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The blunted insulin release after exercise and the relationship with gastric inhibitory polypeptide and glucagon-like peptide-1

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The blunted insulin release after exercise and the relationship with Gastric Inhibitory Polypeptide and Glucagon-like Peptide-1

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ABSTRACT

Introduction: During exercise training, an increase in insulin sensitivity is accompanied by a decrease in plasma insulin concentrations during an oral glucose tolerance test (OGTT). The purpose of this study was to investigate the role incretin hormones play in the blunted insulin release after exercise. Gastric inhibitory polypeptide (GIP) and Glucagon-like peptide-1 (GLP-1) are incretin hormones that cause insulin release after glucose ingestion. We hypothesized that insulin, GLP-1, and GIP concentrations during an OGTT would be lower after exercise compared to after 7 days of inactivity.

Methods: Nine healthy men (n = 5) and women (n = 4) currently engaged in endurance exercise (23 ± 2 y) underwent 5 d of exercise at ~75% VO_2peak for 60 min. An OGTT was performed immediately post-exercise (IPE), one day after exercise (Day 1), and one week later (Day 7). Subjects were inactive between Day 1 and Day 7 (no exercise exceeding the intensity of activities of daily living).

Results: No change in body mass or body composition occurred during the study period. Glucose area under the curve (mmol/L x min) was significantly lower ($P < 0.05$) on Day 1 (160 ± 65) compared with IPE (233 ± 65) and was similar on Day 7 (186 ± 94). Although insulin sensitivity, as calculated by insulin-glucose index (pmol/L · min x mmol/L · min x 10^4), did not reach significance between Day 1 (569 ± 328) and Day 7 (1,023 ± 810), the plasma insulin response curve was significantly higher during the Day 7 OGTT compared with IPE ($P < 0.05$). Similarly, plasma GIP concentrations during the Day 7 OGTT were significantly higher than Day 1 ($P < 0.05$). No differences occurred within GLP-1 areas or OGTT responses.

Discussion: These data suggest that GIP and GLP-1 do not play significant roles in the blunted insulin release after exercise. The mechanism for the blunted insulin response may be important for furthering treatment of Type 2 diabetes and should continue to be studied, perhaps by focusing on other factors from the gut that influence insulin secretion.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Immediately after exercise, higher plasma glucose and insulin responses during a 75-g oral glucose tolerance test suggest impairment in insulin sensitivity (41). Within 10-16 h, the relative insulin resistance is followed by improved insulin sensitivity and glucose tolerance. This improved insulin action appears to last for three, but not 5 days (41).

Although the mechanism for a lower insulin secretion in response to glucose after exercise is unclear, there are factors in addition to glucose that stimulate the release of insulin from the pancreas. Increases in plasma amino acid concentrations, fat ingestion, and the release of gut hormones have all been shown to stimulate insulin release (44, 83, 86). Two gut hormones that elicit the incretin effect are Glucagon-like Peptide-1 (GLP-1) and Gastric inhibitory polypeptide (also referred to as Glucose-dependent insulinotropic Peptide, or GIP).

The importance of gut hormones in modifying insulin release became clear after it was noted that oral glucose administration causes a higher insulin response compared to an intravenous glucose infusion (61, 78). Shuster and others (78) found that insulin secretion, C-peptide, and GIP were all significantly higher in response to oral glucose ingestion compared to intravenous infusion in normal subjects with the same basal plasma glucose. The lower insulin response as a result of exercise is also more pronounced with oral glucose administration.

The insulin responses to glucose, arginine, and fat ingestion are all blunted in endurance trained people (44). Although the plasma insulin concentrations continued to

rise when intake of fat was combined with glucose and amino acid infusion, and fat ingestion, the plasma concentration of GIP decreased suggesting other factors regulating the release of insulin. Perhaps other gut hormones such as GLP-1 are responsible for the reduced release of insulin in endurance trained people.

