106).

### 2.4.1 GLP-1

The two major molecular forms of GLP-1 are GLP-1 (7-36) and GLP-1 (7-37) amides. The majority of the circulating biologically active form of GLP-1 is found as GLP-1 (7-36) while lesser quantities of bioactive GLP-1 (7-37) are still detectable (107). Rapidly after their secretion, they are both degraded by DPP-4 to GLP-1 (9-36) and GLP-1 (9-37), respectively. The two latter are biologically inactive forms of GLP-1. Glucagon-like peptide-1 7-36 amide (GLP-1 7-36) enhances glucose-stimulated insulin secretion in humans (108).

GLP-1 plays other roles, including inhibition of glucagon secretion (109), slowing gastric emptying, and reducing food intake (110) (Figure 2-2). In animal models, GLP-1 exerts direct effects on muscles to enhance glucose uptake (111).

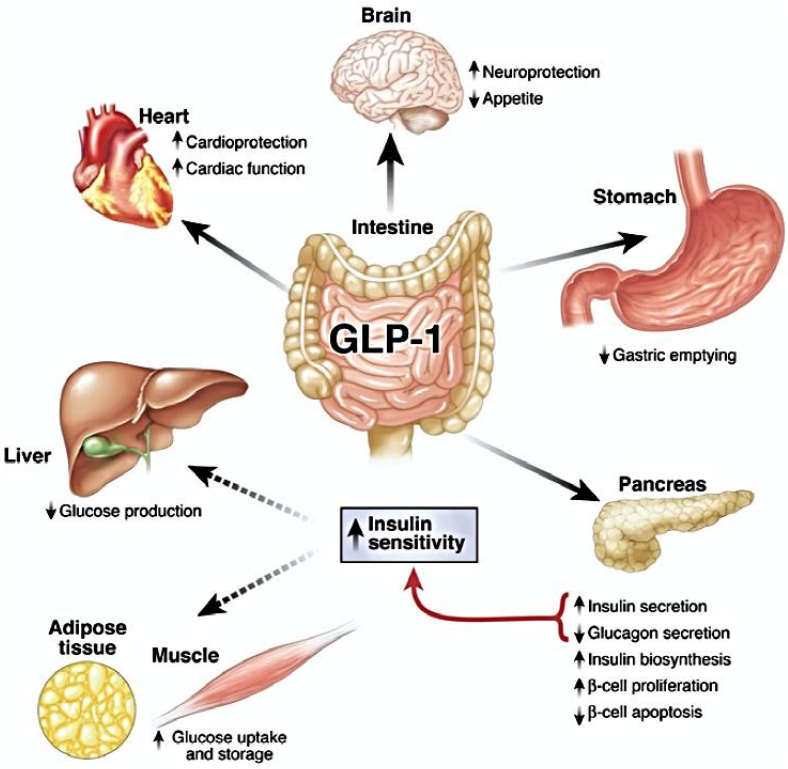



***Figure 2-2 GLP-1 actions in peripheral tissues***
Adopted from Biology of incretins: GLP-1 and GIP (112)[1]

Although both glucose and protein can stimulate the L cells, the majority of these nutrients are absorbed in the proximal GI tract (113, 114). In contrast, fatty acids reach the distal intestine at higher concentrations (115) and their placement into the ileum has been shown to stimulate GLP-1 release (116).

GLP-1 secretion occurs in a biphasic pattern. There is an early phase of 10-15 min that is followed by a second longer 30-60 minute phase (117). The short half-life of GLP-1, due to

[1] Reprinted from Gastroenterology, 132(6): 2131-57, Laurie L. Baggio, Daniel J. Drucker, "Biology of Incretins: GLP-1 and GIP", 2007, with permission from Elsevier.

degradation by DPP-4 enzyme, is less than 2 minutes (118). In humans, fasting plasma concentrations of active GLP-1 typically range between 5 to 10 pmol/L, and this amount increases about two to three-fold postprandial (107, 119, 120). The peak values highly depend on nutrient composition and the size of the meal (120, 121). Some uncertainty exists regarding the effect of nutrient ingestion on postprandial GLP-1 secretion in individuals with T2D compared to healthy subjects (i.e., reduced or unchanged) (122, 123).

Leptin has been known as GLP-1 stimulator. Leptin resistance could be the factor behind reduced GLP-1 secretion in obese individuals (124). The reasons behind impaired postprandial GLP-1 secretion in T2D are still unknown.

**GLP-1 receptor**

GLP-1 receptor (GLP-1R) belongs to the same family of glucagon and GIP receptors (125). GLP-1R is found in a variety of different locations throughout the human body, including α- and β-cells of pancreatic islets, the lungs, heart, kidney, stomach, intestines, adipose tissue, and several regions of the central nervous system including the brain stem and hypothalamus (126). Due to the intact function of GLP-1R in T2D, GLP-1R agonists can be used as an effective approach for enhancement of glucose control in patients with T2D. Whether exercise has any effects on expression or function of GLP-1R is unknown.

## Biological actions of GLP-1

### Pancreas

GLP-1R agonists cause several biological actions (Figure 2-2). In the pancreas, they stimulate glucose-dependent insulin secretion (108, 127, 128). GLP-1 also inhibits the secretion of glucagon (129). In experimental rodent models with diabetes, GLP-1R agonists improve glucose tolerance, enhance β-cell proliferation and neogenesis, and inhibit β-cell apoptosis (130). These all lead to an increase in β-cell mass.

### Gastrointestinal system

GLP-1R agonists inhibit meal-stimulated gastric acid secretion and gastric emptying (131, 132). Decreasing the speed of gastric emptying weakens the meal-associated increase in blood glucose concentrations. This happens by slowing the transit of nutrients from the stomach to the small intestine. This effect has been shown to contribute to the normalization of blood glucose concentrations in T2D after administration of exogenous GLP-1 (131, 132). This GLP-1 associated decrease in meal-related glycemic excursion is often associated with a reduction in postprandial insulin concentrations (132-134). In humans, Erythromycin, an antibiotic that opposes the deceleration of gastric emptying effect of GLP-1, has been shown to increase insulin secretory responses to meal. This further supports the glucose-lowering effects of GLP-1 (135).

**Cardiovascular system**

GLP-1R are expressed in human and the rodent hearts, although the identity of specific cells that express these receptors is unknown. Intravenous administration of GLP-1R agonists increases systolic and diastolic blood pressure as well as heart rate in rodents (136). It has been suggested that central GLP-1 activates the sympathetic nervous system to modify cardiovascular function (136). A study of Nystrom et al. (137) showed improved endothelial function in patients with type 2 diabetes following GLP-1 infusion. GLP-1 has also been reported to exhibit cardioprotective effects in experimental models. These effects include increased cardiac output, improved left ventricular function and systemic hemodynamics, increased myocardial insulin sensitivity, and glucose uptake (138). GLP-1 also reduces the infarct size and increases left ventricular function plus myocardial glucose uptake after ischemia-reperfusion in rats (139, 140).

Human studies also show positive effects of GLP-1 on the cardiovascular system. In a study by Nikoladias et al. (141), a 72-hour GLP-1 infusion in patients with acute myocardial infarction and angioplasty was shown to improve regional and global left ventricular function and was also associated with lower in-hospital mortality rates and hospitalization duration.

**Muscle, adipose tissue, and liver**

Little research has been done in this area, especially on human subjects. In muscles, GLP-1 increases the incorporation of glucose into glycogen (142). It also inhibits the hepatic glucose production (143) and stimulates glucose uptake in muscle and adipose tissues (144, 145). GLP-1 has also been shown to increase glucose metabolism and glycogen synthase activity

in rat and human skeletal muscles (142). The improving effects of incretins on muscle glucose uptake has been suggested to be insulin independent and also in distinction from their typical incretin effect (146).

### 2.4.2 GIP

As mentioned earlier in the incretin hormones section (section 2.3), GIP is secreted by K-cells, which are located largely in the duodenum and proximal jejunum and to a smaller extent throughout the entire small intestine (102, 104). Once ingested, glucose and fat are among the main stimulators for GIP secretion. The rate of nutrient absorption is a stronger factor in stimulation of GIP compared to nutrient presence itself. Consequently, in individuals with intestinal malabsorption, or in those taking medication that reduces the nutrient absorption (e.g., Gymnemic acid), the secretion of GIP decreases (147, 148).

In humans, fat is the main stimulator for GIP secretion. Based on the assay used to measure human total or intact GIP, the basal circulating concentrations range from 0.06 to 0.1 nmol/L. These could increase to 0.2-0.5 nmol/L postprandially (120, 149). GIP concentrations in patients with T2D have been reported as normal or slightly increased (120, 150). The half-life of GIP in healthy individuals and those with T2D is 7 and 5 minutes, respectively (151). GIP is inactivated by DPP-4 in both healthy individuals and those with diabetes (151, 152). In comparison to GLP-1, GIP has been shown to be less susceptible to deactivation by DPP-4 in humans. During an intravenous infusion of intact exogenous GLP-1 and GIP hormones, it was found that 40% of GIP remains active compared to 20% GLP-1 (151, 152). This might explain the longer half-life of GIP compared to GLP-1. It has been suggested that the kidney plays the major

role in clearance of GIP (153). The elimination rate of GIP is similar in healthy, obese, and T2D individuals (154).

**GIP receptor**

The GIP receptor (GIPR) is expressed in a variety of different organs throughout the body including the pancreas, stomach, small intestine, adipose tissue, heart, endothelial cells, lung, and kidney (155) (Figure 2-3). It has been suggested GIPR becomes downregulated due to hyperglycemia (156). Vilsboll et al. (157) have found that although late phase insulin secretion in response to GIP is lost in T2D, the first phase insulin response is still present. The authors suggest that post-receptor mechanisms, not the downregulation of GIPR, are involved with the loss of late phase insulin secretion. The GIPR are also expressed in pancreatic alpha cells where they increase glucagon secretion (158). This makes GIP a less attractive potential treatment for T2D. Whether exercise exerts any effects on GIPR expression or post-receptor mechanisms involved in its function is unknown.

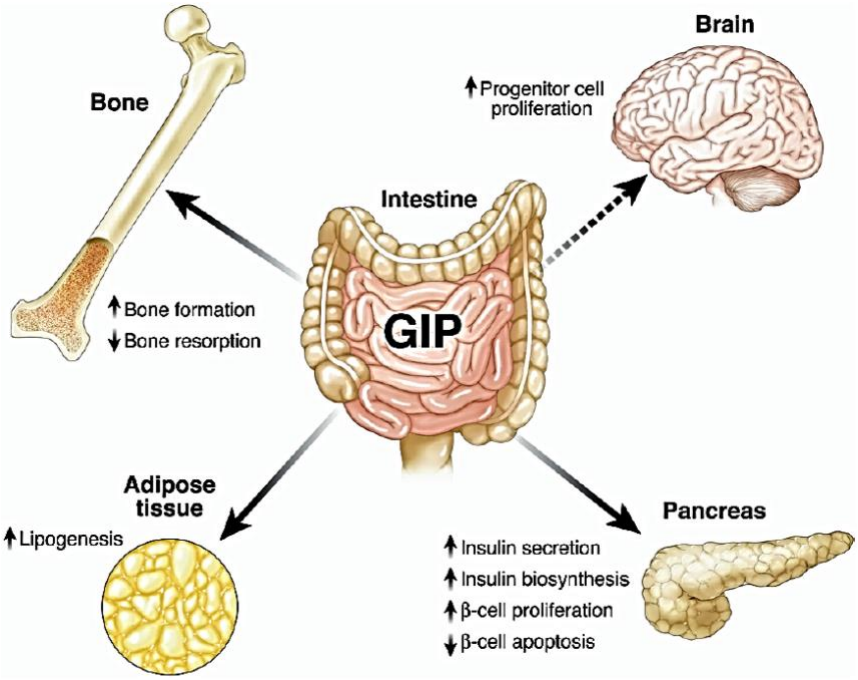



**Figure 2-3 GIP actions in peripheral tissues**
*Adopted from Biology of incretins: GLP-1 and GIP (112)*[2]

**Biological actions of GIP**

**Pancreas**

The molecular mechanisms by which GIP exerts its glucose-dependent insulin secretion significantly overlap with the ones for GLP-1 (159, 160). GIP also up-regulates β-cell insulin gene transcription and biosynthesis (161). It has been shown that the elimination of GIP receptor (GIPR) signalling in rodents could impair oral glucose tolerance and glucose-stimulated insulin

[2] Reprinted from Gastroenterology, 132(6): 2131-57, Laurie L. Baggio, Daniel J. Drucker, "Biology of Incretins: GLP-1 and GIP", 2007, with permission from Elsevier.

secretion (162-166). Although less is known regarding the effects of GIP on people with T2D, a study on diabetic rats showed that two weeks of infusion with GIP could significantly reduce β-cell apoptosis (167). It has also been reported that in contrast to GLP-1, GIP infusion could increase glucagon secretion (168). By simultaneous infusion of GLP-1 and GIP, GIP increased the glucagon concentration that was decreased by GLP-1 (168).

**Adipose tissue**

GIPR has been shown to be expressed in rat adipocytes (169) and is believed to be involved in the control of lipid metabolism and development of obesity. Fat ingestion is a major stimulant for GIP secretion and in some obese individuals, plasma GIP concentrations are increased (170, 171). It has been suggested that GIP might also have lipolytic effects by making rats less resistant to diet-induced obesity increased adipocyte mass (172). Furthermore, compared to normal rats, the rats with the GIPR knockout gained less weight, showed reduced adiposity, and improved glucose tolerance and insulin sensitivity (172).

**2.5 DPP-4**

Depeptidyle peptidase-4 (DPP-4), also known cluster of differentiation 26 (CD26), is an enzyme that modifies or inhibits the activity of some oligopeptides and proteins. DPP-4 quickly metabolises GLP-1 (7-36) and GLP-1 (7-37) to GLP-1 (9-36) and GLP-1 (9-37), respectively. It also inactivates GIP (1-42) into biologically inactive GIP (3-42) (11, 152, 173) (Figure 2-4). DPP-4 is found in a variety of different organs, and more importantly on the surface of endothelial cells directly adjacent to the GLP-1 secretory cells (106). Following secretion and before entering portal circulation, about 75% of GLP-1 is degraded by DPP-4 located in capillaries. As a result,

only about 25% of GLP-1 reaches the liver, where another 40-50% is further degraded by DPP-4. Consequently only 10-15% of GLP-1 enters the systemic circulation (174). In rats, more than 50% of intravenous bolus GLP-1 is deactivated within two minutes of peptide administration. In contrast, in DPP-4-deficient rodents GLP-1 remains active (152, 175).

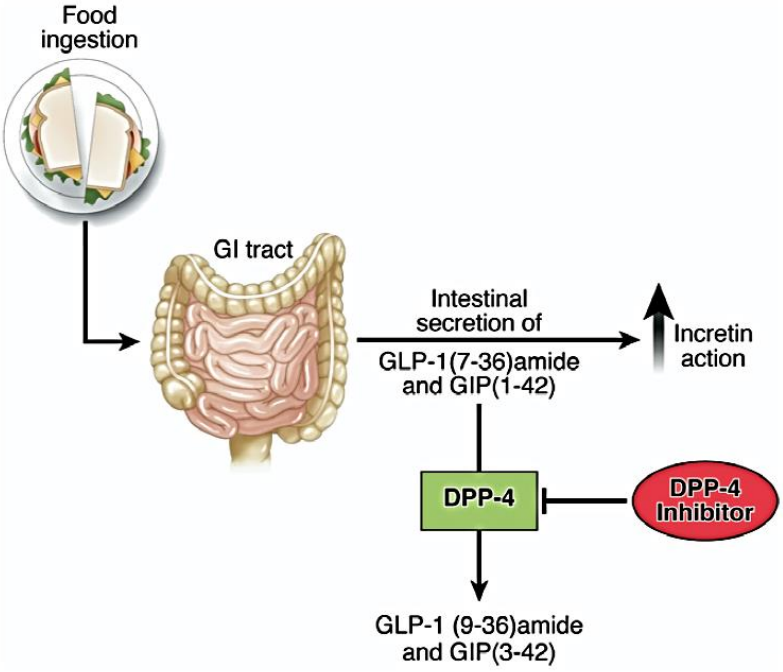



**Figure 2-4 DPP-4 effect on incretin hormones**
*Adopted from Biology of incretins: GLP-1 and GIP (112)*[3]

## 2.6 LITERATURE REVIEW SUMMARY

This literature review shows how both insulin action and/or secretion might be impaired in people with T2D. The effects of exercise on glycemic control were discussed for both healthy people and T2D. Despite well-known effects of exercise on muscle, liver, cardiovascular system,

[3] Reprinted from Gastroenterology, 132(6): 2131-57, Laurie L. Baggio, Daniel J. Drucker, "Biology of Incretins: GLP-1 and GIP", 2007, with permission from Elsevier.

adipose tissues and so on, there is little research on the effects of exercise on gut hormones such as incretins. Previous research has shown that exercise can increase incretins in healthy subjects but the amount of literature on T2D is scarce.

Although the incretin mimetic medications and DPP-4 inhibitors are increasingly popular for controlling glucose in patients with T2D, more research is needed to investigate the effects of exercise on the endogenous forms of these hormones. The following chapters aim to examine if exercise can affect endogenous incretin hormones in people with T2D and others.

# Chapter 3

## Effects of exercise on incretin hormones; a meta-analysis

### 3.1 ABSTRACT

**Background**: Despite the increasing approval of pharmacological approaches to manipulate incretin hormones, very little is known regarding the effects of other front-line therapies (e.g., exercise) on incretins.

**Objective**: The purpose of this systematic review was to investigate the effects of an acute bout of aerobic exercise on plasma concentrations of incretins (i.e., GLP-1, GIP). Pre-post exercise designs as well as randomized trials and crossover studies were considered.

**Methods**: Online databases (PubMed, EMBASE and Medline) were searched in the first week of March 2017. Eligible studies included human participants of different obesity or glucose control status who participated in a single bout aerobic exercise. Plasma concentrations of GLP-1 and/or GIP before and after exercise and/or control conditions, or following a subsequent meal were compared.

**Results**: 29 studies met the inclusion criteria. Eight studies provided meals with different compositions and variable timings between the end of exercise and the beginning of the meal. Among those eight studies, four did not have blood samples drawn before or after exercise. Hence, twenty-one studies were used in quantitative analyses. GLP-1 significantly increased following exercise only in normal weight participants (Standardized mean difference (SMD) = 0.46, 95% CI, 0.19 to 0.73). There were no changes observed in obese individuals. Furthermore,

there was no difference between exercise and control conditions and also for pre versus post comparisons on GIP.

**Conclusion**: A single bout of acute aerobic exercise increases plasma concentrations of GLP-1 in normal weight individuals but not in participants with obesity. Further investigations are necessary to study exercise effects in T2D.

## 3.2 INTRODUCTION

There is a firm base of evidence supporting effects of incretin hormones on appetite and blood glucose regulation (176-179). The two main incretin hormones are glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). Soon after their secretion (i.e., less than 2 minutes) majority of the intact forms of GLP-1 and GIP are partially degraded by dipeptidyl peptidase peptide-4 (DPP-4) (112). Only 10-15% of the intact GLP-1 reaches pancreatic beta cells (180). Studies have suggested a negative correlation between body mass index (BMI) and incretin effect (181). The prescription of incretin mimetics and DPP-4 inhibitors has become increasingly popular as two pharmacological approaches to control blood glucose in patients with type 2 diabetes (T2D) (182). Incretin mimetics also promote weight loss in obese individuals (183).

While the pharmacological manipulation of incretins has been approved in many countries, little is known about the effects of other front-line therapies on incretins. Exercise has long been used as a first-line treatment for controlling blood glucose (5, 24, 184) and weight maintenance/loss (185). Despite some evidence suggesting that exercise increases

plasma concentrations of incretins in normal weight participants, data is inconsistent regarding the effects of exercise in people with obesity or T2D. The inconsistent findings may in fact be due to the characteristics of the populations studied, or in part be due to the small sample sizes in most studies to date, or because of methodological differences in the research protocols.

Therefore, the primary objective of this study was to systematically review the acute effects of aerobic exercise on plasma concentrations of incretin hormones (i.e., GLP-1 and GIP) versus control condition. When appropriate, meta-analyses were performed to summarize the available evidence. Depending on the studies identified, planned subgroup analyses included the comparison of the incretin changes among different populations (e.g., normal weight vs. obese) or exercise interventions (e.g., postprandial vs. fasting exercise).

## 3.3 METHODS

### 3.3.1 Search strategy

In the first week of March 2017, a literature search of online databases (PubMed, EMBASE and Medline) was performed. No search limits were applied regarding the date or language of publication. The search was performed to identify publications that included keywords and MeSH terms relating to exercise and incretin hormones (see Supplementary Table 3-1). The references of eligible articles were searched by hand to find additional articles which the online literature search may have missed.

Two authors (SRTE and NGB) independently reviewed the search results to find eligible articles. Records found through the literature search were assessed to determine if they met the following inclusion criteria:

- **Population**: Human participants of different obesity or glucose control status (e.g., normal weight, overweight or obese, as well as normoglycemic, prediabetes, and T2D). There was no exclusion criterion relating to the duration or severity of diabetes or medication use.
- **Intervention**: A single bout of aerobic exercise defined in terms of intensity, type, and duration. The aerobic exercise included any types of activity that predominantly utilized aerobic metabolic pathways. Hence, activities such as sustained running, cycling, swimming, and rope skipping were considered as aerobic exercise. Furthermore, where there was an anaerobic component in the exercise protocol (e.g., interval training), studies were included as long as there was a primary (i.e., >50%) aerobic contribution to these protocols.
- **Comparison**: Exercise versus control (randomized, non-randomized, or crossover trials), or pre-exercise versus post-exercise.
- **Outcome**: Eligible studies were required to report the plasma concentrations of incretin hormones (i.e., GLP-1 or GIP) before and after exercise and/or control conditions.

### 3.3.2 Data extraction

From the eligible studies, relevant data were extracted independently by SRTE and MM. The authors were not blinded to information such as authors, institutions of origin, and journal

of publication. Incretin-related outcomes of interest were included in the meta-analysis when reported in three or more eligible studies. These outcomes included active GLP-1, total GLP-1, and/or GIP plasma concentrations before exercise (i.e., baseline), immediately following the end of exercise, and after 30 minutes of the time exercise was ceased. The post 30-minute time-point was included since it was more commonly reported and also was less likely to be affected by other confounders (i.e., post exercise meals). When a control, non-exercise condition was provided, these outcomes were extracted according to the same schedule of blood sampling.

Where it was not specified in studies whether active (7-36) or total (9-36) form of GLP-1 was measured, the hormone was reported as not specified (NS). When a DPP-4 inhibitor was added to blood samples at the time of drawing samples, the measured incretin was considered as active form. Since most of the studies did not clarify whether active (1-42) or total (3-42) form of GIP was being measured, all of the GIP concentrations were included as "GIP".

In addition, information regarding study participants (age, sex, BMI), exercise interventions (intensity, type, and duration), the timing of exercise in relation to meals, and the methodological quality were extracted. Exercise was considered to have taken place during the postprandial state when it occurred within four hours after the previous meal (186). The next six-hour period following postprandial state was defined as the postabsorptive state (187). Hence, 10 hours after the last meal was the beginning of the fasting state.

When information was displayed within figure but not available in the numerical form, means and standard errors were approximated from the figure using a graph digitizing software

(Plot Digitizer, Sourceforge.net). Missing standard deviations in two studies (188, 189) were extrapolated based on the standard deviations from the other time points.

Where incretin concentration was not reported based on the international system (SI) of units, the conversion bellow was used to convert them to SI units:

Nanogram/liter or picogram/milliliter x 0.3032 = picomole/liter

### 3.3.3 Risk of bias

The inclusion of non-randomized pre-post designs or randomized crossover trials can potentially increase the risk of specific biases and threaten internal validity. Since majority of the identified studies were randomized cross-over trials, the assessment of risk of bias was performed by answering the following questions, many of which were developed from section 16.4.3 (Assessing the risk of bias in crossover trials) in the Cochrane Handbook (190): 1- Was the order of conditions (exercise vs. control) randomized in cross-over trials? 2- Was the carry-over effect of the last bout of exercise minimized by allowing at least 72 hours between the exercise and control conditions? 3- Was the number of participants who dropped-out reported? 4- Did the analysis of a crossover trial take advantage of the within-person design? The answers to these four questions were coded independently as “yes”, “no” or “unsure” by two authors (SRTE and MM) and disagreements were resolved by discussion with NGB.

Funnel plots were created by plotting the standard error on the y-axis and the mean difference on the x-axis for visual inspection for asymmetries to evaluate publication biases. In addition, regression of the effect size on the standard error was used to assess publication bias (i.e., Egger’s linear regression method) (191). For the exercise vs control comparisons, sensitivity analyses were conducted by excluding non-randomized studies.

### 3.3.4 Statistical analysis

Statistical analyses were performed using Review Manager Software (RevMan 5.3, Cochrane Collaboration, Copenhagen, Denmark). Standardized mean differences were calculated using a fixed effect model. Heterogeneity was estimated by the Chi-square test of heterogeneity. The percent of total variability that is attributable to heterogeneity (i.e., not to chance) was expressed as the I-squared ($I^2$).

As the primary analyses of interest, incretin concentrations were compared between exercise and control conditions immediately after exercise was ceased. Concentrations were also compared pre-exercise. Separate analysis was conducted to compare incretin concentrations pre- versus post-exercise, pre- versus 30 minutes post exercise completion, and post versus 30 minutes post exercise. Since the within-person standard deviations (SD) or standard errors (SE) were unavailable for most of the studies, we were unable to use them for our analysis. We therefore inputted the mean and standard deviation at each time point.

For each of the analyses, studies were divided to normal weight and obese subgroups. We also conducted subgroup analyses categorize studies according to the timing of exercise (i.e., fasting vs postprandial). Finally, we conducted sensitivity analyses to examine if results differed when looking at studies that specified they measured active forms of the incretins.

Where there was more than one exercise condition in a cross-over design study the number of participants (N) was divided between those conditions and they were each entered in the analysis as separate entries. In cases where there was an odd number of participants assigned to two exercise conditions, a coin was flipped to assign numbers to each condition.

## 3.4 RESULTS

### 3.4.1 Description of studies

The literature search retrieved 1388 records. Duplicates were removed to decrease the number of potentially relevant studies to 1210. After screening titles and abstracts, 88 studies were deemed appropriate for full-text review. Twenty-nine studies met our inclusion criteria after reviewing full texts. The majority of the full texts were excluded from the analysis because their exercise protocol involved more than a single bout of exercise (i.e., regular exercise training) and did not examine the acute effect of exercise. A flow diagram of the search results is displayed in Figure 3-1.

Of the 29 included studies four did not have blood samples taken immediately before and after exercise (192-195), but had samples acquired throughout oral glucose tolerance or meal tests conducted in the hours following exercise. There was a wide variety in regard to the timing of the meal tests and also their compositions (which is known to affect incretins) therefore a meta-analysis did not seem appropriate. Hence, only a qualitative summary is provided.

Two studies included participants with type 1 diabetes (196, 197), one was performed on subjects with chronic cardiac failure (198) and one was done in prepubertal children (199). These four studies were not included in quantitative analyses. Overall, twenty studies, with a total of 271 participants, were used in the quantitative analyses.

An article by Adam et al. (200) included normal weight and obese subgroups. The obese group was followed during a three-month weight loss program. Although the area under the curve (AUC) for GLP-1 concentrations was reported in obese group before and after weight loss

program and also for normal weight participants at the beginning of the study, the active GLP-1 concentrations at different time points were only reported for the normal weight group. Thus, only the results from normal weight participants were included in the meta-analysis.

Twelve studies with a total of 163 participants introduced a control (no-exercise) condition in addition to exercise. All of these studies, except one (201), were crossover trials in which participants completed both the exercise and control conditions. Nineteen studies with a total of 241 participants were included in the pre- vs post-exercise analyses. Among the twenty-one studies included in the quantitative analyses, seven studies performed the exercise in the fasting state (201-207). One trial involved both fasting and postprandial conditions (208). Nyhoff et al. (188) applied exercise intervention 4.5 hours after lunch. Participants in the study of Howe et al. (209) performed exercise at least 4 hours postprandial. Both of these studies were considered as postabsorptive state. Participants in the remaining twelve studies performed the exercise in the postprandial state. Table 3-1 summarizes characteristics of the included participants, of the exercise interventions, and whether exercise was performed in a fasted or postprandial state.

### 3.4.2 Effects of exercise on GLP-1

Compared to the control condition, plasma concentrations of GLP-1 were significantly higher at the end of exercise in normal weight participants (SMD = 0.46, 95% CI, 0.19 to 0.73, p for heterogeneity = 0.66, I2 = 0%, see Figure 3-2.b.). GLP-1 was also increased following exercise in obese individuals (SMD = 0.42, 95% CI, 0.00 to 0.84, p for heterogeneity = 0.05, I2 = 54%, see Figure 3-2.b.). The present heterogeneity was explained by the results from study of Nyhoff et al. (188). This study performed exercise in the postabsorptive phase, and also tended to have

baseline differences between exercise and control. By removing Nyhoff study from the analyses the effect of exercise was no longer significant in the obese subgroup. The comparison between exercise and control condition was also performed for the incretin concentrations at the baseline (i.e., pre-exercise) and did not reveal statistically significant differences in both normal weight (SMD = 0.03, 95% CI, -0.23 to 0.30, p for heterogeneity = 0.94, I2 = 0%, see Figure 3-2.a.) and obese participants (SMD = 0.27, 95% CI, -0.14 to 0.68, p for heterogeneity = 0.23, I2 = 27%, see Figure 3-2.a.).

Pre- versus post-exercise comparison for the concentrations of GLP-1 showed a significant increase in normal weight participants (SMD = 0.71, 95% CI, 0.48 to 0.93, p for heterogeneity = 0.008, I2 = 52%, see Figure 3-3) but not in obese subjects (SMD = -0.04, 95% CI, -0.44 to 0.36, p for heterogeneity = 0.92, I2 = 0%, see Figure 3-3).

Despite an apparent decrease in GLP-1 thirty minutes post exercise, compare to the immediately post exercise values, there was a significant heterogeneity in the normal weight group (SMD = 0.29, 95% CI, 0.05 to 0.54, p for heterogeneity = 0.008, I2 = 52%, see Figure 3-4). This was explained by the results from study of Larson-Meyer et al. (210). Removing Larson-Meyer's study from the analysis showed that GLP-1 concentrations remained steady 30 minutes after the end of exercise. In the obese subgroups, GLP-1 was maintained at a level similar to the post exercise values (SMD = 0.25, 95% CI, -0.21 to 0.72, p for heterogeneity = 0.56, I2 = 0%). Although studies reporting active GLP-1 were pooled with the ones measuring total GLP-1, running separate meta-analysis for active and total GLP-1 did not reveal any meaningful differences. In the trials that compared exercise versus a control condition, active GLP-1 was

significantly higher immediately following exercise compare to the control in normal weight but not in obese participants.

In the article by Hazell et al. (211) the changes in total GLP-1 concentrations were reported relative to baseline, but baseline values were not reported. Hence, the plasma concentrations of total GLP-1 could not be calculated from that study.

### 3.4.3 Effects of exercise on GIP

The effects of exercise on concentrations of GIP before and immediately after the end of exercise are shown in Figure 3-5. Exercise did not have any significant effects on GIP both in normal weight ($p = 0.74$) and obese ($p = 0.66$) participants (see Figure 3-5). Among five studies that reported GIP concentrations (188, 198, 201, 207, 212), only the studies of O'Connor et al. (212) and Koivisto (207) reported GIP levels thirty minutes after the end of the exercise. They did not find any significant changes of GIP concentrations compared to baseline values. Further comparison of exercise with control condition did not reveal any significant differences in plasma GIP between different conditions.

### 3.4.4 Incretin responses to meals ingested following exercise

Eleven studies further investigated the effects of exercise on responses to meal after exercise termination (188, 189, 192-195, 201, 203, 208, 212, 213). There were different intervals between the end of exercise and the beginning of meals and also various meal compositions and volumes. Therefore, no quantitative analyses were performed for this variable.

In five studies (188, 189, 193, 208, 213) either a test drink or a meal was provided within sixty minutes from the end of exercise. There was no significant difference in incretin concentrations between exercise and control conditions. Three studies (192, 194, 195) conducted exercise the evening before a next day OGTT. None of those studies found any significant difference between effects of exercise and control conditions on incretins.

In study by Brown et al. (203) on normal weight participants, despite having conditions with different beverages, there was no control (no-exercise) condition to be compared with exercise on meal responses. Normal weight runners in the study of O'Connor et al. (212) received either 75 grams glucose (in 250 ml water) or water right after the end of the exercise. On a separate control (no-exercise) condition they received glucose. Despite increased active GLP-1 in all conditions, there was no significant difference in active GLP-1 between the exercise and control conditions. GIP concentration increased to a similar extent after ingesting glucose in both the exercise and control conditions. Blom et al. (201) investigated the effect of exercise versus control (no-exercise) on GIP concentrations of normal weight participants. A glucose beverage (0.7 g of glucose per kg body weight, given as a 30% solution in $H_2O$) was ingested by participants immediately after exercise and 2, 4, and 6 hours later. Postprandial GIP concentrations were significantly higher in control condition compared to exercise.

### 3.4.5 Methodological quality and risk of Bias

Overall the included studies received positive scores specially for the first two questions on methodological quality and risk of bias. Sensitivity analyses were performed to examine whether there was a significant difference between randomised and nonrandomised trials. No meaningful difference was found. The incomplete data and how they were treated (i.e., missing

blood samples) was reported for two studies (203, 213). Since, the acute glucose lowering effects of exercise can last for up to 72 hours, the risk of carry-over effect was considered high if there was less than 72 hours between exercise and control conditions. Only one study had wash-out period that might have potentially been less then 72 hours for some of its' participants (205).

Funnel plots were created for each outcome measure to investigate the risk of publication bias. Visual inspection of funnel plots and Egger's linear regression approach did not reveal any significant risk of publication bias (plots not shown).

**3.5 DISCUSSION**

The primary findings of the current study were that in comparison to control condition, exercise significantly increased GLP-1 concentrations in normal weight but not in individuals with obesity.

Although there have been other systematic reviews (214-217) examining the effects of exercise on appetite regulating hormones, including incretins, they each included fewer studies with incretins as outcomes (i.e., from 2-7 studies) than 29 studies included in present review. The current study exclusively focuses on incretin hormones (i.e., GLP-1 and GIP). To the best of our knowledge, only one review differentiated between total and active forms of GLP-1 (215). The time-to-peak responses following exercise and absolute concentrations of active and total GLP-1 are expected to be different (218). However, under normal circumstances the changes in their plasma concentrations are associated with a similar pattern. Hence, we pooled the studies measuring active and total GLP-1 together for our meta-analyses.

We observed a pattern of increased GLP-1 immediately post exercise (Figures 3-2 and 3-3), however there was some heterogeneity in our findings. Subgroup analyses according to weight status suggested that obesity could reduce the effect of acute exercise on GLP-1 (Figure 3-2). Difference between normal weight and obese participants may be due to differences in study protocols. However, our findings are in line with the results from a study by Adam et al. (200). In Adam's study, in contrast to responses in obese group (before weight loss) acute exercise only increased GLP-1 in normal weight group.

In a large study by Faerch et al. (219) conducted in normal weight, overweight and obese participants, GLP-1 responses to an oral glucose tolerance test (OGTT) were about 8% and 20% lower in overweight and obese groups, respectively. Previous studies have shown a negative correlation between BMI and incretin secretion (181). Although in current review GLP-1 did not increase at the end of exercise in obese participants, similar to the control (no exercise) condition, it was kept relatively stable in post-exercise compared to pre-exercise.

While current review attempted to include participants with different glucose control status, only one of our randomised clinical trials (193) performed on type 2 diabetes participants was identified and met our inclusion criteria at the time of literature search. In a recent randomised clinical trial published after our literature search (218) we showed that in T2D patients walking two bouts of 90 minutes on a treadmill does not have any significant effects on GLP-1 and GIP concentrations immediately after the end of the exercise.

Another potential limitation of the current review is that we did not differentiate between aerobic exercise of different intensities. While some studies used moderate intensity, others utilized high intensities of exercise. There were also two studies that used sprint interval

exercise as their intervention. We also did not include trials that used resistance exercise. Nevertheless, we were able to explain the heterogeneity among studies by comparing studies according to participants' BMI and timing of the meal (i.e., fasting versus postprandial).

Since the present review focussed on the acute effects of exercise (i.e., a single bout), future reviews should include studies with longer duration exercise training. Furthermore, performing systematic reviews that focus on differences between various exercise modes, intensities and frequencies might help to identify more effective forms of exercise for increasing incretin concentrations.

In summary, the current review showed a single bout of exercise significantly increases GLP-1 secretion in normal weight but not obese participants. The practical implications of these findings remain to be further elucidated, as do the effects of longer-term exercise training.