Few studies have investigated the gut hormones response to exercise. An early study on GIP demonstrated lower plasma concentrations of GIP after exercise compared to non-exercised individuals in response to glucose ingestion (9). O'Connor and colleagues (64) detected increases in plasma GLP-1 during and after a 120 minute running bout. De Luis et al. (16) found resting GLP-1 levels to decrease after weight loss induced by physical activity and hypocaloric diet. Previous literature on GIP indicates that significant changes in GIP levels do not occur during and after exercise (33, 36, 64). Because of the limited research on GLP-1 and GIP related to exercise training and the blunted response of insulin, it was the purpose of this study to investigate the role of the gut hormones in the blunted insulin response after exercise. We hypothesized that plasma gut hormone concentrations will be significantly higher seven days after cessation of exercise compared to immediately after exercise and one day after exercise similar to the trend of insulin concentrations.

Thesis Organization

This thesis is organized into a General Introduction, Manuscript, and General Conclusions. The manuscript is formatted according to the Journal of Applied Physiology specifications. The primary author for this paper is Alison R. Glidden, a Master's student in the Kinesiology department at Iowa State University. Dr. Douglas S. King, a Professor of

Kinesiology at Iowa State University, contributed to the experimental design, data analysis, and preparation of the manuscript.

Review of Literature

Introduction

Insulin has many metabolic roles, most importantly to regulate glucose homeostasis. Regulating and maintaining normal glucose levels is imperative in preventing metabolic diseases, namely diabetes mellitus (35). The concept of exercise-induced insulin sensitivity with improved glucose tolerance has shed light on the importance of physical activity in treating and preventing disease (43). The mechanism responsible for the decrease in insulin secretion in response to exercise is still unclear. Factors that contribute to the release of insulin include blood glucose concentrations, plasma amino acid concentrations, fatty acids, catecholamines, the nervous system, and gut hormones. The interest in gut hormones on their effect on insulin secretion became heightened after the discovery of the incretin effect. The incretin effect demonstrates that oral glucose ingestion elicits a higher insulin response compared to intravenous infusion of glucose (78). This review focuses on the stimulation of insulin release by two incretins, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (glucose-dependent insulinotropic peptide or GIP).

Insulin and Disease

Insulin is a hormone secreted from the pancreas to help regulate levels of blood glucose by causing glucose uptake into cells of the muscle, adipose, and liver. Insulin also helps to regulate fat and protein synthesis and without normal functioning of insulin to regulate its

effects on metabolism, disorders such as diabetes mellitus, hyperlipidemia, metabolic syndrome, and obesity can appear (35). The most common metabolic disorder is diabetes with diabetes mellitus (Type 2 diabetes) being the most common form of diabetes. Type 2 diabetes is characterized by a cellular resistance to insulin, meaning that although the pancreas is secreting insulin, the cells are not responding and therefore the metabolic effects of insulin are not taking place (24). The effects of diabetes mellitus can also occur as a result of worse insulin secretion in response to glucose. The ability of insulin to function effectively (characterized by insulin resistance and sensitivity) can be measured with an Oral Glucose Tolerance Test (OGTT) which can then be used to diagnose disorders such as diabetes mellitus (38). An OGTT consists of giving 75 g of glucose and monitoring plasma glucose levels for the three hours following ingestion. If plasma glucose concentrations exceed normal levels (at one hour, above 200 mg/dl; at two hours, above 140 mg/dl), impaired glucose tolerance is suspected (1).

Recently, the incretins have become an important topic of study with a potential to help treat Type 2 diabetics because diabetic patients have a reduced incretin effect (61) and the hormones have an important contribution to the enteroinsular axis to regulate blood glucose homeostasis. Because of problems with incretin degradation in the blood, two main therapeutic strategies are under clinical investigation and trial. The first possible therapy is using receptor agonists or incretin mimetics (27). A GLP-1 mimetic, exenatide is found naturally occurring in the Gila monster and has been shown to produce similar effects as GLP-1 while being resistant to the enzyme dipeptidyl peptidase IV (DPP IV) that breaks down the incretins. The second idea of therapy aims to inhibit the DPP IV enzyme which rapidly breaks down the incretins into their noninsulinotropic metabolites (17, 20, 21). The

DPP IV inhibitor has shown hints of preserving endogenous incretin activity, however the long term affects of DPP IV inhibitors is unknown. The DPP IV enzyme is linked to inhibition of tumor growth and therefore inhibition of this enzyme may lead to progression of cancer (50, 71, 89). As noted, insulin and its stimulators are important hormones for metabolic regulation and therefore it is important to understand all components related to their regulation.