**Table 3-1 Characteristics of included studies**

| Source | Participant characteristics | | | | Outcome | Fasting vs. postprandial vs. post-absorptive exercise | Exercise characteristics | | |
|---|---|---|---|---|---|---|---|---|---|
| | Group | Sample size (M/F) | Age, y | BMI ($kg/m^2$) | | | Type | Duration (min) | Intensity |
| Adam2004 (200) | NW<br>Ob | 28(12/16)<br>27 (21/6) | 35 (12.7)<br>47.1 (11.9) | 22.9 (1.4)<br>30.9 (2.7) | Active GLP-1 | Fasting | Cycling | 60 | 25% POWERmax |
| Bailey (2015) (189) | | 12 (12/0) | 21.6 (2) | 23.5 (2) | Total GLP-1 | Postprandial | Treadmill | 50 | MIE: 70% $VO_{2max}$<br>HIIE: 90% $VO_{2max}$ |
| Beaulieu (2015) (213) | | 6 (6/0) | 25 (3) | NR | Active GLP-1 | Postprandial | Treadmill | 20 | 30-s sprints: 4 min recovery |
| Blom (1985) (201) | Control<br>Exercise | 5 (5/0)<br>5 (5/0) | 28 (4.9)<br>23.2 (6.0) | NR<br>NR | GIP | Fasting | NR | NR | 75% Max $O_2$ uptake |
| Brown (2016) (203) | | 13 (0/13) | 23 (4) | 23.1 (2.9) | Active GLP-1 | Fasting | Cycling | 60 | 65% $VO_{2peak}$ |
| Campbell (2015) (196) | | 10 (10/0) | 27 (3.2) | 25.5 (0.95) | Total GLP-1 | Postprandial | Treadmill | 45 | 70% $VO_{2peak}$ |
| Dekker (2010) (192) | | 9 (9/0) | 59 (2) | 34 (2) | Active GLP-1<br>GIP | NR | Treadmill | 60 | 55% $VO_{2peak}$ |
| Eshghi (2013) (193) | | 10 (8/2) | 58 (6) | 28.6 (5.3) | Total GLP-1<br>GIP | Postprandial | Treadmill | 15<br>15<br>5 | 3.5 kph<br>Bellow VT<br>Above VT |
| Gonzalez (2013) (208) | | 11 (11/0) | 23.2 (4.3) | 24.5 (2) | Active GLP-1 | Fasting and Postprandial | Treadmill | 59 | 60% $VO_{2peak}$ |
| Hazell (2017) (211) | | 10 (10/0) | 29 (6) | 23.7 (2.2) | Total GLP-1 | Postprandial | Cycling | 40<br>40<br>37 | MICT: 65% $VO_{2max}$<br>HICT: 85% $VO_{2max}$<br>SIT: 30-s sprints, 4 min recovery |

| Source | Participant characteristics | | | | Outcome | Fasting vs. postprandial vs. post-absorptive exercise | Exercise characteristics | | |
|---|---|---|---|---|---|---|---|---|---|
| | Group | Sample size (M/F) | Age, y | BMI ($kg/m^2$) | | | Type | Duration (min) | Intensity |
| Heden (2013) (194) | NW<br>Ob | 13 (7/6)<br>13 (6/7) | 26.0 (2)<br>25.4 (1) | 23.0 (0.5)<br>34.6 (1) | Active GLP-1<br>GIP | NR | Treadmill | 60 | 55% - 60% $VO_{2peak}$ |
| Howe (2016) (209) | | 15 (0/15) | 31.1 (6.7) | 21.1 (1.7) | Active GLP-1 | Postabsorptive | Treadmill | 45.7 (10.8)<br>33.6 (5.6) | MIE: 60% $VO_{2max}$<br>HIE: 85% $VO_{2max}$ |
| Kawano (2013) (204) | | 15 (15/0) | 24.4 (1.7) | 22.1 (2) | Active GLP-1 | Fasting | Cycling<br>Skipping | 40<br>40 | 63.9% (7.5) $VO_{2max}$<br>64.8% (6.9) $VO_{2max}$ |
| Koivisto (1985) (207) | | 8 (8/0) | 25 (1) | NR | GIP | Fasting | Cycling | 120 | 55% $VO_{2max}$ |
| Larson-Meyer (2012) (210) | | 9 (0/9) | 23.7 (2.4) | 19.8 (1) | Total GLP-1 | Postprandial | Treadmill | 60 | 70% $VO_{2max}$ |
| Maffeis (2013) (199) | | 10 (NR) | 9.0 (0.9) | 26.4 (2.2) | Total GLP-1<br>GIP | Fasting | Cycling | 30 | 4 x RMR |
| Marchbank (2011) (206) | | 12 (12/0) | 26.0 | 23.7 (1.5) | Active GLP-1 | Fasting | Treadmill | 20 | 80% $VO_{2max}$ |
| Martins (2007) (220) | | 6 (6/0) | 25.9 (4.6) | 22 (3.2) | Total GLP-1 | Postprandial | Cycling | 60 | MIIC: 65% $HR_{max}$ |
| Martins (2015) (221) | | 12 (5/7) | 33.4 (10) | 32.3 (2.7) | Total GLP-1 | Postprandial | Cycling | 27 (6)<br>18 (3)<br>9 (2) | MIIC: 70% $HR_{max}$<br>HIIC: 85-90% $HR_{max}$<br>S-HIIC: 85-90% $HR_{max}$ |
| Nicholls (1992) (198) | | 10 (10/0)<br>18 (16/2) | 65.0 (NR)<br>64.5 (NR) | NR<br>NR | GIP | Postprandial<br>Fasting | Treadmill | NR<br>NR | Modified Naughton exercise test |
| Numao (2013) (195) | | 11 (11/0) | 27.0 (1.0) | 21.5 (0.5) | Active GLP-1<br>GIP | NR | Cycling | 32 (2) | 50% $VO_{2peak}$ |

| Source | Participant characteristics | | | | Outcome | Fasting vs. postprandial vs. post-absorptive exercise | Exercise characteristics | | |
|---|---|---|---|---|---|---|---|---|---|
| | Group | Sample size (M/F) | Age, y | BMI ($kg/m^2$) | | | Type | Duration (min) | Intensity |
| Nyhoff (2015) (188) | | 11 (0/11) | 24.3 (1.4) | 37.3 (2.1) | Active GLP-1 GIP | Postabsorptive | Treadmill | 400 kcal | ModEx: 55% $VO_{2max}$<br>IntEx: 80% $VO_{2max}$ |
| O'Connor (1995) (222) | | 26 (23/3) | 37 | NR | Total GLP-1 | NR | Marathon | 239 (169 – 307) | NR |
| O'Connor (2006) (212) | | 6 (6/0) | 35.5 (5) | NR | Total GLP-1 GIP | Postprandial | Treadmill | 120 | 60% $VO_{2max}$ |
| Stevenson (2009) (223) | | 8 (0/8) | 23.8 (7.2) | 21.3 (1.9) | Active GLP-1 | Postprandial | Treadmill | 60 | 50% $VO_{2peak}$ |
| Stevenson (2015) (197) | | 10 (10/0) | NR | NR | GLP-1 | Postprandial | Treadmill | 45 | 70% $VO_{2peak}$ |
| Ueda (2009a) (224) | | 10 (10/0) | 23.4 (4.3) | 22.5 (1) | Total GLP-1 | Postprandial | Treadmill | 30 | MIE: 50% $VO_{2max}$<br>HIE: 75% $VO_{2max}$ |
| Ueda (2009b) (225) | NW<br>Ob | 7 (7/0)<br>7 (7/0) | 22.4 (4.2)<br>22.9 (3.4) | 22.4 (2.4)<br>30 (3.1) | Active GLP-1 | Postprandial | Treadmill | 60 | MIE: 50% $VO_{2max}$ |
| Unick (2010) (205) | | 19 (0/19) | 28.5 (8.3) | 32.5 (4.3) | Total GLP-1 | Fasting | Treadmill | NR | 70-75% age-predicted $HR_{max}$ |

M, male; F, female; Y, years; SD, standard deviation; BMI, body mass index; $VO_{2max}$, maximal oxygen consumption; $HR_{max}$, maximum heart rate; MIE, moderate interval exercise; HIIE, high intensity interval exercise; MICT, moderate intensity continuous training; HICT, high intensity continuous training; SIT, sprint interval training; MIIC, moderate intensity interval cycling; HIIC, high intensity interval cycling; S-HIIC, short high intensity interval cycling; ModEx, moderate exercise; IntEx, interval exercise, NR, not reported; NW, normal weight; Ob, obese.

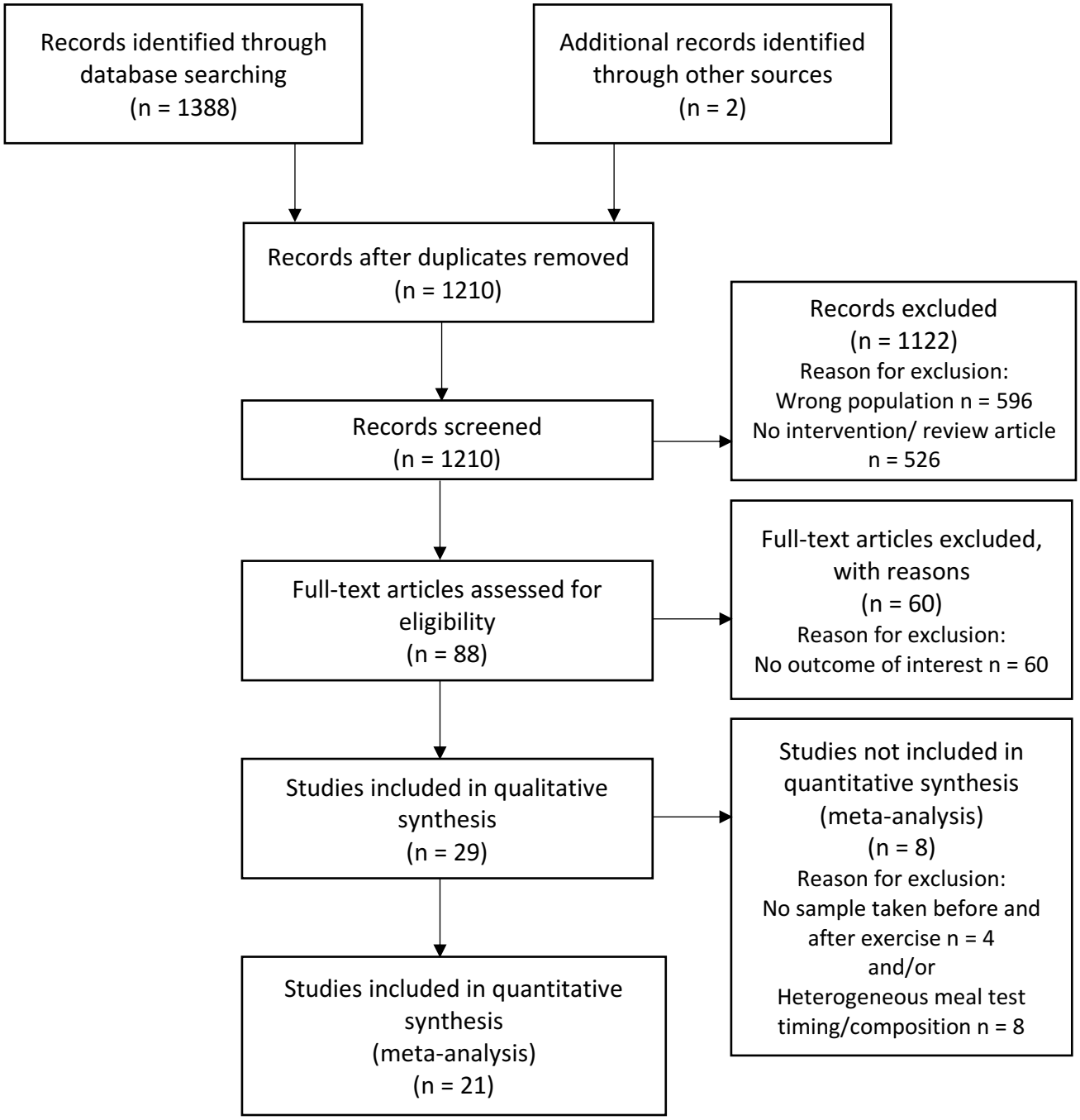
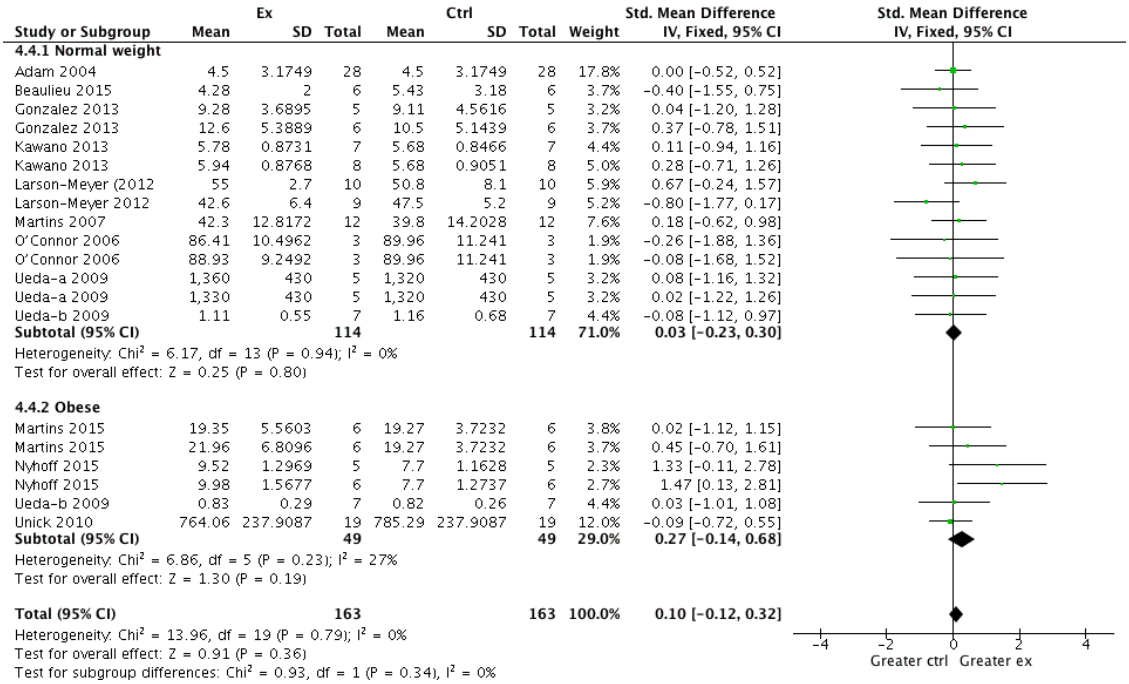



**Figure 3-1 PRISMA flow diagram (226)**

**a.**




**b.**

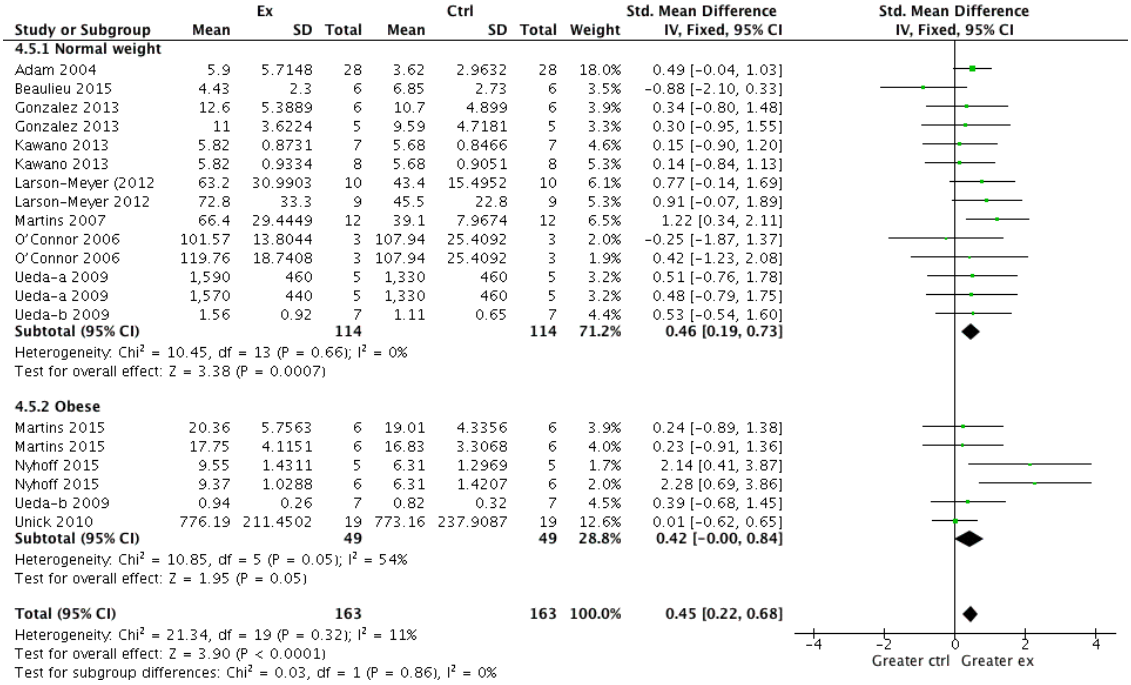



**Figure 3-2 GLP-1 before (a) and immediately following (b) exercise versus control conditions**

CI, confidence interval; SD, standard deviation.

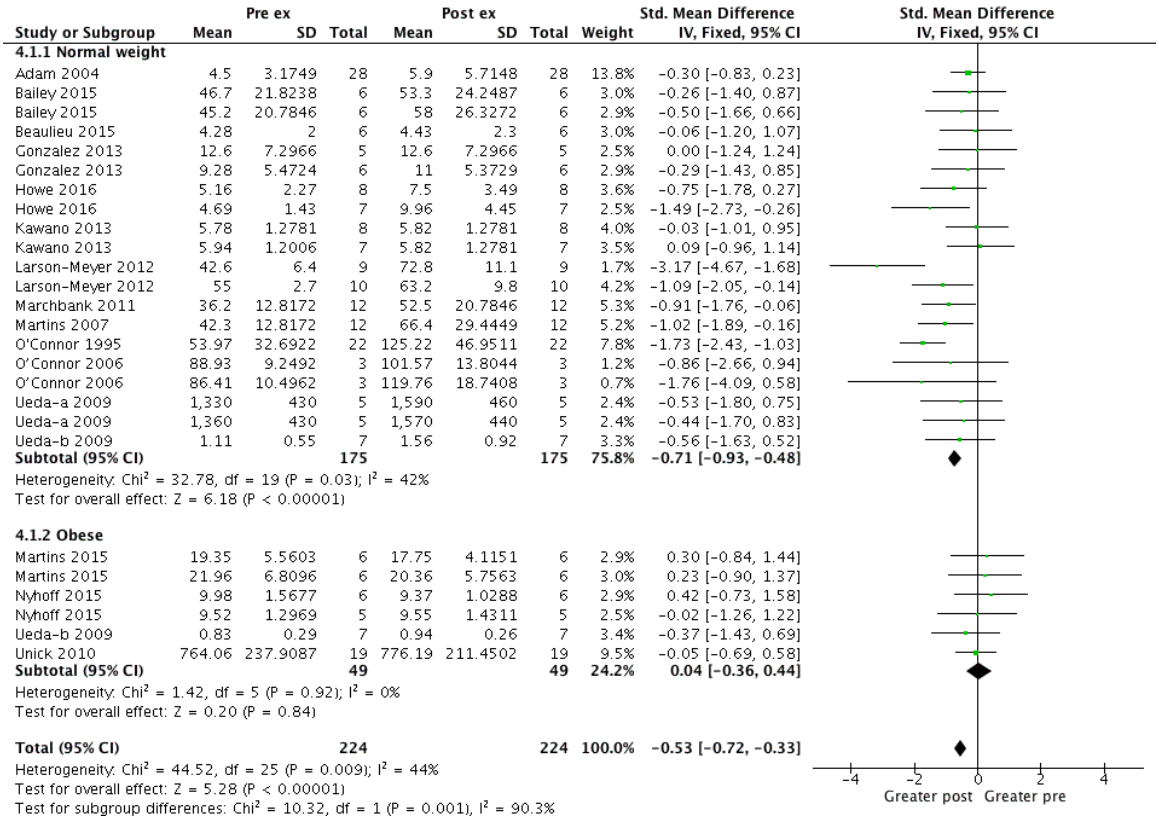



**Figure 3-3 GLP-1 pre-exercise versus post-exercise**

CI, confidence interval; SD, standard deviation; Pre, pre-exercise; Post, post-exercise.

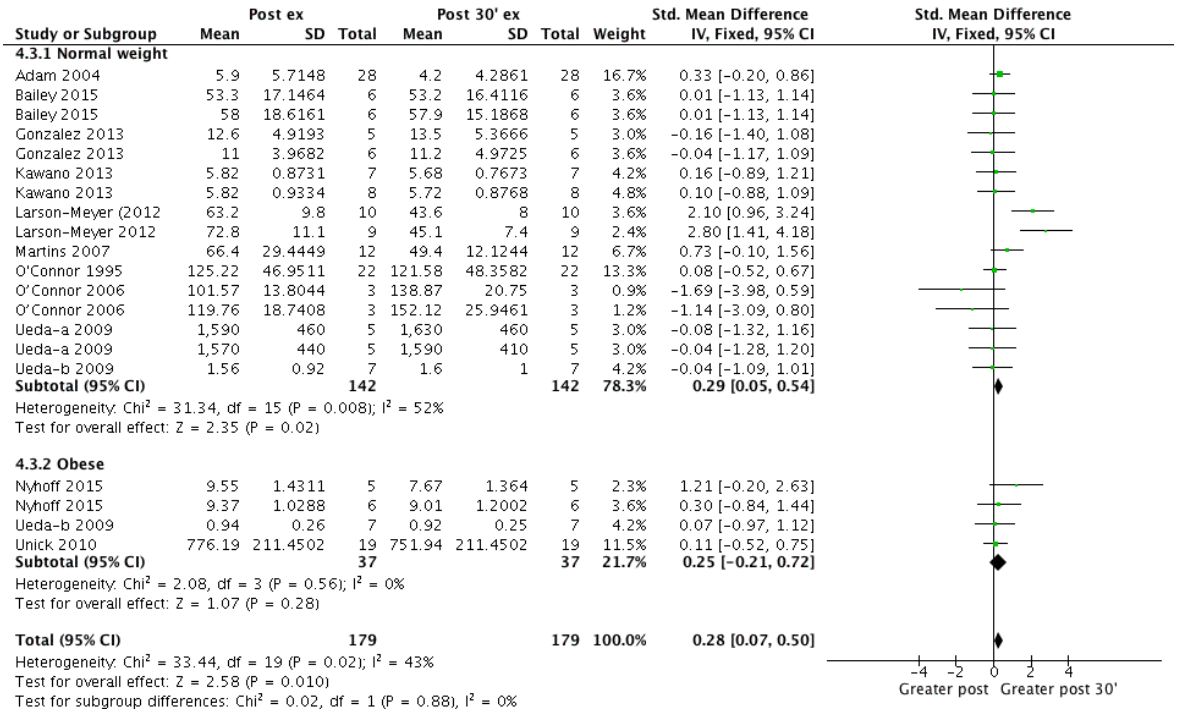



**Figure 3-4 GLP-1 post exercise versus 30 minutes post-exercise**

CI, confidence interval; SD, standard deviation; Pre, pre-exercise; Post, post-exercise.

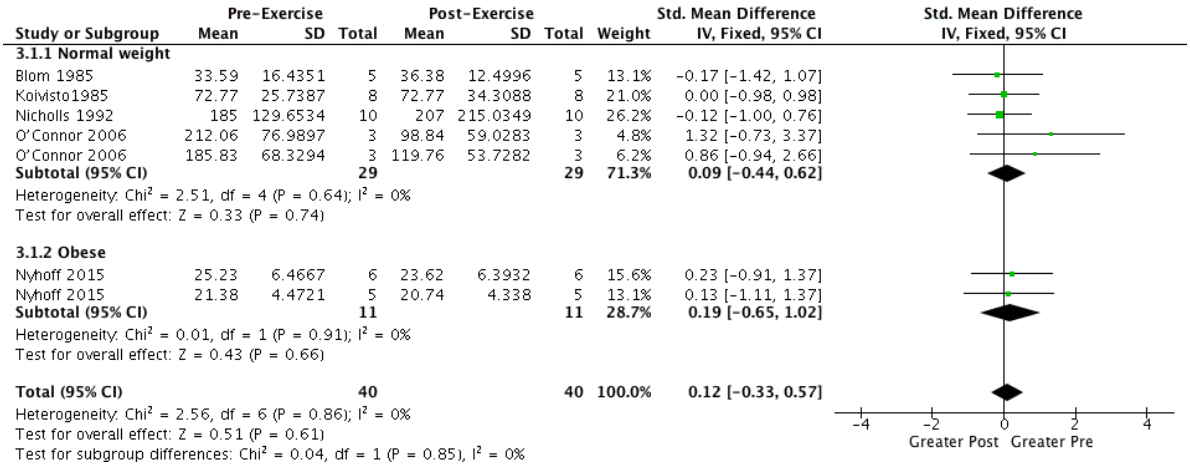



**Figure 3-5 GIP pre-exercise versus post-exercise**

CI, confidence interval; SD, standard deviation; Pre, pre-exercise; Post, post-exercise.

# Chapter 4

## [4]Effects of Aerobic Exercise with or without Metformin on Incretins in Type 2 Diabetes


**ABSTRACT**

**OBJECTIVE:** Despite positive effects of incretins on insulin secretion, little is known about the effect of exercise on these hormones. Metformin can affect incretin concentrations and is prescribed to a large proportion of people with diabetes. We, therefore, examined the effects of aerobic exercise and/or metformin on incretin hormones.

**METHODS:** Ten participants with type 2 diabetes were recruited for this randomized crossover study. Metformin or placebo was given for 28 days, followed by the alternate treatment for 28 days. On the last 2 days of each condition, participants were assessed during a non-exercise day and a subsequent exercise day. Aerobic exercise took place in the morning and blood samples were taken in the subsequent hours (before and after lunch).

**RESULTS:** Aerobic exercise did not increase total plasma glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotropic polypeptide (GIP) in the pre- or post-lunch periods (all $p>0.1$). GLP-1 was higher in the pre-lunch ($p=0.016$) and post-lunch ($p=0.018$) periods of the metformin conditions compared with the placebo. Total plasma GIP was higher in the pre-lunch period ($p=0.05$), but not in the post-lunch period ($p=0.95$), with metformin compared with placebo.


[4] A version of this chapter has been published:
Eshghi, S.R., G.J. Bell, and N.G. Boule. Can J Diabetes, 2013 Dec;37(6):375-80. doi: 10.1016/j.jcjd.2013.07.030

**CONCLUSIONS:** In contrast to our hypothesis, aerobic exercise did not acutely increase total GLP-1 and GIP concentrations in patients with type 2 diabetes. Metformin, independent of exercise, significantly increased total plasma GLP-1 and GIP concentrations in these patients.

## 4.1 INTRODUCTION

Diabetes mellitus is caused by defective insulin secretion, defective insulin action or both (1). It has been known for over 40 years that insulin secretion is greater in response to oral glucose compared to an intravenous glucose load that leads to a similar plasma glucose profile (227). This is known as the incretin effect, and the two main incretin hormones are considered to be glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP; also called gastric inhibitory polypeptide) (228, 229). The secretion of these incretins, especially GLP-1, is impaired in type 2 diabetes (120, 122, 181). Pharmacological interventions targeting these hormones have been approved recently in Canada (230). Some of these drugs are classified as GLP-1 receptor agonists (e.g., Exenatide and Liraglutide) or dipeptidyl peptidase-4 inhibitors (e.g., Sitagliptin, Saxagliptin and Linagliptin) that exert their effects through a DPP-4 resistant analogue to GLP-1 (231) or by increasing GLP-1 half-life, respectively (152).

Compared to the knowledge accumulated on these pharmacological interventions, very little is known about the effects of exercise on GLP-1 or GIP. This is important since exercise is considered to be a first line intervention for the prevention and management of type 2 diabetes (24-26). Previous research has consistently shown that exercise can increase incretins in healthy subjects (17, 18). However, to our knowledge, no studies in patients with type 2 diabetes (who have impaired incretin responses) have been published.

In addition to exercise, metformin is also considered a first line therapy for type 2 diabetes (232) and this is notable because a large proportion of individuals with diabetes are treated with metformin. While Canadian data is scarce, the estimated number of metformin prescriptions in the U.S. has increased from about 38 million in 2006 to over 48 million in 2010 (Top 10 for generic drugs) (233). In type 2 diabetes, metformin can inhibit the DPP-4 activity in fasting state and thus increase the incretins' concentrations (234, 235) and has been shown to increase plasma concentrations of GLP-1 in rats (236). It is unknown if the combination of metformin and exercise leads to greater incretin responses than following metformin alone. Interestingly, we recently observed that the combination of metformin and exercise acutely increased glucagon concentrations (23); a finding that might be explained by changes in incretin hormones concentrations (157, 237, 238).

Therefore, the objectives of the current study were to examine the effects of exercise, metformin and their combination on plasma concentrations of GLP-1 and GIP before and after meal ingestion. It was hypothesized that both exercise and metformin would increase GLP-1 and GIP in people with type 2 diabetes.

## 4.2 METHODS

### 4.2.1 Participants

Ten volunteers (eight men and two postmenopausal women) with type 2 diabetes were recruited for this study, which was approved by the University of Alberta Health Research Ethics Board. Complete details have been previously published (23). Briefly, participants met the following eligibility criteria: 1) between 30 and 65 years of age; 2) not taking glucose-lowering medication or insulin; 3) no changes in physical activity over the last 3 months and not planning

on changing medication, physical activity, or diet over the course of the study; and 4) HbA1c ≤8%, resting blood pressure ≤140/90 mmHg, LDL cholesterol ≤3.5 mmol/L, and total: HDL cholesterol ≤5.0.

### 4.2.2 Study design

The study used a 2 x 2 factorial design during which each participant was exposed to 4 conditions: 1) metformin and no exercise, 2) metformin and exercise, 3) placebo and no exercise, and 4) placebo and exercise. The order of the metformin versus placebo conditions was randomly assigned by personnel not involved with the study, and allocation was concealed in sealed envelopes until participants completed the study. Participants, study personnel, and investigators were blinded to the order of the placebo/metformin conditions. Metformin or placebo was given for 28 days, immediately followed by the alternate condition for 28 days. On the last 2 days of each condition (days 27 and 28), participants returned to the laboratory for a non-exercise and exercise session, respectively. The order of these sessions was not randomly determined and exercise was always performed on day 28 since the acute glucose-lowering effect of exercise may persist for at least 24 h (239). The experimental design of this study and a portion of the methods have been previously published but there is no duplication in any of the dependent variables reported in this paper (23).

### 4.2.3 Study protocol

As previously described (23) a baseline exercise stress test was performed to determine the participants' peak oxygen uptake ($VO_{2peak}$) and ventilatory threshold. Participants were given either metformin or placebo pills and were asked to maintain their routine physical activity and dietary habits. Each participant consumed 500 mg of metformin with breakfast during the first

week of the intervention followed by a 500-mg increase in each of the subsequent weeks until 1,000 mg were consumed with breakfast and supper during week 4 (total: 2,000 mg/day).

On days 27 and 28 of the metformin and placebo conditions, participants arrived in the laboratory at 8:00 A.M. after a 12-h fast. Participants ate a standardized breakfast (549 kcal; 56% carbohydrate, 30% fat, 14% protein) and took their assigned pills. At 10:00 A.M., an intravenous catheter was inserted into an antecubital vein kept patent with 0.9% sterile saline. On day 27 of the metformin and placebo conditions, the participants remained at rest for the duration of the testing period. At 10:45 A.M. of day 28 during both conditions, participants performed a series of exercises that were selected to represent different intensities, modes, and energy systems. They began with 20 consecutive maximal leg extensions and flexions on a Cybex II isokinetic dynamometer.

After a 5-min rest period, the first of three aerobic exercise bouts began. Each bout was separated by a 5-min rest period. During the first exercise bout, all participants walked at 3.5 km/h and 0% grade for 15 min. This corresponded to the estimated average walking speed for individuals with type 2 diabetes in free-living conditions (240). The second bout also lasted 15 min and was completed at a speed and grade equivalent to an exercise intensity below each participant's measured ventilatory threshold. The third bout was completed at an intensity above their ventilatory threshold and lasted 5 min. The above exercise protocol was developed as part of our original study which was designed to examine the effect of metformin on various types and intensities of exercise. The mean energy expenditure over the 35 minutes of aerobic exercise was 4.9 metabolic equivalents (METs) (23). Consequently the total energy expenditure from this exercise would be consistent with a 50-minute brisk walk at 3.5 METs (241), which would meet

the recent Canadian Diabetes Association minimum recommendation for aerobic exercise if performed 3 times per week (26).

Approximately 20 min after exercise (at 11:59 A.M.), a blood sample was taken immediately before the standardized meal (556 kcal; 59% carbohydrate, 22% fat, 19% protein). Participants remained in the laboratory, and blood samples were taken at 12:30 and 1:00 P.M. Additional blood samples had been taken throughout the day (23), however only the above 3 samples have been analysed for glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) due to budgetary constraints. These samples were targeted as they were thought to best represent the acute effect of exercise and meal ingestion.

Each blood sample was first transferred into a 10-mL EDTA vacutainer tube. Tubes were centrifuged after which the plasma was aliquoted into tubes prior to cooling and storage in a freezer at -20°C until assays were completed. Total GLP-1 and total GIP were measured according to the manufacturers' procedures using commercially available enzyme-linked immunosorbent assay (ELISA) kits (EMD Millipore, Billerica, MA, U.S.A.). All samples were run in duplicate in each assay. The mean coefficient of variation for GLP-1 and GIP was 3.6% and 2.7%, respectively.

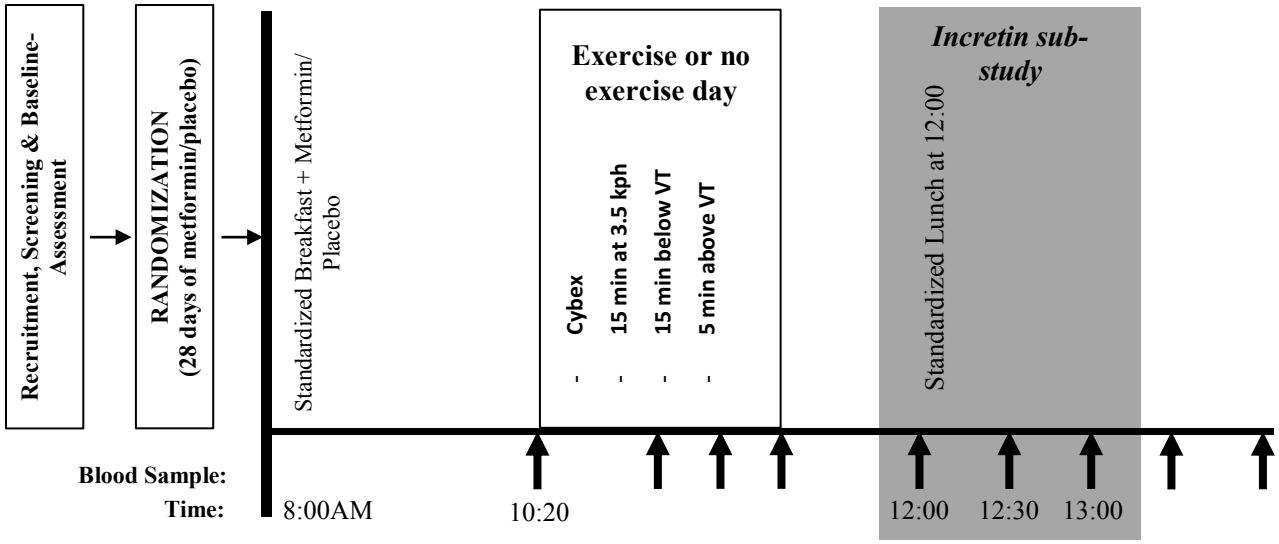



**Figure 4-1 Study experimental design**
Adopted from Metformin and exercise in type 2 diabetes (Boule 2011) (23)
kph, kilometers per hour; VT, ventilatory threshold

### 4.2.4 Statistical analyses

Analyses were conducted using repeated-measures ANOVA with treatment order added as a between-subject factor. To simplify the interpretation, the testing days were broken down into two periods: pre-lunch, and post-lunch. The number of within-factors and levels varied among these periods [e.g., post lunch was a 2 × 2 × 2 factorial ANOVA to examine the effect of exercise, metformin, and time (i.e., 30 and 60 minutes post lunch), respectively]. The mean ± standard errors of the mean data are presented. Statistical tests were two-tailed, and P values of ≤0.05 were considered significant. Statistical analyses were performed with SPSS 21 (SPSS Inc., Chicago, IL).

## 4.3 RESULTS

Baseline characteristics are presented in Table 4-1. Participants had an average body mass index (BMI) of 28. 6 ± 5.3 kg/m$^2$ and relatively well controlled glycemia (glycated hemoglobin =

6.5 ± 0.6% and fasting glucose 7.3 ± 0.6 mmol/L). Some reported mild to moderate gastrointestinal side effects during the 4-week metformin intervention; but all participants except one (final metformin dosage, 1,500 mg/day) were able to tolerate the maximum dosage of 2,000 mg/day during the last week of the intervention. Half of the participants started with 28 days of metformin.

The first 15-min bout was performed at 33.9 ± 5.4% of $VO_{2peak}$, the second 15-min bout averaged 67.2 ± 7.3% of $VO_{2peak}$, and the remaining 5-min bout averaged 79.4 ± 8.8% of $VO_{2peak}$.

### 4.3.1 Plasma Total GLP-1 concentration

Plasma total GLP-1 concentrations pre-lunch were unaffected by exercise ($p = 0.48$) but were significantly higher in both non-exercise and exercise days with metformin compared to similar days during the placebo conditions ($p = 0.02$, see Figure 4-1). Plasma concentrations of total GLP-1 remained higher in both metformin conditions during the 1-h post-lunch period ($p = 0.02$).

### 4.3.2 Plasma GIP concentration

Plasma total GIP concentrations pre-lunch were also unaffected by exercise ($p = 0.38$) but were significantly higher in both non-exercise and exercise days with metformin compared to the placebo conditions ($p = 0.05$, see Figure 4-1). Plasma GIP concentrations increased post lunch in both metformin and placebo conditions, but GIP concentration was no longer significantly different between metformin and placebo conditions 1-h after lunch.