Pancreas Physiology

The pancreas has two types of cells (endocrine and exocrine) that connect to form an organ located on the dorsal and ventral side of the duodenum. The exocrine cells are composed of acinar and ductal cells and function to release and transport digestive enzymes into the small intestine. The endocrine cells form the islets of Langerhans and function to regulate glucose homeostasis (88). There are roughly one million islets of Langerhans (4, 67) in a human adult and each islet consists of approximately two thousand cells. Four different types of cells are contained within each islet being the alpha (α) cell secreting glucagon, the beta (β) cell secreting insulin, delta (δ) cells secreting somatostatin, and the pancreatic polypeptide (F) cells secreting pancreatic polypeptide (PP). Each hormone has its own specific purpose when secreted into the circulation, but controlled by their regulatory mechanisms together make up the hormonal milieu that regulates glucose homeokinesis. Some regulation of the hormones of the endocrine pancreas is done by cell-to-cell interaction (45, 58). Somatostatin's role in regulation is inhibiting both insulin and glucagon secretion. An example of this cell-to-cell regulation is the delta-to-beta cell axis in which somatostatin is released from the delta cell into the circulation around the islet and

reaches the somatostatin receptor on the beta cell to inhibit insulin secretion. This type of regulation is thought to occur throughout the endocrine axis of the pancreatic islets (45). Effects of the other hormones of this axis include glucagon functioning to stimulate insulin and PP secretion and inhibit somatostatin while insulin acts to decrease glucagon and stimulate somatostatin (58).

Insulin Synthesis and Release

Insulin begins as a single polypeptide chain called preproinsulin located on the ribosomes in the cytosol of the beta-cell. A signal peptide (N-terminal) sends the growing amino acid chain into the rough endoplasmic reticulum (RER) for preparation for secretion (84). In the RER, the signal peptide is removed forming proinsulin to be transported to the Golgi apparatus. By moving through the Golgi apparatus (and packaged into forming β-granules from sections of cisternae enclosing the proinsulin) proinsulin is converted to insulin and C-peptide. The cleavage of proinsulin to form insulin and C-peptide is completed with the help of proprotein-converting endopeptidases which allow for removal of amino acids to form an insulin molecule with its α– and β–chain aligned and C-peptide to become the lumen of a mature β-granule (66) and ending in an individual secretory granule. The contents of a mature granule are either released from the β-cell via exocytosis when stimulated by factors that encourage insulin release or degraded after some time via crinophagy or autophagy (75).

Insulin Stimulation

Substances including amino acids, fatty acids, and hormones stimulate insulin secretion; however glucose is the main factor to control insulin release (30). Glucose stimulates the release of insulin from the β-cell when it is metabolized by the mitochondria (46). The metabolism of glucose causes changes in the ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). The increase in this ratio leads to closure of the cell membrane's ATP-sensitive potassium (K^+) channels thus causing membrane depolarization and the opening of the voltage-dependent calcium channels (VDCC). The channels open creating an influx of intracellular calcium (Ca^{2+}) which triggers insulin secretion and plays an important role in fueling insulin secretion (5). Two hormones from the gut that stimulate insulin release are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). These hormones initiate insulin secretion by binding to their receptors on the β-cell membrane which activate cyclic AMP (cAMP) levels to turn on cAMP-dependent protein kinase (PKA). The receptors are coupled to G_s-proteins and activation of PKA leads to the production of second messenger pathways to increase Ca^{2+} concentrations (80). The influx of Ca^{2+} through the VDCC causes insulin secretion from vesicles via exocytosis. Insulin may also be secreted due to phosphorylation of vesicular proteins by PKA (39). This abbreviated processes described here, however, does not account for the entire regulatory physiological steps required to induce insulin release, and exact mechanisms are still being investigated. Hormones and catecholamines such as norepinephrine inhibit insulin secretion in much the same way as stimulators. They bind to receptors that are coupled to $G_{i/o}$-proteins that inhibit adenylyl cyclase which alter the channels of K^+ and Ca^{2+} (76). Insulin secretion is also influenced by the autonomic nervous