## 4.4 DISCUSSION

The current study was unique in that for the first time the effects of exercise and/or metformin on incretin hormones in type 2 diabetes was investigated. As opposed to previous studies in healthy participants (17, 242), our study in people with type 2 diabetes did not observe an increase in GLP-1 and GIP following exercise. However, we did confirm that the administration of metformin increased the concentration of total GLP-1 and GIP. This occurred in both exercise and non-exercise conditions. This observation is consistent with Ryskjaer et al. (243) that found higher total GLP-1 and GIP concentrations in diabetic patients receiving metformin in comparison to a group that did not receive metformin.

Most of the studies on the effect of physical activity on incretin hormones have been conducted with healthy subjects, athletes or in obese participants. O'Connor et al. showed prolonged exercise (running on a treadmill for 2 h at 60% $VO_{2max}$) with and without subsequent glucose ingestion can significantly elevate GLP-1 and GIP concentrations in healthy people (17). In marathon runners, the GLP-1 increased after a race (18). One hour cycling on 65% HRmax in 12 healthy normal weight volunteers raised GLP-1 as well (242). The GLP-1 concentrations began to increase during exercise and stayed higher than the baseline after exercise (242). Although GLP-1 concentrations were significantly lower in obese people compare to lean individuals, a 3-month exercise and diet-induced weight loss program increased GLP-1 concentrations that were similar in obese and lean individuals (200). In contrast with the aforementioned studies in non-diabetic subjects, we were unable to detect a significant effect of exercise on incretin hormones in patients with type 2 diabetes. This observation suggests that acute exercise might be unable to increase the low incretin concentrations in this population. The mechanism in which exercise

can increase incretins concentration has been recently addressed by Ellingsgaard et al. (19). They found that interleukin-6 released from contracting skeletal muscle stimulates GLP-1 secretion from the intestine and the pancreas. Although speculative, it may be that the chronically elevated interleukin-6 that are associated with type 2 diabetes may interfere with this response.

In contrast to exercise, positive effects of metformin on incretin hormones have been investigated in different populations. Although, treating subjects with metformin reduce plasma or serum DPP-4 activity (234, 244, 245), it does not directly inhibit DPP-4 activity in vitro (236, 246). Maida et al. (247) suggested that metformin modulates different components of the incretin axis, and enhances expression of the GlP-1 receptor. In a study of Migoya et al, metformin increased inactive form of GLP-1 (9-36) in mice with diet-induced obesity. In healthy humans, metformin increased total GLP-1, but not GIP (248). Similarly, a single dose of metformin can increase total GLP-1 in non-diabetic subjects (236) and chronic therapy with metformin leads to increased plasma concentrations of total GLP-1 in obese non-diabetic and diabetic participants (249, 250).

In regard to the effect of meals on plasma concentration of GLP-1, a similar GLP-1 concentration during both the metformin and placebo conditions in the pre-lunch compared to the post-lunch period is in contrast with the currently accepted meal-related GLP-1 response. Although, these findings are comparable with the ones from study of Toft-Nielsen et al. who reported an impaired response of GLP-1 related to meal ingestion in type 2 diabetes patients (122).

The effect of metformin on increasing GIP before lunch did not persist into the 1h post-lunch period where the increase in GIP occurred in both metformin and placebo conditions.

Vollmer et al. showed an exaggerated GIP response to meal ingestion in patients with type 2 diabetes (123). In another study, metformin could not increase GIP concentrations in healthy human subjects (248). In the current study, metformin was still an effective agent to raise GIP concentrations before meal ingestion.

A limitation of our study was the inability to measure active versus inactive GLP-1 and GIP. At the time of the sample collection, a DPP-4 inhibitor was not added. Therefore, we could only measure total GLP-1 and GIP. Nevertheless, studies have shown that the active and total forms of GLP-1 and GIP follow the same trends (although the active form quantities are lower) (248, 251). While our sample size was small (n=10) we still were able to detect the meaningful effect of metformin. The effect of exercise was small and inconsistent and even though a larger sample size would have provided more power to detect statistical significance, it is unlikely that the effect would be clinically meaningful. Finally, we cannot claim that all exercise interventions are ineffective at raising incretins in type 2 diabetes, particularly longer term exercise training which may be associated with greater metabolic adaptations.

As we previously reported from this cohort (23), adding an acute bout of exercise to 28 days of metformin treatment increased the glucose and glucagon response to a subsequent meal. The present analyses suggest that this may be in part due to the inability of exercise to increase GLP-1 and GIP in patients with type 2 diabetes (treated with or without metformin), a finding that is contrary to previous observations in people with normal glycemia. On the other hand, we confirm that metformin can significantly increase total GLP-1 and GIP concentrations.

**ACKNOWLEDGMENTS**

Funding for this study was provided by the Alberta Diabetes Institute, the University of Alberta EFF-SAS and Faculty of Physical Education and Recreation of the University of Alberta.

**CONFLICT OF INTEREST**

No potential conflicts of interest relevant to this article were reported. N.G.B. and G.J.B. contributed to the design of the original study, collected data, analyzed data and critically revised the manuscript. S.R.T.E. contributed to the design of the current study, analyzing the blood samples, analyzed data and wrote the manuscript. All authors contributed to discussion and reviewed and edited the manuscript. The metformin and placebo were graciously provided by Apotex Inc. Canada. The authors would like to thank the study participants for their time and efforts.

| | Baseline |
|---|---|
| **Sex (men/postmenopausal women)** | **8/2** |
| **Age (years)** | **58 ± 6** |
| **BMI ($kg/m^2$)** | **28.6 ± 5.3** |
| **Weight (kg)** | **86.9 ± 18.7** |
| **A1C (%)** | **6.5 ± 0.6** |
| **Fasting glucose ($mmol \times L^{-1}$)** | **7.3 ± 0.6** |
| **$Vo_{2peak}$ ($ml.kg^{-1}.min^{-1}$)** | **30.2 ± 5.1** |

**Table 4-1 Participant characteristics**
Data are reported as mean ± SEM.

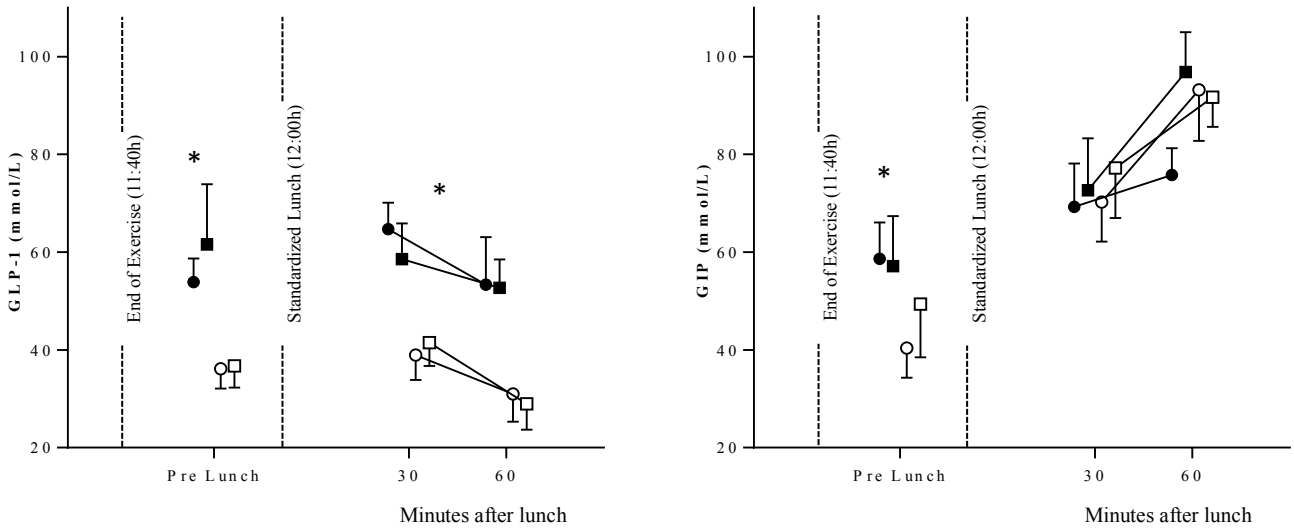



**Figure 4-2 The effect of exercise and metformin on GLP-1 and GIP**

Data are reported as mean ± SEM. Analyses were adjusted for treatment order (i.e., metformin first vs. placebo first). N = 9 or 10. ●, metformin + no exercise; ■, metformin + exercise; ○, placebo + no exercise; □, placebo + exercise; *, Significant main effect of metformin ($p<0.05$)

# Chapter 5

## [5]Glycemic and metabolic effects of two long bouts of moderate intensity exercise in men with normal glucose tolerance or type 2 diabetes


**ABSTRACT**

**Background:** The glycemic and insulinemic responses following 30-60 minutes of exercise have been extensively studied and a dose response has been proposed between exercise duration, or volume, and improvements in glucose tolerance or insulin sensitivity. However, few studies have examined the effects of longer bouts of exercise in type 2 diabetes (T2D). Longer bouts may have a greater potential to affect glucagon and interleukin-6 (IL-6) and incretin hormones [i.e., glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)].

**Aim:** To examine the effect of two bouts of long duration, moderate intensity exercise on incretins, glucagon and IL-6 responses before and after exercise, as well as in response to an oral glucose tolerance test (OGTT) conducted the following day.

**Methods:** Twelve men, six with and six without T2D, participated in two separate conditions (i.e., exercise vs. rest) according to a randomized crossover design. On day 1, participants either rested or performed two 90 minute bouts of treadmill exercise (separated by 3.5 hours) at 80% of their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ventilatory threshold. All participants received standardized meals on day 1. On day 2 of each condition, glucose and hormonal responses were measured during a 4-hour OGTT.

**Results:** On day 1, exercise increased IL-6 at the end of the first bout of exercise (exercise by time interaction p=0.03) and GIP overall (main effect of exercise p=0.004). Glucose was reduced to a greater extent in T2D following exercise (exercise by T2D interaction p=0.03). On day 2, GIP and active GLP-1 were increased in the fasting state (p=0.05 and p=0.03, respectively), while plasma insulin and glucagon concentrations were reduced during the OGTT (p=0.01 and p=0.02, respectively) in the exercise compared to the rest condition for both healthy controls and T2D. Postprandial glucose was elevated in T2D compared to healthy control ($p<0.05$) but was not affected by exercise.

**Conclusions:** Long-duration, moderate-intensity aerobic exercise can increase IL-6. On the day following exercise, fasting incretins remained increased but postprandial insulin and glucagon were decreased without affecting postprandial glucose. This long duration of exercise may not be appropriate for some people and further research should investigate why next day glucose tolerance was unchanged.

## 5.1 INTRODUCTION

Exercise recommendations for the prevention and treatment of type 2 diabetes (T2D) emphasize exercise prescriptions designed to target insulin sensitivity or body composition **(5, 252)**. These outcomes have been extensively studied and it is generally recognized that typical exercise-induced changes in body composition are modest and that changes in insulin sensitivity

are short lived **(5, 252)**. Evidence to support, adapt and fine-tune these recommendations are rapidly accumulating. The most recent (November 2016) position statement of the American Diabetes Association on Physical Activity/Exercise and Diabetes **(252)** currently recommends:

- To enhance insulin action: daily exercise, or at least not allowing more than 2 days to elapse between exercise sessions.
- For optimal glycemic and health outcomes: adults with T2D should ideally perform both aerobic and resistance exercise training.
- To prevent or delay the onset of T2D in populations at high risk and with prediabetes: structured lifestyle interventions that include at least 150 min/week of physical activity and dietary changes resulting in weight loss of 5–7%.

While exercise interventions based on this paradigm clearly contribute to meaningful reductions in the incidence of diabetes **(253, 254)** or hyperglycemia **(91, 255)** an unintended consequence of this success may have been a substantially smaller emphasis on the effects of exercise on other pathophysiologic disturbances present in T2D. For example, Defronzo **(30)** proposed an "ominous octet" of potential pathophysiologic targets that also includes an increased glucagon secretion and a decreased incretin effect. The effects of exercise on many of these other outcomes are largely unknown in people with T2D.

Insight regarding how exercise could potentially affect glucagon or incretins in type 2 diabetes may be obtained from studies in other populations. For example, repeated long-bouts (e.g., two bouts of 90 minutes) of moderate-intensity exercise performed on the same day have been shown to lead to reductions in glucagon and other counter-regulatory hormones, as well as

reductions in sympathetic nerve activity, that persist until at least the next day in people with type 1 diabetes (T1D) **(256)** and in healthy participants **(257)**. This has been studied as part of the concept known as hypoglycemia-associated autonomic failure or HAAF **(258)**. Reduced glucagon responses may be problematic in T1D who can experience hypoglycemia in response to exercise or excess insulin and has been studied more extensively. However, T2D and impaired glucose tolerance (IGT) are characterized by impaired postprandial suppression of glucagon and could potentially benefit from non-pharmacological reductions in glucagon **(44, 259, 260)**.

Incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are secreted from the gastrointestinal tract into the portal circulation in response to nutrients. In a nutrient-dependent manner, incretins have been shown to contribute to lowering blood glucose by increasing insulin secretion, decreasing glucagon secretion and decreasing the rate of gastric emptying (as reviewed by (261)). On the other hand, GIP can increase glucagon secretion when glucose is low (262). The GLP-1 receptor has been found in cardiac muscle, smooth muscle of the vasculature and perhaps skeletal muscle (263, 264). These findings, combined with the established heart rate increasing effect of GLP-1 (265), suggest that incretins could play a role in cardiometabolic responses to exercise.

Ellingsgaard et al. (19) have shown that increased interleukin 6 (IL-6) during exercise could stimulate secretion of GLP-1 from intestinal L cells and also pancreatic alpha cells. As reviewed by Pedersen (266), it is likely that increased circulating IL-6 during exercise is secreted directly by skeletal muscle and is proportional to the amount of glycogen depletion. In vivo human studies in healthy, obese or T2D have often not observed an effect of exercise on incretin concentrations (267, 268); whereas a study in healthy runners observed increased GLP-1 following a marathon

(18). It is unclear if this difference among human studies is due to differences in exercise duration or volume.

The effects of exercise clinical relevant outcomes such as glycated hemoglobin have been extensively studied **(8, 91)**. The objective of this study was to examine the effect of two bouts of long duration, moderate intensity exercise on biomarkers such as such as plasma glucagon, IL-6, GLP-1 and GIP. It was hypothesized that, compared to rest, two bouts of long duration (i.e., 90 minutes) moderate-intensity exercise would increase plasma IL-6 and incretin hormone concentrations in T2D and in healthy participants. On the day after the exercise or rest conditions, participants returned to the laboratory for a 4-hour oral glucose tolerance test (OGTT) and it was hypothesized that two bouts of long duration moderate-intensity exercise would reduce the following day glucagon concentrations immediately before (fasted state) and during the OGTT. These objectives were examined using large amounts of exercise as a proof of concept with the understanding that this large amount of exercise (i.e. 3 hours in a single day) is unlikely for most people and unsafe for some.

## 5.2 METHODS

### 5.2.1 Research design

The experimental design involved two conditions that each required visits to the laboratory on two consecutive days (i.e., a total of 4 visits). On Day 1 of each condition, participants were assigned to either exercise or control (i.e. rest) according to a randomized crossover design (Figure 5-1). On Day 2 of each condition, participants return to the lab following an overnight fast for a 4-hour oral glucose tolerance test (OGTT). The two-day exercise and control conditions were separated by at least two weeks.

### 5.2.2 Participants

Twelve men, six without diabetes and six with physician diagnosed T2D were recruited for this study. Men were selected since they have higher glucagon concentrations in response to various stimuli (e.g., exercise or hypoglycemia) **(269)** and previous exercise studies of this nature have shown larger reductions in counter-regulatory responses in men **(268)**. To minimize heterogeneity in T2D and the risk of hypoglycemia with prolonged exercise, participants were required to be treated with lifestyle intervention(s) and metformin only. In order to be eligible, all participants also had to be non-smokers and not taking any beta-blockers. Furthermore, participants were excluded if they had cardiovascular or orthopedic limitations to exercise, or felt they would be unable to walk for 90 minutes without interruption. Many of the participants with T2D were recruited from our previous exercise studies **(270, 271)** and were purposefully identified due to their above average level of fitness as potential volunteers due to the long bouts of walking required in the present study. Comparable cohorts of men without diabetes had not previously been studied in our lab, therefore recruited a convenience sample of healthy counterparts with similar body mass indices.

This study was carried out in accordance with the recommendations of the Tri-Council Policy Statement on "Ethical Conduct for Research Involving Humans" with written informed consent from all subjects. The protocol was approved by the University of Alberta Health Research Ethics Board.

### 5.2.3 Baseline assessment

Participants attended a baseline visit to measure glycated hemoglobin (A1c; DCA Vantage™ A1C Analyzer, Siemens Medical Solutions, Malvern, PA), resting metabolic rate (RMR)

and perform a graded submaximal exercise test with indirect calorimetry (TrueMax metabolic measurement system, Parvo Medics, Salt Lake City, Utah). Heart rate (HR) was measured using a Polar heart rate monitor (Polar Electro, Finland). The submaximal exercise test was performed according to a modified Balke-Ware treadmill protocol where each participant walked at a self-selected speed, determined as comfortable but brisk, while the grade was increased by 1% each minute. The test was ended shortly after participants reached their individual ventilatory threshold (VT) using the V-slope criteria **(272)** as determined by a trained exercise physiologist.

Once eligibility was confirmed and baseline assessments were performed, a one-month exercise habituation phase was completed by every participant that included three sessions of exercise per week at 80% of their ventilatory threshold. The duration began with 30 minutes and gradually progressed until participants could walk for 90 minutes continuously.

### 5.2.4 Experimental protocol

On Day 1 of each condition, participants arrived at lab at 08h00 after a minimum 10-hour overnight fast. They were asked to avoid vigorous exercise the day before each testing condition. An intravenous catheter was inserted into an antecubital vein and was kept patent with sterile saline. The exercise condition contained two 90-minute bouts of treadmill exercise at intensity of 80% of the previously determined VT, as described in previous studies **(257, 273)**. The first exercise bout began at 09h00 in the fasted state and the second at 14h00. Blood samples were taken immediately before and after each bout of exercise. Indirect calorimetry and heart rate measurements were collected during the first and last 10 minutes of each 90-minute bout of exercise. During the non-exercise condition, participants remained sedentary but the above

measures (except for heart rate) were collected at the same times as during the corresponding exercise condition.

The energy intake required to maintain energy balance during non-exercise condition was estimated based on participants' previously measured resting metabolic rate multiplied by a physical activity level (PAL) of 1.4 (Note: this PAL is typically used to characterize a sedentary lifestyle [8]). As in previous studies of this nature **(274, 275)**, energy intake was kept the same on the exercise and non-exercise conditions. Energy intake was divided in two equal standardized meals (59% carbohydrate, 22% fat, 19% protein) provided 30 minutes after each exercise bout (see Figure 5-1). As such, the first exercise bout was performed in the fasting state and the second bout started 150 minutes after the first meal.

On Day 2, participants returned to the lab after a minimum 10-hour fast. Two fasting blood samples were taken; one 15 minutes before and the other immediately before the beginning of the OGTT containing 75 grams of glucose (Trutol, Thermo Fisher Scientific, Canada). Ten blood samples were collected at specific time points following consumption of the glucose beverage (i.e., 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes). Oxygen consumption and carbon dioxide production were collected for 10 minutes before the OGTT and for the last 10 minutes of each of the next 4-hour periods (see Figure 5-1) using the same metabolic measurement system. Respiratory exchange ratio (RER) was determined as ratio between carbon dioxide production and oxygen consumption, while energy expenditure was calculated assuming non-protein energy equivalents. A metabolic equivalent (MET) was calculated as 1 $kcal \cdot kg^{-1} \cdot hr^{-1}$ **(276)**. Participants were asked to sit continuously throughout the test, with the exception of a bathroom break if required. Appetite rating and heart rate were collected during the same

intervals. Appetite ratings were measured by a 150 mm visual analogue scale and included questions on hunger, fullness, prospective food consumption and desire to eat something sweet, salty or fatty **(277)**. Heart rate was measured using a standard three-lead ECG over the same intervals as for the indirect calorimetry. Heart rate variability (HRV) is a tool that can be used to investigate the sympathetic and parasympathetic function of the autonomic nervous system. Autonomic nervous system activity can be affected by both hypo- and hyper-glycemia. HRV indices included the root mean squared of the successive differences between R-R intervals (rMSSD), the standard deviation of the R-R intervals (SDRR), and the ratio of low frequency spectral power (LF) to high frequency spectral power (HF). For both Day 1 and Day 2, the first and last minutes of the 10-minute indirect calorimetry and heart rate periods were excluded to allow for more stable data. Participants with T2D refrained from taking their metformin dose on all four testing days. The last metformin dose was consumed more than 12 hours prior to first blood sample which was taken on Day 1 of each testing condition.

### 5.2.5 Blood samples

Each blood sample was first collected into a 10-mL EDTA vacutainer tube. Subsequently, 2.0 ml was transferred into a tube with 20 µL of a dipeptidyl peptidase (DPP-4) inhibitor (Millipore, MA, USA), 2.0 mL was transferred into a tube with 6.7 µL aprotinin (Millipore, MA, USA), and 0.25 mL whole blood was transferred into 1.0 mL ice-cold 8% perchloric acid. Aprotinin was added to inhibit proteases known to interfere with the determination of glucagon. The DPP-4 inhibitor was added to prevent degradation of active GLP-1. Perchloric acid was added to deproteinize the samples. The EDTA tubes were centrifuged at 1500 x g for 10 minutes at 4°C. The tubes containing perchloric acid and aprotinin were centrifuged at 2000 x g for 15 minutes

at 4°C. Following centrifugation, the samples were immediately moved to a -80°C freezer until assays were completed.

Nonesterified fatty acids (NEFAs) were analyzed using commercially available kits (Wako Diagnostics, CA, USA), while plasma glucose and lactate were determined enzymatically using spectrophotometric assays. Total GIP and GLP-1, glucagon and insulin were measured using a Multi-Spot® Assay System with a Sector® Imager 2400 (Meso Scale Discovery®, MD, USA). Active GLP-1 was measured separately (Meso Scale Discovery®, MD, USA). Hematocrit was measured only on Day 1 for both exercise and non-exercise conditions. Plasma IL-6 was also measured from Day 1 plasma samples using a high-sensitivity ELISA (Quantikine HS human IL-6, R&D Systems Ltd., Abingdon, UK). Plasma metformin concentrations were assessed by high performance liquid chromatography in all plasma samples from Day 1 as well as fasting samples from Day 2. The concentration of phosphate solution used in the mobile phase was 20 mmol/L. The metformin assay was validated to a lower limit of quantitation of 7.8 ng/mL metformin based on 0.1 mL of human plasma **(278)**. All assays were run in duplicate and the average of the two was reported.

### 5.2.6 Statistical analysis

The primary analyses were conducted using a three-way mixed factorial design ANOVA with *Diabetes* as a between group factor (i.e., T2D vs. healthy control), as well as *Exercise* (i.e., exercise vs. rest conditions) and *Time* (i.e., consecutive blood samples) as repeated measures factors. The number of levels for the time factor differed depending on the time period examined (e.g., Day 1 had four consecutive blood samples). For Day 2, The *Diabetes* by *Exercise* by *Time* ANOVA showed a significant effect of *Time* for all of the blood sample results (all $p<0.01$). Therefore, it was deemed more informative to separate the fasting from the post glucose

beverage results. For the 10 blood samples taken at different intervals postprandially, we considered both the area under the curve (AUC) and incremental AUC (iAUC). The AUC was calculated by the trapezoid method. The iAUC was calculated by subtracting the average of the two fasting values from the AUC. For the variables that were measured at one-hour intervals postprandially (e.g., calorimetry, HRV and appetite) the four postprandial values were averaged. Age was a known confounder for HRV and was significantly associated with our HRV outcomes; we therefore considered age as a covariate for the statistical analyses on HRV. For each ANOVA, we examined interaction effects and main effects, but did not conduct the many possible post-hoc comparisons due to lack of statistical power. Sphericity was tested using Mauchly's test of sphericity. In the events where Mauchly's sphericity test was significant the Greenhouse-Geisser correction was used.

Baseline characteristics were compared between groups using independent t-tests. Secondary analyses also examined the bivariate correlations among variables (e.g., IL-6 vs. GLP-1). Statistical tests were two-tailed, and p values $\leq 0.05$ were considered significant. Statistical analyses were performed with SPSS 21 (SPSS, Inc, Chicago, IL).

## 5.3 RESULTS

### 5.3.1 Participants

All twelve participants (six T2D and six healthy) completed the study. Baseline characteristics are presented in Table 5-1. T2D and healthy participants had an average age of 60.5±8.5 and 42.5±10.5 years ($p<0.01$) and an average body mass index (BMI) of 24.8±4.3 and 26.7±3.2 kg/m$^2$ ($p=0.39$), respectively. All T2D participants had a well-controlled glycemia as

suggested by their A1c (6.4%±0.3%). They were treated with 500 to 1500 mg of metformin per day and the average duration of diabetes diagnosis was 3.9±2.3 years.

### 5.3.2 Day 1

**Energy expenditure and heart rate**

All participants completed both 90-minute exercise bouts without requiring adjustments to the exercise intensity. Indirect calorimetry and heart rate results from the exercise bout and corresponding rest conditions are presented in Table 5-2. As expected, energy expenditure and RER were significantly increased with exercise (main effect of *Exercise* $p<0.001$). Energy expenditure corresponded to approximately one metabolic equivalent (MET) on the rest day and seven METs during exercise with no significant difference between T2D and healthy participants (see Table 5-2 for details). In addition to an increased RER with exercise, a significant *Exercise* by *Diabetes* interaction ($p=0.036$) and an *Exercise* by *Time* interaction ($p<0.001$) were observed for RER. These interactions were the result of RER being lower on the rest day in the healthy participants and after lunch in the exercise condition but greater after lunch in the rest condition. During the exercise bouts, heart rate averaged 121±3 beats per minute during the first 10 minutes of each bout of exercise, or 72±2 % of age predicted maximum heart rate, and drifted upwards throughout exercise (main effect of time $p=0.001$).

**Plasma samples**

Results of blood sample analyses from Day 1 are summarized in Table 5-3 and Figure 5-2. There was a significant *Time* by *Diabetes* interaction ($p=0.03$) suggesting that glucose changed to

a greater extent in T2D over time. In addition, there was a significant main effect of *Exercise* leading to lower overall glucose concentrations in the exercise condition (p=0.02).

There was a significant *Exercise* by *Time* interaction for IL-6 (p=0.03). Visual Inspection of the graph in Figure 5-2 suggests that exercise increased IL-6 compared to rest when performed in the fasting state but not when performed after lunch when IL-6 was increased overall compared to fasting. The IL-6 responses were similar for participants with and without T2D (Figure 5-2). GIP followed a similar pattern during the first exercise bout compared to rest, but increased to a greater extent in healthy participants following lunch, leading to an *Exercise* by *Time* by *Diabetes* interaction (p=0.03). Overall plasma metformin concentrations decreased from 402±120 ng/ml to 191±52 ng/ml (main effect of *Time* p=0.03) throughout Day 1 and were not affected by exercise.

### 5.3.3 Day 2

**Energy expenditure, heart rate and appetite**

There were main effects of *Time* on energy expenditure and RER during the OGTT (both p<0.001). There were no effects of *Exercise* or *T2D* on energy expenditure in the fasting state or postprandially. RER was greater in participants with diabetes but lower after exercise throughout Day 2 (main effect of *T2D* and *Exercise,* both $p \leq 0.01$ see Table 5-4). There was no statistically significant effect of exercise or diabetes and HRV indices during the OGTT. Overall, ratings for prospective food consumption were higher during the OGTT from the exercise condition compared to the rest condition (p=0.03). However, postprandial fullness decreases following exercise in T2D only (Diabetes by Exercise interaction p=0.04 for fasting; p=0.056 for the mean postprandial values). Participants with T2D had a lower desire to eat something sweet in the

fasting (p=0.01) and postprandial state (p=0.03), but a Diabetes by Exercise interaction (p=0.045) indicated that exercise tended to increase the desire to eat something sweet in T2D while decreasing this rating in healthy participants during the OGTT.

**Plasma samples**

There were significant main effects of *Time* on all energy substrates and hormones on Day 2. Therefore, analyses were conducted separately for the fasting and postprandial values. There was a main effect of *Diabetes* on fasting, AUC and iAUC glucose (all $p<0.05$) but no main effect of *Exercise* on the AUC and iAUC. However, there was a main effect of *Exercise* on fasting glucose (p=0.05), with a 0.5mmol/L and 0.1mmol/L decrease fasting glucose in the morning following exercise in the T2D and healthy control group, respectively (note: The *Diabetes* by *Exercise* interaction was not significant, p=0.35).

Exercise increased fasting glucagon in the healthy control group but not in T2D (*Exercise* by *Diabetes* interaction, p=0.02), whereas the postprandial iAUC for glucagon was reduced by exercise (main effect of E*xercise*, p=0.01). Fasting insulin was not affected by exercise but iAUC and AUC insulin were reduced (main effect of *Exercise*, p=0.08, p=0.01 and p=0.001, respectively). In terms of the insulin:glucagon ratio, both the iAUC and AUC were reduced following exercise (main effect of *Exercise* p= 0.04 and p=0.004, respectively).

Fasting active GLP-1 and GIP concentrations showed a small increase with exercise (main effect of *Exercise*, both $p<0.05$). Exercise on the previous day did not affect postprandial incretin hormones during the OGTT. Upon arrival on Day 2, fasting plasma metformin concentrations were very low and similar between exercise and rest (i.e., control) conditions (76±17 ng/ml to 83±18 ng/ml, respectively, main effect of *Time,* p=0.03).

### 5.3.4 Bivariate Correlations

There was no significant bivariate correlation between changes in IL-6, insulin or glucagon and changes in active GLP-1 or GIP, either when examining the participants with and without T2D together or separately on day 1. On day 2, there was an inverse association (r=-0.60, p=0.038) between the exercise induced changes in lactate and heart rate variability as assessed by RMSSD. No associations were found between incretins and glucagon or insulin.

## 5.4 DISCUSSION

To our knowledge, no other study in T2D has examined the glycemic, hormonal and metabolic responses to exercise of such a high volume in a single day (i.e., 3 hours walking). Although other studies have suggested a dose-response relationship between exercise duration and improvements in glucose tolerance or insulin sensitivity **(279-281)**, we did not observe any improvements in glucose tolerance following 3 hours of exercise.

Unlike other studies in T2D or obesity which utilized shorter bouts of exercise **(267, 268, 282)**, we observed elevated incretin hormones, particularly GIP, immediately after exercise (Figure 5-2). This increase persisted to the following day in the fasted state but not during the OGTT. It is unclear if these increases are practically meaningful as the increases were small in absolute terms and occurred at times when incretins were low. The increase was nonetheless consistent as we were able to detect these differences with a small sample size. In the participants with T2D who had relatively well-controlled glycemia, postprandial plasma incretin concentrations were not lower in T2D compared to healthy controls. While earlier studies suggested lower incretins in T2D, recent meta-analyses suggests that this is not always the case for both GIP **(283)** and GLP-1 **(284)**. Another possibility to explain the strong incretin response

(particularly for GLP-1) during the OGTT in our participants with T2D was that they were prescribed metformin, an oral hypoglycemic medication that has been shown to increase incretins **(267)**. However, the last metformin dose had been consumed at least 36 hours before the OGTT and metformin concentrations had been reduced less than 5% of the concentrations we observed in the hours following a morning dose of metformin **(267)**. However, it is not known if the effect of long term metformin treatment could have persisted beyond 36 hours.

According to Ellingsgaard et al. **(19)** an increased GLP-1 following exercise may be due to increased IL-6. Importantly, the increase in plasma IL-6 during exercise can be directly attributed to secretion from skeletal muscle and IL-6 is thought to be secreted in proportion to glycogen depletion (as reviewed by **(266)**). We observed that plasma IL-6 only increased compared to rest during the first exercise bout, which was performed in the fasting state and not during the second bout performed after lunch. The design of the present study does not allow us to conclude if the absence of an effect of exercise on plasma IL-6 after lunch was due to the meal itself or to a reduced effect when sequential exercise bouts are performed. However, a recent study found that consuming a carbohydrate beverage during 120 minutes of cycling abolished leg IL-6 release even though muscle glycogen was reduced to a similar extent compared to fasting exercise **(285)**. IL-6 was also increased by lunch itself, which is consistent with previous studies **(286, 287)**. Therefore, it appears that exercise-induced IL-6 secretion requires, or at least is more pronounced, with fasting exercise protocols.

A notable finding in the present study was that, in accordance with the hypothesis, two long bouts of exercise enhanced the postprandial suppression of glucagon (i.e., reduced iAUC). The postprandial suppression of glucagon is thought to be impaired in people with T2D **(30)**.

While both insulin and glucagon were lowered by exercise during the OGTT, insulin was reduced to a greater extent as reflected as a decrease in both the iAUC and AUC for the insulin:glucagon ratio. Insulin acts to suppress glucagon secretion; therefore, the observation of a lower glucagon in the presence of lower insulin is noteworthy since previous studies using hyperinsulinemic clamp protocols reported a reduced glucagon following exercise when insulin was maintained in the exercise and rest conditions **(256, 257, 273)**. The mechanism by which this form of exercise suppresses postprandial glucagon concentration in T2D cannot be elucidated from this study, and is indeed a topic of continued interest and debate **(30, 288)**. Despite postprandial glucagon being reduced in our participants with T2D, postprandial hyperglycemia was not improved. While this may be disappointing from a clinical perspective, the similar concentrations of plasma glucose in both conditions may be considered fortuitous to examine changes in glucoregulatory hormones without needing to clamp glucose at a fixed concentration.

From a theoretical perspective, the absence of a glucose lowering effect of exercise during an OGTT performed on the following day in T2D was unexpected. It is generally believed that the glucose lowering effect of exercise is proportional to the duration or volume of exercise **(280, 281)**. Although our study had a small sample size, the absence of the expected glucose lowering effect of exercise is unlikely to be due low statistical power as the post OGTT glucose AUC was slightly higher (1%) in the exercise condition. The reasons for the unchanged glucose were unclear, although postprandial insulin was reduced suggesting improved insulin sensitivity. Other studies have documented an absence of improvement in OGTT following longer bouts of exercise. For example, Tremblay et al, observed an increase in glucose AUC during an OGTT performed 16 hours after 90 minutes of cycling at 67% of $VO_{2max}$ **(289)**. They attributed this

increased to an increased adipose tissue lipolysis, increased NEFA and a decreased glucose oxidation **(289)**. This explanation is consistent with our observation of a decreased RER (i.e., indicating a decreased carbohydrate oxidation) and a tendency for increased NEFA (p=0.09) during the OGTT from the exercise condition.

A primary limitation of the present study was the small sample size. As a result, our study was underpowered to detect potentially meaningful effects of exercise or diabetes. Our randomized crossover design helped to reduce the impact of this limitation on statistical power when comparing the exercise and rest conditions. However, the between participant comparison of T2D (n=6) to healthy control (n=6) was particularly underpowered for some outcomes.

The validity of conclusions regarding comparisons between T2D and healthy controls was further impaired by these small subgroups which were not matched for possible confounders (e.g., age, BMI and fitness). BMI and exercise induced energy expenditure ended up being relatively similar between the healthy and T2D participants; however, the healthy control group was younger and likely had different body composition (e.g., more fat free mass). Age was not associated with most outcomes in our study with the notable exception of HRV. HRV was lower in our T2D participants but these differences were no longer significance after adjusting for age. Although not statistically significant, there were trends to suggest an increase in indices of HRV following exercise in T2D but not in healthy participants. In addition to detecting differences in glucose, our study was able to detect other expected differences between healthy participants and T2D (e.g., RER **(290)** and glucagon **(44)**). Nonetheless, the primary contributions to be retained from this article should be in regard to the exercise vs control comparison.

The participants with T2D that were recruited for our study were likely more fit, more physically active, and leaner than many people with type 2 diabetes. Such participants were selected to increase the likelihood of completing the exercise protocol and reduce the risk of injury. However, this selection also introduces potential bias. The phenotypic differences in our participants could influence many of the hormonal and metabolic responses to exercise. For example, participants with lower fitness or greater adiposity may have a different inflammatory profile or different inflammatory response to exercise **(291, 292)**. This could potentially reduce generalizability of our result.

Another limitation of the study was the reliance on plasma concentrations of hormones taken from peripheral blood samples. These concentrations from the systemic circulation often do not reflect the exposure of other organs to these hormones (e.g., pancreas or liver). In addition, our multiplex hormone assay used has limitations in regard to specificity. For example, the glucagon assay has been shown to have some cross-reactivity with glicentin (or to a lesser extent oxyntomodulin) **(293)**.

In conclusion, exercise can affect a variety of pathological features that can contribute to hyperglycemia. Potential benefits include decreasing postprandial hyperglucagonemia and increasing incretin concentrations. The exercise protocol (i.e., two 90 minute bouts of exercise) used in this study is likely not feasible for most people. Larger samples sizes and closer matching of participant characteristics would be required to more carefully address differences between participants with normal glucose tolerance and those with type 2 diabetes. Future studies should seek to better understand if similar results can be obtained with shorter exercise protocols, as well as the persistency of the observed changes.