system (especially during the cephalic phase of nutrient ingestion) (3) with the parasympathetic branch releasing acetylcholine to increase activity of M_3 receptors (which are coupled to G_q-proteins) to potentiate insulin release and the sympathetic branch releasing epinephrine to inhibit insulin secretion (87).

GLP-1 and GIP Physiology

The role hormones play to regulate insulin release became evident after the relationship between the gastrointestinal tract and the pancreas became evident. A higher elicited response of insulin was discovered when glucose is ingested orally compared to the same amount infused intravenously. The concept gave rise to what is known as the incretin effect with two gut hormones responsible for this, GIP and GLP-1 (28). The gut hormones are in the glucagon peptide family. The hormone GIP is formed within the intestinal K cells from a protein precursor (proGIP) made of a signal peptide, an N-terminal peptide, GIP, and a C-terminal peptide. Cleavage at the arginine on each side of the GIP forms a biologically active 42 amino acid peptide (85) which is released from the K cells located in the duodenum and proximal jejunum with small amounts also through the entire small intestine (59). The formation and cleavage of the proglucagon gene produces glucagon and major proglucagon-derived fragment (MGPF) in the α-cells and GLP-1, GLP-2, and glicentin in the L cells of the ileum and colon. After the translational release of GLP-1, the 30 amino acid peptide is released from the L cells of the ileum and colon (25) as well as from parts of the central nervous system including the hypothalamus, pituitary, and the reticular nucleus.

Release of GIP and GLP-1 is stimulated by oral nutrient ingestion of fat and carbohydrate and by proteins for GLP-1 only (32) and contact of the peptides with the

intestinal mucosa is also adequate to cause release of the hormones from their corresponding cells. Lipid ingestion stimulating the incretins release is supported in a dose-dependent relationship (91) with GLP-1 tending to have a greater sensitivity to intraluminal lipid content. Inhibition of both peptides has been shown after release of somatostatin release from nearby δ-cells via a paracrine interaction within the gut (17). GIP has also been shown to be stimulated by the autonomic nervous system (3), sympathetic nervous system (β-adrenergic stimulation) (26), glutamine, cAMP, and phorbol 12-myristate 13-acetate (PMA) (69) while α-2 adrenergic activation was shown to decrease GIP levels (73). A negative feedback mechanism of GIP was also evident when an increase in hypothalamic insulin levels decreased the release of GIP after a glucose load (90). GLP-1 release can be stimulated by neural control as well (via activation of vagal cholinergic muscarinic receptors and β-adrenergic receptors), and by GIP, acetycholine, neuromedin C (64), and Gastrin-Releasing Peptide (GRP) (13) and GLP-1 is inhibited by sympathetic efferents, or α-2 receptors (34).

Table 1. Summary of factors influencing incretin release.

	GIP Release		**GLP-1 Release**	
	Stimulate	*Inhibit*	*Stimulate*	*Inhibit*
Nutrient:	Fat and CHO presence on lumen Protein (animals only) Glutamine phorbol 12-myristate 13-acetate (PMA)		Fat, CHO, Protein presence on lumen Plasma glucose (after oral ingestion only)	
Hormonal:	Gastrin-releasing peptide	Somatostatin	GIP (high concentrations only) Acetylcholine Gastrin-releasing peptide	Somatostatin
Neural:	β-2 adrenergic receptors	α-2 adrenergic receptors	β-2 adrenergic α-1 receptors vagal cholinergic muscarinic (M3) receptors	α-2 adrenergic receptors
Other:	cAMP (calcium dependent)		cAMP (calcium dependent) Neuromedin C	