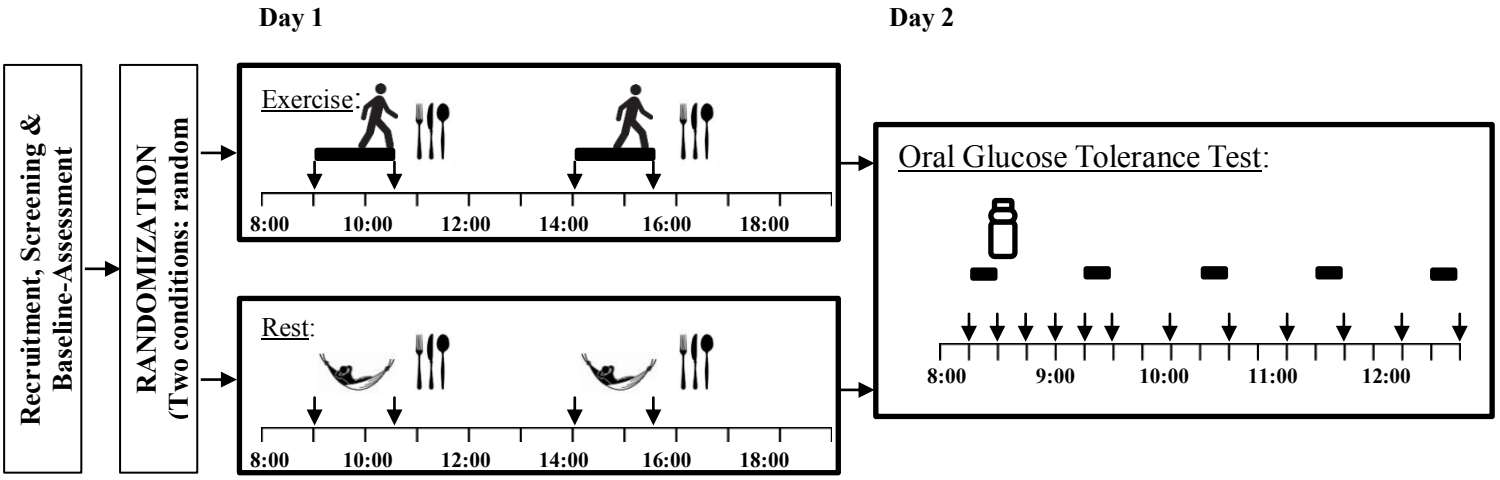


**Figure 5-1 Study experimental design**

Legend: = Exercise, = Rest (Control), = Standardized meal, ↓ = Blood samples,

▬ = heart rate variability and indirect calorimetry.

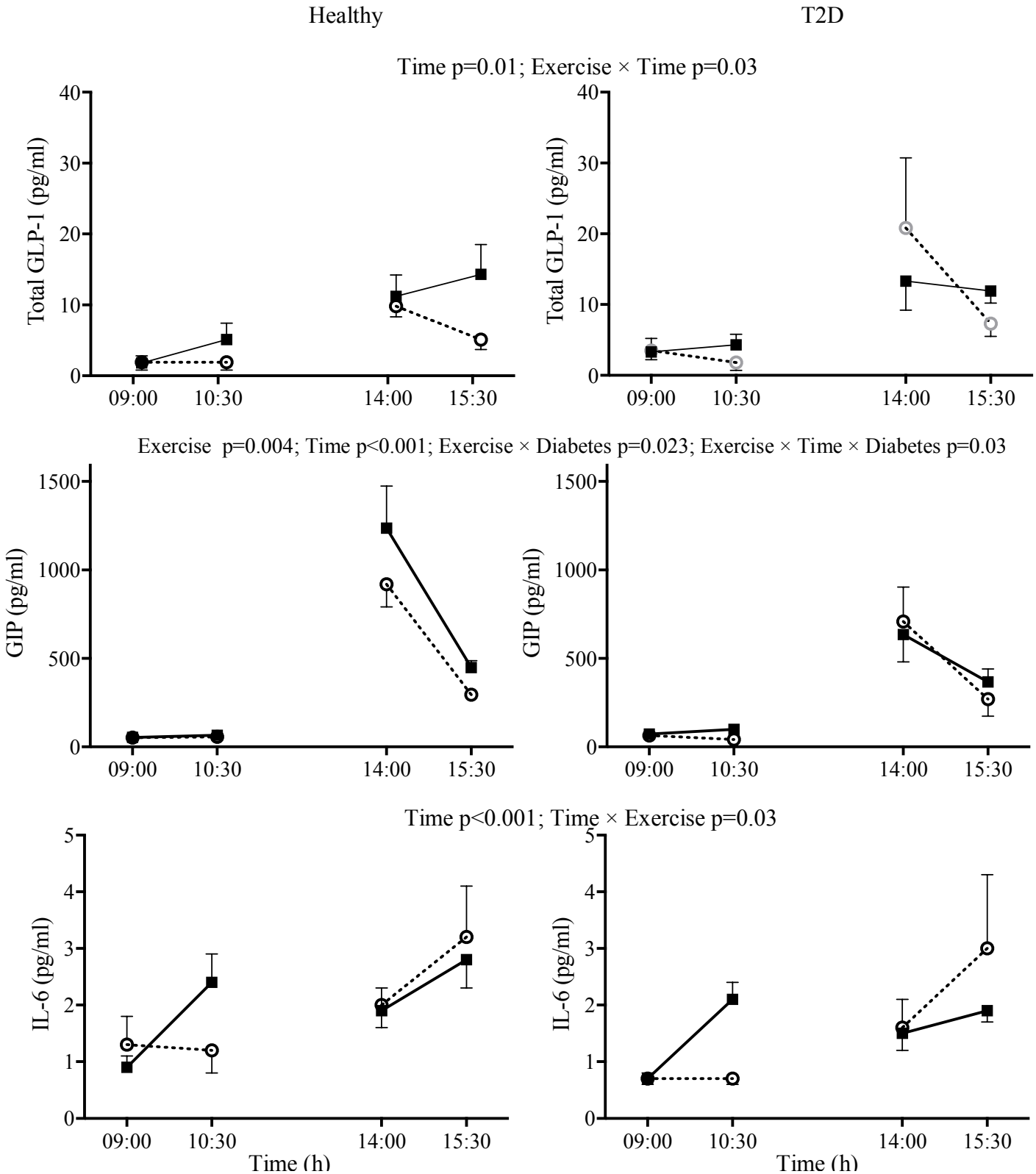


**Figure 5-2 Day 1 plasma concentrations for total interleukin-6, Total GLP-1, and total GIP**

in response to two 90-minute bouts of exercise (▪) vs rest (○) in healthy participants (left panels) and in type 2 diabetes (right panels). Data shown as mean±SEM.

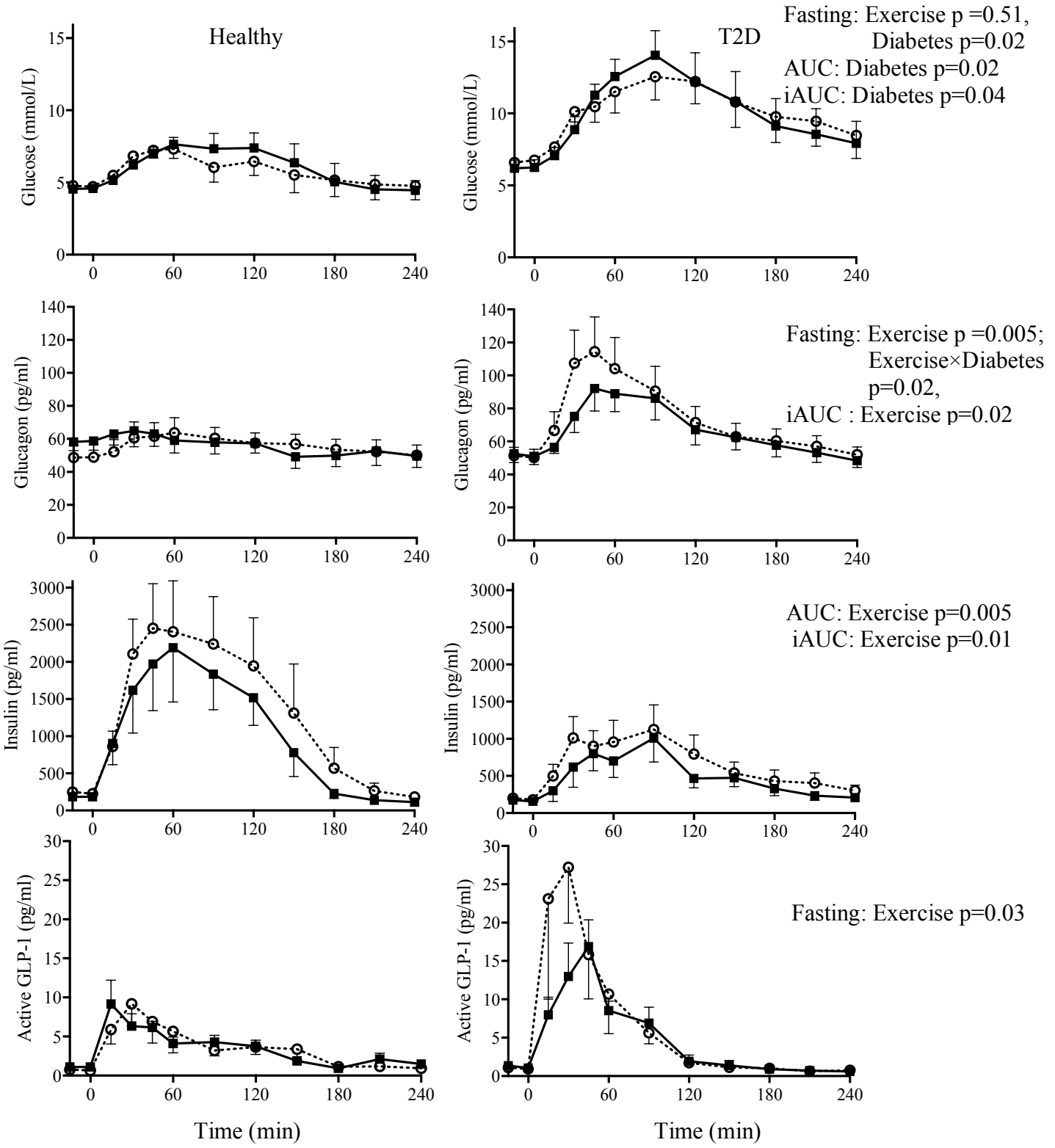


**Figure 5-3 Day 2 fasting plasma concentrations (-15 and 0 minutes) and responses to an oral glucose tolerance test for glucose, glucagon, insulin, and Active GLP-1**

the day after two 90-minute bouts of exercise (▪) vs rest (○) in healthy participants (left panels) and in type 2 diabetes (right paanels). Results from 2×2 ANOVA showing main effects of exercise vs. rest, diabetes vs. control and their interaction Data shown as mean±SEM.

| | T2D | Healthy | p |
|---|---|---|---|
| n | 6 | 6 | NA |
| Age (y) | 60.5±8.5 | 42.5±10.5 | <0.01 |
| BMI (kg/m$^2$) | 24.8±4.3 | 26.7±3.2 | 0.39 |
| Body weight (kg) | 75.5±16.2 | 81.6±10.2 | 0.45 |
| Duration of T2D (y) | 3.9±2.3 | NA | NA |
| A1c (%) | 6.4±0.3 | 5.6±0.1 | <0.01 |
| VO2@VT (ml/kg/min) | 27.8±4.8 | 38.3±8.5 | <0.05 |
| SBP (mmHg) | 125±14 | 131±11 | NS |
| DBP (mmHg) | 75±10 | 71±5 | NS |

**Table 5-1 Baseline characteristics**

T2D = type 2 diabetes, BMI = body mass index, A1c = glycated hemoglobin, NA = not applicable, NS = Not significant, VT = ventilatory threshold, SBP = systolic blood pressure, DBP = diastolic blood pressure, RMR = resting metabolic rate. Data presented as mean ± SD.

| | | Healthy | | | | T2D | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 9h00-9h10 | 10h20-10h30 | 2h00-2h10 | 3h20-3h30 | 9h00-9h10 | 10h20-10h30 | 2h00-2h10 | 3h20-3h30 | p |
| RER ($VCO_2/VO_2$) | Ex | 0.87±0.04 | 0.84±0.02 | 0.90±0.02 | 0.85±0.03 | 0.89±0.03 | 0.82±0.03 | 0.89±0.03 | 0.84±0.04 | Ex,Time<0.001<br>Ex×T2D=0.036<br>Ex×Time<0.001 |
| | Rest | 0.76±0.03 | 0.74±0.05 | 0.83±0.03 | 0.80±0.03 | 0.79±0.04 | 0.79±0.05 | 0.86±0.05 | 0.84±0.06 | |
| EE (METs) | Ex | 7.25±1.81 | 7.59±1.65 | 7.29±1.25 | 7.08±1.14 | 6.78±1.06 | 6.86±1.01 | 6.56±0.98 | 6.49±1.19 | Ex<0.001 |
| | Rest | 0.93±0.15 | 0.91±0.11 | 1.12±0.14 | 1.01±0.10 | 0.86±0.18 | 0.94±0.11 | 1.08±0.11 | 1.01±0.16 | |
| HR (bpm) | Ex | 120±13 | 135±23 | 139±23 | 145±25 | 121±6 | 139±9 | 129±9 | 141±11 | Time=0.001 |
| | Rest | NA | NA | NA | NA | NA | NA | NA | NA | |

**Table 5-2 Indirect calorimetry and heart rate on Day 1**

at the beginning and at the end of two bouts of moderate-intensity exercise or rest (i.e., rest).

T2D = Type 2 diabetes, Ex= exercise, RER= Respiratory exchange ratio, EE = Energy expenditure, METs = metabolic equivalent or kilocalories divided by kilograms of body mass and hours ($kcal \cdot kg^{-1} \cdot hr^{-1}$), HR = Heart rate, bpm = beats per minute, Ex = exercise, NA = not available. Data presented as mean ± SD.

| | | Healthy | | | | T2D | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 9h00 (Pre-1st bout) | 10h30 (Post-1st bout) | 2h00 (Pre-2nd bout) | 3h30 (Post-2nd bout) | 9h00 (Pre-1st bout) | 10h30 (Post-1st bout) | 2h00 (Pre-2nd bout) | 3h30 (Post-2nd bout) | P= |
| Glucose (mmol/L) | Ex | 4.7±0.2 | 4.3±0.3 | 4.8±0.3 | 3.8±0.2 | 6.1±0.5 | 5.2±0.2 | 8.8±1.1 | 4.3±0.3 | Ex=0.02<br>Time,T2D<0.001<br>Time×T2D=0.03 |
| | Rest | 4.5±0.3 | 4.7±0.2 | 5.1±0.8 | 4.5±0.5 | 6.2±0.6 | 6.2±0.5 | 9.3±1.1 | 6.9±1.0 | |
| Lactate (mmol/L) | Ex | 0.9±0.2 | 1.1±0.2 | 1±0.2 | 1±0.1 | 1±0.1 | 1.2±0.1 | 1.1±0.1 | 1.2±0.2 | Ex×Time=0.05 |
| | Rest | 1±0.2 | 0.7±0.0 | 1.1±0.1 | 0.8±0.1 | 1±0.1 | 0.8±0.1 | 1.2±0.1 | 1±0.1 | |
| NEFA (mEq/L) | Ex | 0.4±0.1 | 1.2±0.3 | 0.2±0 | 1.1±0.2 | 0.5±0.1 | 1.4±0.2 | 0.3±0.1 | 1.1±0.2 | Ex,Time<0.001<br>Time×T2D<0.05<br>Ex×Time<0.001 |
| | Rest | 0.5±0.1 | 0.5±0.1 | 0.2±0.0 | 0.3±0.0 | 0.4±0.1 | 0.6±0.1 | 0.2±0.0 | 0.2±0.0 | |
| Insulin (pg/ml) | Ex | 227±52 | 121±36 | 1793±475 | 138.8±61 | 178±55 | 211±65 | 981±282 | 219±44 | Time<0.001 |
| | Rest | 216±44 | 189±52 | 1859±599 | 342±123 | 182±51 | 143±48 | 2283±928 | 701±244 | |
| Glucagon (pg/ml) | Ex | 54.7±6.5 | 81±14.7 | 79.1±5.5 | 114±16.1 | 71.3±6.7 | 78.9±6.3 | 97.3±19 | 103.6±9.9 | Ex,Time≤0.001 |
| | Rest | 50.2±6.9 | 49.8±7.7 | 77.7±6.2 | 62.8±6.6 | 58.9±5.9 | 47.3±9.3 | 86.4±13.8 | 69.2±4.6 | Ex×Time<0.05 |
| Active GLP-1 (pg/ml) | Ex | 1.1±0.2 | 1.0±0.2 | 7.0±0.5 | 3.9±1.2 | 2.0±0.5 | 1.3±0.3 | 7.0±1.7 | 5.1±0.5 | Time=0.001 |
| | Rest | 0.8±0.3 | 0.7±0.2 | 5.7±1.4 | 2.9±0.9 | 2.2±0.6 | 1.0±0.3 | 16.2±8.8 | 4.3±0.8 | |

**Table 5-3 Concentrations of energy substrates and hormones on Day 1**
before and after two 90-minute moderate-intensity exercise bouts or rest.
T2D = Type 2 diabetes, Ex= exercise, NEFA = non-esterified fatty acids. Results presented as mean±SEM.

| | | Fasting | | | Mean Postprandial | | | ΔPostprandial | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Healthy | T2D | P= | Healthy | T2D | P= | Healthy | T2D | P= |
| RER | Ex | 0.77±0.04 | 0.73±0.03 | T2D<0.01 | 0.82±0.04 | 0.76±0.02 | T2D=0.01 | 0.04±0.03 | 0.03±0.01 | |
| ($VCO_2/VO_2$) | Rest | 0.80±0.03 | 0.76±0.02 | Ex=0.08 | 0.84±0.03 | 0.80±0.02 | Ex<0.01 | 0.05±0.02 | 0.04±0.02 | |
| EE | Ex | 0.85±0.12 | 0.90±0.11 | | 0.91±0.08 | 0.95±0.12 | | 0.05±0.10 | 0.05±0.05 | |
| (METs) | Rest | 0.85±0.13 | 0.88±0.08 | | 0.91±0.07 | 0.92±0.11 | | 0.06±0.08 | 0.03±0.07 | |
| HR | Ex | 60±5 | 59±15 | | 64±4 | 61±13 | | 4±2 | 2±2 | |
| (bpm) | Rest | 56±5 | 61±15 | | 59±4 | 62±13 | | 3±1 | 1±4 | |
| RMSSD | Ex | 51±23 | 34±15 | | 40±10 | 28±11 | | -11±12 | -6±10 | |
| | Rest | 60±20 | 27±14 | | 49±16 | 26±11 | | -10±5 | -1±9 | |
| SDRR | Ex | 80±38 | 46±17 | | 66±19 | 54±26 | | -14±23 | 8±18 | |
| | Rest | 74±25 | 46±20 | | 70±20 | 48±27 | | -1±19 | 2±13 | |
| LF/HF | Ex | 1.52±1.14 | 0.91±9.34 | | 1.61±0.72 | 1.32±0.94 | | -0.04±1.12 | 0.41±1.17 | |
| | Rest | 0.77±0.27 | 1.80±1.03 | | 1.27±0.84 | 1.46±1.28 | | 0.53±0.66 | -0.34±1.53 | |

**Table 5-4 Indirect calorimetry and heart rate variability on Day 2**
during fasting and following an oral glucose tolerance test (OGTT).