Once released from their corresponding cells, GIP and GLP-1 both break down rapidly. The enzyme dipeptidyl peptidase IV (DPP IV) cleaves the N-terminal of both hormones indicated in vitro (55) and in vivo (20). The abundance of DPP IV deriving locations include the kidney, intestinal brush border membranes, hepatocytes, and throughout the vascular bed (54) causing the incretins to be broken down rapidly with a plasma half-life of exogenous GIP shown to be about 7 minutes while the half-life of exogenous GLP-1 is as short as 1 to 2 minutes (52). More importantly, endogenous hormones are more rapidly degraded (particularly GLP-1) as DPP IV is found in the endothelium of the capillaries neighboring the L-cells causing more than half of GLP-1 to

be degraded before it even enters systemic circulation (29). The degradation of both exogenous (18, 19) and endogenous (7) GIP and GLP-1 has been shown to be successfully repressed with the presence of DPP IV inhibitors which can adequately preserve the effects of the hormones (21).

Role of GIP and GLP-1 on Insulin Secretion

Although GIP and GLP-1 have various functions as hormones, their role promoting insulin secretion is one of the most important. Both peptides are glucose-dependent in their insulin-secreting effects and use a similar pathway to cause insulin secretion. The intracellular pathway utilizes G_s protein-coupled receptors for GIP and GLP-1 on the β-cell that are activated to initiate a rise in adenylyl cyclase activity and cAMP leading to an increase in PKA activity and intracellular Ca^{2+} concentrations and ultimately insulin release via exocytosis (22, 47, 72). The influences of GIP and GLP-1 have been verified with studies in rats with either missing GLP-1 or GIP receptors or when administering the peptide receptor antagonist to prevent the influence of the hormones on insulin release. After nutrient ingestion, the results of insulin release were analyzed and indicated attenuated insulin secretion resulting in hyperglycemia and glucose intolerance (25, 57, 70).

The incretins have also been thought to be a cause of insulin secretion during the cephalic phase of nutrient ingestion. Ahren and Holst (3), however, found contraindicating results when circulating levels of GIP and GLP-1 failed to increase during the initial 10-minute preabsorptive period after meal ingestion. The study suggested that GIP is responsible for the insulin response at 15 minutes post meal ingestion via neural mediation. GIP and GLP-1 help insulin secretion further by aiding in decreasing β-cell apoptosis, and

enhancing β-cell insulin gene transcription, insulin biosynthesis, and β-cell proliferation (23, 81) with GLP-1 additionally inhibiting glucagon secretion (68). It is also important to note that a GLP-1 receptor agonist (exendin-4) has been detected to produce similar effects of GLP-1, specifically increasing insulin secretion and β-cell mass as well as peripheral insulin sensitivity (60). The use of the agonist from the Gila monster may be a helpful substitute for GLP-1 as it has been shown to be active for up to 12 hours in vast contrast to the rapidly degrading GLP-1 hormone. Additionally, preventing the breakdown of the gut hormones with the application of a DPP IV inhibitor has also been shown to preserve the insulinotropic effects of the incretins (21).

Metabolic Effects of Insulin

After the stimulation and release from the pancreas, insulin initiates its effects on metabolism with insulin receptors present on target tissues and in high concentrations on muscle, adipose, and liver tissue. Insulin binds to a receptor on the target tissue initiating a series of events that result in the activation of phosphoinositide 3-kinase (PI 3-kinase). It is the activation of PI 3-kinase that plays a large role in the metabolic effects of insulin (77) including glucose uptake (82), glycogen synthesis (14), decreased lipolysis (65), and protein synthesis (53). In skeletal muscle and adipose tissue, the cascade of events that take place lead to the uptake of glucose via a facilitative glucose transporter called GLUT 4 by causing the glucose transporters located within the cellular storage to translocate to the cell membrane to allow for glucose uptake (15). Additional effects from insulin include fat synthesis, amino acid uptake, and inhibition of muscle and liver glycogenolysis (28).