T2D = Type 2 diabetes, Ex= exercise, RER= Respiratory exchange ratio, EE = Energy expenditure, METs = metabolic equivalent or kilocalories divided by kilograms of body mass and hours ($kcal \cdot kg^{-1} \cdot hr^{-1}$), HR = Heart rate, bpm = beats per minute, SDRR = standard deviation of the R-R intervals, rMSSD = root mean squared of the successive differences between R-R intervals, LF/HF the ratio of low frequency spectral power to high frequency spectral power, Mean Postprandial = average from 10 minutes at the end of each of the four 1-hour postprandial periods, ΔPostprandial= Mean Postprandial minus Fasting. Data presented as mean ± SD.

| | | Fasting | | | iAUC | | | AUC | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Healthy | T2D | P= | Healthy | T2D | P= | Healthy | T2D | P= |
| Glucose | Ex | 4.6±0.1 | 6.2±0.5 | T2D<0.05 | 372±171 | 1006±264 | T2D<0.05 | 1469±190 | 2499±345 | T2D<0.05 |
| (mmol/L) | Rest | 4.7±0.2 | 6.7±0.6 | | 269±138 | 902±193 | | 1408±185 | 2504±304 | |
| Lactate | Ex | 0.8±0.1 | 0.8±0 | T2D<0.05 | 38.8±5.3 | 51.9±11.5 | | 221.4±15.0 | 241.3±9.8 | Ex=0.05 |
| (mmol/L) | Rest | 0.7±0.1 | 1.0±0.1 | Ex×T2D<0.05 | 49.6±7.4 | 26.6±12.2 | | 222.9±10.0 | 264.8±15.3 | |
| NEFA | Ex | 0.7±0.1 | 0.6±0.1 | | -73.4±23.2 | -55.7±8.9 | | 90.3±14.2 | 76.5±13.1 | |
| (mEq/L) | Rest | 0.6±0.1 | 0.5±0.1 | | -75.4±12 | -48.1±6.5 | | 66.7±5.2 | 72.0±17.0 | |
| Insulin | Ex | 185±44 | 167±50 | | 210236±57957 | 81304±22318 | Ex=0.01 | 254710±67373 | 121330±30230 | Ex<0.01 |
| (pg/ml) | Rest | 237±53 | 189±51 | | 273119±84174 | 117050±29503 | | 329907±94510 | 162452±37315 | |
| Active GLP-1 | Ex | 1.1±0.2 | 1.2±0.4 | Ex<0.05 | 571±105 | 844±202 | | 837±75 | 1134±182 | |
| (pg/ml) | Rest | 0.7±0.1 | 1.0±0.5 | | 680±192 | 1309±537 | | 854±184 | 1557±505 | |

**Table 5-5 Concentrations of energy substrate and hormones on Day 2**
during fasting and following an oral glucose tolerance test (OGTT).
T2D = Type 2 diabetes, Ex= exercise, AUC = area under the curve, iAUC = incremental area under the curve, T2D = type 2 diabetes, Ex = Exercise NEFA = non-esterified fatty acids. For all outcomes there was a sigificant main effect of Time. Data presented as mean ± SEM.

# Chapter 6

## 6.1 DISCUSSION

The increasing prevalence of T2D has raised the importance of research on different treatments and their mechanisms of action. Incretin therapies, which include incretin mimetics and DPP-4 inhibitors, have become increasingly popular for treatment of T2D. However, at the time of conducting this research, the effects of exercise on incretins was unknown in this population. The current chapter will do the following:

- **Briefly describe the key findings of each study;**
- **Integrate the findings of these studies;**
- **Discuss the overall limitations and future directions of research in this area.**

## 6.2 SUMMARY AND INTEGRATION OF FINDINGS

The current literature described inconsistent findings regarding the effects of exercise on incretins. This thesis was focussed on investigating the effects of a single bout of aerobic exercise on the plasma concentrations of incretins.

### 6.2.1 Acute effects of exercise on incretin hormones

Overall h**ypothesis statement:** The overall hypothesis was that aerobic exercise increases plasma concentrations of incretins, both immediately post-exercise and subsequent to meal consumption.

Despite present evidence regarding the effects of acute exercise on increasing incretin plasma concentrations in normal weight healthy individuals (206, 212, 220, 222), the literature

was limited in obesity (188, 221) and T2D. Studies have shown that incretin effect (i.e., beta cell function following an oral glucose) is significantly reduced in obesity (181) and T2D (33). **Study 1 (Chapter 3)** demonstrated that acute aerobic exercise is capable of increasing GLP-1 concentrations immediately at the end of exercise in normal weight healthy individuals. The increases were observed both in comparison to control (i.e., non-exercise) conditions and to the baseline concentrations (i.e., before exercise).

Our review showed that GLP-1 was not increased following exercise in individuals with obesity and similar to the control condition was maintained in a relatively steady level. Previous studies have shown a negative correlation between BMI and GLP-1 effect (i.e., insulin response to GLP-1) (122, 123, 181). Further studies are still necessary to investigate this phenomenon.

**Studies 2 and 3** further investigated the effects in T2D. In **study 2**, although GLP-1 concentrations were significantly elevated after taking metformin, exercise did not affect GLP-1 concentrations. There was also no immediate difference in incretins following exercise (i.e., two bouts of 90 minutes treadmill walk) and control condition in **study 3**. Although they were significantly increased the next day following exercise in the fasting state. There is still controversy regarding whether concentrations of incretins are lower, higher or not-changed in T2D. Current literature suggests that late postprandial effect of incretins on insulin (i.e., post 60 minutes of ingestion) is impaired in individuals with T2D (122).

Figure 6.1 schematically summarizes the effect of acute aerobic exercise on GLP-1 in different groups based on the results of **studies 1-3 (chapters 3-5)**. GLP-1 units used in this figure are arbitrary. Given the controversy regarding the concentrations of GLP-1 in T2D compared to the healthy individuals, they are both presented with similar baseline values.

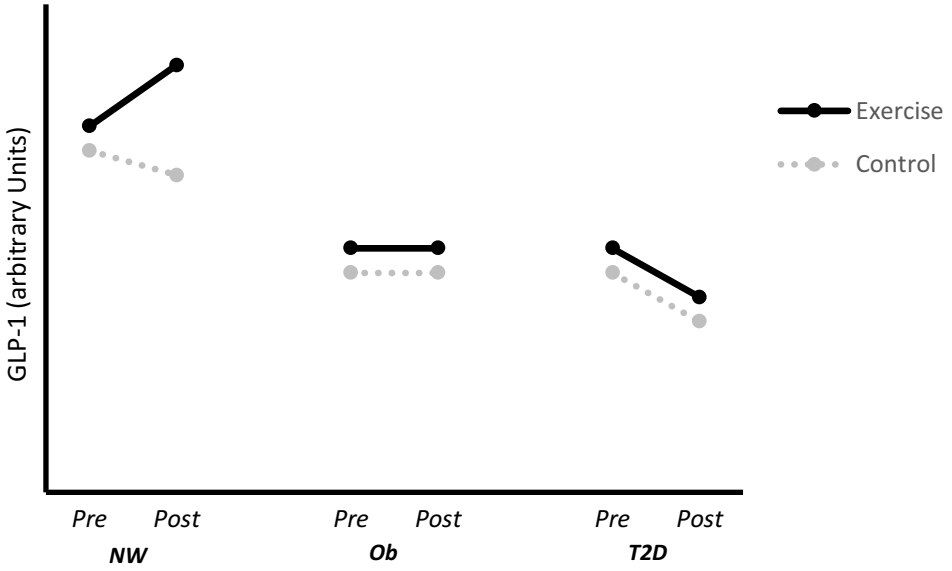


**Figure 6-1 Schematic representation of the acute effect of exercise on GLP-1**
Pre, pre-exercise; Post, post-exercise; NW, normal weight; Ob, obese; T2D, type 2 diabetes.

Additionally, the effects of acute exercise on GIP was investigated in current dissertation. In **study 2** GIP was only increased in metformin condition before lunch. No significant difference remained postprandially between the metformin and placebo groups. GIP was also elevated following the first meal at the beginning of the second exercise bout compared to the control condition, but only in healthy participants **(study 3)**. Although **study 1** did not reveal any significant effects of acute aerobic exercise on GIP, studies have investigated the effects of longer durations of exercise training on this hormone. Different exercise protocols including longer durations and different intensities have also been studied. Kelly et al. (294) investigated the effects of a 12-week aerobic exercise training protocol in individuals with obesity and impaired glucose tolerance. Exercise along with an eucaloric diet did not change GIP responses to a 3-hour OGTT. In another study, Nyhoff et al. (295) compared moderate

intensity exercise and high intensity interval training effects in females with obesity. GIP responses did not differ between different exercise conditions. While exercise alone does not seem to affect GIP, losing weight induced by nutritional counselling and exercise has been shown to significantly increase GIP and betta cell function in T2D (296).

In summary, while a single bout of aerobic exercise could significantly increase GLP-1 in healthy individuals with normal glucose tolerance, it does not have any significant impacts in people with T2D. Furthermore, GLP-1 concentrations did not seem to be affected by single bouts of exercise in participants with obesity.

### 6.2.2 Effects of exercise on incretin responses to subsequent meal

**Hypothesis statement:** We hypothesized that a single bout of aerobic exercise would increase incretin responses to a subsequent meal.

The peak secretion of incretins is normally observed following ingestion. Hence, performing studies to investigate the impacts of exercise on post-meal incretin concentrations was deemed important. There have been discrepancies in the literature regarding the effects of exercise on incretin responses to a subsequent meal. While some studies showed no difference between exercise and control conditions (188, 189, 208), Blom et al. (201) suggested that GIP concentrations were significantly lower during a meal consumed following exercise. Ellingsgaard et al. (297) injected IL-6, in doses similar to concentrations measured during exercise, prior to oral or intraperitoneal glucose tolerance tests. IL-6 only improved oral but not intraperitoneal glucose tolerance. The authors suggested that this might indicate enhancement of incretin axis. Administration of 40 and 400 ng of IL-6 not only increased plasma insulin in a dose- and glucose-dependent fashion but also increased plasma GLP-1.

**Study 1 (Chapter 3)** provided a systematic review of the current literature. Quantitative meta-analyses did not seem appropriate for the postprandial outcomes due to the low available data and high heterogeneity in study designs. One of the major limitations of these studies is the presence of a variable duration between the end of exercise and the beginning of meal consumption. The second limitation that makes it difficult to compare these studies is utilizing meals with different compositions. Overall, there was no clear effect of acute exercise on incretin responses to meals in normal weight individuals or ones with obesity. **Study 2 (Chapter 4)** investigated the effects of aerobic exercise on incretin responses following a standardized meal in men with T2D. Despite the significant impact of metformin, exercise did not affect incretins compared to control conditions. **Study 3 (Chapter 5)** focused on the effects of a next day OGTT. Three hours of treadmill walking had no effect on next day incretin responses.

Overall, despite some inconsistencies in the current literature, acute aerobic exercise does not seem to have any significant effects on incretin responses to a subsequent meal.

### 6.2.3 Acute effects of exercise on insulin and glucagon

Although not the primary outcome of the current dissertation, the effects of acute exercise on insulin and glucagon were studied as secondary outcomes. As reviewed by Way et al. (298), there is a firm base of evidence supporting impacts of exercise on increasing insulin sensitivity in T2D. Results of **studies 2 and 3 (Chapters 4 and 5)** further supported this claim. Despite similar glucose profiles, plasma insulin was significantly lower following exercise compared to the control conditions in **study 2**. In **study 3**, exercise lowered insulin iAUC in response to a next day OGTT. Additionally, long bouts of aerobic exercise reduced the next day glucagon (i.e., a counter-regulatory hormone) responses to OGTT in T2D. The reduced glucagon

response following this form of long-duration exercise was previously shown in people with T1D (256) and in healthy individuals as well (257), however our results are the first to expand these findings to T2D.

### 6.2.4 Acute effects of exercise on IL-6

Researchers have been long looking for a mechanism by which exercise could affect incretin secretion. Decades before suggesting IL-6 secretion from contracting muscles, an "exercise factor" was thought to play the messenger role between muscle and peripheral organs (e.g., liver, pancreas and adipose tissue). The exercise factor idea was further supported by showing how muscle electrical stimulation could initiate responses similar to a healthy contracting muscle in patients with spinal cord injury (299). Recently, IL-6 was introduced as a myokine that is secreted from working muscles. Fischer (300) showed that among different characteristics of exercise, duration of exercise plays the most important role in controlling IL-6 released from muscles. Following two long bouts of aerobic exercise in **study 3 (chapter 5)**, we observed that IL-6 was significantly elevated following the first bout, which was performed in the fasting state. However, we did not find any differences between exercise and control conditions in changing plasma concentrations of IL-6 following a meal (second exercise bout). Due to the design of **study 3**, it is difficult to conclude whether the absence of IL-6 elevation following postprandial exercise is due to the meal or to other confounding factors. However, consuming a carbohydrate beverage during a long bout of aerobic exercise has been shown to abolish leg IL-6 release (285). This might suggest a possible effect of food consumption on reducing exercise-induced IL-6 release.

In a study by Ellingsgaard et al. (19), IL-6 secreted from contracting muscles was shown to increase insulin secretion by enhancing GLP-1 secretion. IL-6 has been since suggested as one of the potential mechanisms behind the effects of exercise on incretins. This phenomenon (i.e., effects of exercise on IL-6) was investigated throughout **study 3 (Chapter 5)** in human participants with T2D. Despite increases in IL-6, there was no significant increase in GLP-1 following exercise. Hence, the results of **study 3** are not fully in-line with the findings from Ellingsgaard (19).

### 6.2.5 Effects of Metformin on incretins

Studies have shown that long term treatment with metformin can significantly increase GLP-1 plasma concentrations in healthy people with obesity (249) and also in individuals with concomitant obesity and diabetes (250). Although metformin increases GLP-1, it has no effect on postprandial GIP concentrations (248). Despite the fact that the current dissertation focused on the effects of exercise, metformin was consumed by participants with T2D recruited in s**tudies 2 and 3 (Chapter 4-5)**. Hence, here we briefly summarize the effects of metformin on the study outcomes.

In **study 2 (Chapter 4)**, metformin raised GLP-1 both prior to and following a meal consumed 20 minutes after exercise. Additionally, GIP was increased before the meal in the metformin condition. In **study 3 (Chapter 5)**, metformin was skipped by participants with T2D the night before first day of every testing condition. Nonetheless, the increasing effects of metformin on incretin concentrations could not be ruled out in that study and could explain why incretin concentrations were often similar between participants with and without T2D.

## 6.3 LIMITATIONS

The main limitation of the current dissertation is the small sample size in **studies 2 and 3**. Although there was a tendency towards significant differences for some outcomes, low statistical power may have prevented our trials to reach statistical significance for those outcomes. Since **study 2** was conducted using the plasma samples acquired from a previously conducted clinical trial in our laboratory, there was no possibility to increase sample size. Additionally, having a challenging design for the **study 3** (i.e., walking for three hours on a treadmill), we only recruited 12 participants (6 healthy, 6 T2D) which further limited our power.

Studying the effects of exercise on incretins in people with T2D, the current dissertation only focused on acute aerobic exercise. Our findings are therefore not generalizable to other forms (e.g., resistance), intensities (e.g., high intensity), modes (e.g., interval), or durations (e.g., chronic training) of exercise. For instance, some studies suggest that regular exercise training could increase GLP-1 even in participants with obesity (202, 301, 302). For instance, Adam et al. (202) showed that after three month of weight loss by exercise training, GLP-1 significantly increased in individuals with obesity.

Although measuring plasma concentrations of incretins is a simple feasible approach, it does not reflect concentrations elsewhere in the body (i.e., portal circulation). Plasma concentrations may not be a good representative of incretin sensitivity of tissues. Additionally, measuring plasma concentrations of active GLP-1, one is dealing with extremely low levels and detecting any significant differences would demand larger study sample sizes. Using commercially available ELISA assays with far from ideal sensitivities would increase the chance of missing measurable differences in such low concentrations. Since most of the T2D

participants recruited for **studies 2 and 3** were relatively fit individuals with well-controlled diabetes, the results of these studies may not be generalizable to those with more advanced T2D.

### 6.4 PRACTICAL SIGNIFICANCE

The current dissertation further supports the role of exercise in increasing GLP-1 concentrations in healthy individuals. It has been suggested that reduced incretins in T2D is a secondary event in pathogenesis of T2D (122). Hence, from this specific aspect, exercise could potentially contribute to a better glucose control and reduce the risk of developing diabetes. Additionally, BMI, poor glycemic control and high HbA1c have a negative correlation with GLP-1's effect on insulin in T2D (122, 123, 181, 303, 304). Therefore, exercise could still indirectly provide benefits for T2D prevention by improving these factors.

Insulinotropic effects of GIP are lost in T2D (305). Hence, increasing GIP concentrations does not seem to have any meaningful clinical significance on improving insulin secretion in T2D. On the contrary, the insulinotropic effects of GLP-1 have been shown to be intact in T2D. Therefore, elevations in GLP-1 could possibly improve the glucose status of patients with diabetes. Although our studies did not reveal any significant effects of acute exercise on GLP-1 concentrations in this population, different exercise programs (e.g., chronic aerobic exercise) might still be capable of increasing this hormone.

### 6.5 FUTURE DIRECTIONS

The current thesis examined the acute effects of aerobic exercise on incretin hormones. Conducting further research on the influences of chronic exercise training would shed light on

whether or not exercise training could be recommended to increase incretin secretion and/or activity.

Whether performing exercise in fasting versus postprandial conditions would exert different effects on incretins is currently unknown. While we tried to compare the timing of exercise in our **study 3**, having both of these conditions in a single day followed by a next day OGTT makes it difficult to completely compare exercise timing effects. Future studies could further investigate this area.

Additionally, different modes, frequencies and intensities of exercise require further investigation. The main advantage of moderate intensity aerobic exercise used in the present dissertation is its feasibility and safety for patients with different BMI and glycemic statuses. Furthermore, performing moderate intensity exercise compare to higher intensities gives participants a chance to increase the duration of exercise. This would consequently increase IL-6 released from contracting muscles. Whether different intensities of exercise could potentiate different mechanisms to influence incretin concentrations demands further research.

While IL-6 secreted from contracting muscles has been recently suggested as a potential mechanism for GLP-1 secretion, further human studies need to confirm this phenomenon. Although this was investigated for the first time in **study 3**, we did not find any correlations between increases in IL-6 and changes in incretin concentrations. Future human studies could look into this relationship more specifically.

### 6.6 CONCLUSION

In summary, the findings of the present dissertation suggest that GLP-1 concentrations are increased immediately after a bout of aerobic exercise in normal weight healthy individuals,

but that the effects of exercise on GLP-1 become blunted in obese participants. In T2D, large uncommon volumes of aerobic exercise may be required to increase GLP-1. Exercise can also increase the next day fasting concentrations of GLP-1 and GIP. However, the observed increases in incretins were relatively minor and might not have a meaningful clinical effect. Whether other types of exercise trainings (e.g., long term, high intensity) could have different results is subject to further investigation.

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