Insulin and Exercise

Immediately after aerobic exercise, plasma glucose and insulin responses during an oral glucose tolerance test are higher, suggesting an impairment in insulin sensitivity (41). Within 10-16 hours, the relative insulin resistance is followed by improved insulin sensitivity and glucose tolerance lasting for a few days without additional exercise. Improvements in insulin sensitivity from endurance training were found by King et al (42) when using a hyperinsulinemic, euglycemic clamp procedure. Higher glucose disposal in response to the same amount of insulin was observed in trained subjects compared to untrained subjects, indicating higher insulin sensitivity. Improved insulin sensitivity was also seen when using a hyperglycemic clamp procedure, where the trained subjects had significantly higher glucose disposal rates, and with lower plasma insulin concentrations indicating improved insulin action (43). Although trained individuals indicate higher insulin sensitivity compared to untrained individuals, the effect elicited is due to an exercise effect rather than a training effect (43). When trained individuals underwent 14 days of inactivity, their insulin response was significantly higher than one day after exercise with no change in glucose disposal. The effect of exercise on insulin sensitivity was also demonstrated in a middle-aged, moderately exercising population in which they participated in 5 days of exercise followed by 7 days of inactivity with glucose tolerance testing within the period of inactivity (41). The significant increase in glucose tolerance lasted 3 days but was diminished by 5 days after exercise again demonstrating a short term effect of physical activity on increased insulin sensitivity rather than an effect from training.

The benefit of physical activity on insulin sensitivity has lead to research with treating metabolic diseases. Relative risk of Type 2 diabetes decreased markedly with

increasing levels of physical activity noted by increasing levels of energy expenditure with involvement in aerobic activities such as walking, stairs, and recreational sports (31). Specifically, for each 500 kcal increase in energy expenditure, the risk of non-insulin dependent diabetes mellitus (NIDDM) decreased by 6 percent with the most protective effect of physical activity being in persons at the highest risk for NIDDM. Resistance exercise has also shown to benefit insulin action in both healthy adults and adults with NIDDM. In healthy adults participating in a training program for 16 weeks including a variety of muscular strength and endurance training exercises at moderate to vigorous intensity, an increase in muscular strength is accompanied by an increase insulin sensitivity during the hyperinsulinemic-euglycemic clamp procedure (56). Similar results have also been shown after strength training for as little as 20 weeks (79). An increase in insulin sensitivity with resistance exercise also occurs in Type 2 diabetic adults. During a resistance training program of 4 to 6 weeks at 5 days per week using approximately the same moderate to vigorous strength and endurance exercises, an increase in glucose disposal rates during the hyperinsulinemic-euglycemic clamp procedure was significant (37).

For aerobic exercise recommendations concerning intensity, research has indicated that both low and high intensity aerobic exercise elicit similar effects to increase insulin sensitivity (11). A general guideline states that a minimum intensity of moderate exercise (55 percent of VO_2max) at an average of 3 to 4 sessions per week for 40 minutes over 20 weeks is enough to elicit increased insulin sensitivity (92). While an increase in insulin sensitivity may be similar across low to high exercise intensities, it is also indicated that the exercise effects on glucose tolerance tend to be more exaggerated as exercise intensity

increases (12). Specifically, exercise intensity is a better predictor of increased insulin action rather than exercise volume but overall the effect of exercise on insulin secretion suggests important implications for treating and managing metabolic diseases.

Exercise and Incretins' Role on Insulin

Oral glucose administration causes a higher insulin response compared to an intravenous glucose infusion (62, 78) suggesting an important role of the incretin hormones in helping to control insulin release. In addition, the attenuation of the insulin response to glucose after exercise is more pronounced when glucose is administered orally compared with intravenous administration. These findings suggest that the blunted insulin response that appears one day after exercise could be accounted for by changes in concentrations of GIP and GLP-1 which would alter the stimulation of insulin release under certain conditions.

The effects of exercise on the release of gut hormones as well as their relationship with insulin secretion after exercise has only been studied to a small extent. The effects of exercise on gut hormones present contraindicating results on the pattern of secretions during and after bouts of physical activity. Martins et al. found GLP-1 levels to be significantly increased during and after one hour of exercise (49) agreeing with data from O'Connor and colleagues whom detected increases in GLP-1 during and after a 120 minute running bout (64). While some studies have reported results on GLP-1 release in response to exercise and training, much of the gut hormone research is focused on weight loss and satiety levels. Adam and others found GLP-1 release to be stimulated in lean subjects with 60 minutes of physical activity compared to rest and further studied the effects of activity on obese subjects observing that before weight loss GLP-1 levels were not different during

physical activity compared to rest. After significant weight loss the subjects' GLP-1 levels were increased with physical activity (2). De Luis et al. found resting GLP-1 levels to decrease after weight loss induced by physical activity and hypocaloric diet (16). Previous literature on GIP indicates results showing no significant changes in GIP levels during and after exercise (33, 36, 64). Although it is helpful to know the effects of exercise on the gut hormone release, it would be even more so to determine the cause of less insulin release after exercising. An early study on GIP demonstrated lower levels of GIP after exercise compared to non-exercised individuals in response to glucose ingestion (9) hinting at the important effects related to insulin release with exercise. Together, these ideas highlight the implications of gut hormones in modifying insulin release when Shuster and others found that in normal subjects with the same basal plasma glucose, insulin secretion, C-peptide, and GIP were all significantly higher in response to oral glucose ingestion compared to intravenous infusion (78). Using the few studies that give evidence to relationship between gut hormones and blunted insulin release following exercise, future research is needed to confirm the influence of GIP and GLP-1 on insulin.

Further studies have hinted of the relationship between the increased glucose disposal with a modest increase in insulin resulting from GLP-1 specifically. While these studies have not examined the influence of exercise, the effects appear to be possibly related. While we know that GLP-1 causes an increase in insulin, we also know that GLP-1 is broken down rapidly from GLP-1-(7-36) to its metabolite GLP-1-(9-36). It is the differences in these two forms that could be related to the blunted insulin response with an increase in glucose disposal. Meier et al. found that when the GLP-1-(9-36) amide was infused the effects were a higher glucose uptake independent of changes in insulin,

glucagon, or gastric emptying (51). The effects are in contrast to the infusion of GLP-1-(7-36) which displayed a significant increase in insulin concentration along with the increase in glucose disposal. This study indicates that while intact GLP-1 acts in an insulinotropic manner, its metabolite acts as an insulinomimetic. Although GLP-1-(7-36) is more powerful in its effects of increasing glucose disposal, its previously speculated inactive metabolite is now giving rise to indicate its own independent actions.

While GLP-1 plays an important role in insulin sensitivity, we have to acknowledge additional mechanisms that may contribute to the exercise effect. During exercise, there is an increase in catecholamine levels (i.e. epinephrine and norepinephrine) due to sympathetic nervous system activation. Increases in catecholamine levels in turn stimulate lipolysis (8) which leads to an increase in plasma free fatty acid content and therefore intramyocellular triglyceride content (10) with both contributing to an increase in insulin resistance (seen immediately post exercise). Insulin resistance can then be reversed when plasma free fatty acids are lowered. Exercise-induced rises in free fatty acids have been shown to decrease about 40 percent 13-16 hours post exercise and down to 10 percent of exercise levels between 21 and 24 hours post exercise (48). The ability of exercise to help decrease lipid-induced insulin resistance remains unclear however the increase in fatty acid oxidation (40) as well as the augmented storage of fatty acids as intramyocellular triglyceride (6, 74) are two possible reasons as to why exercise induces insulin sensitivity after 24 hours in relation to free fatty acids. Finally, muscle glycogen concentrations are related to insulin sensitivity after exercise as exercise depletes muscle glycogen stores and also withholding dietary carbohydrate consumption after exercise shows an increase in insulin sensitivity the following day (63).

Summary and Future Directions

While hormones are only a small component of regulating insulin secretion, understanding the different mechanisms for the release of insulin is important during different circumstances, such as during exercise, in obese individuals, and diseased populations. If these pathways and regulators are known, we can begin to treat and manage metabolic diseases in a much more efficient manner. The initial investigations and clinical trials of using DPP IV inhibitors and exendin-4 have indicated promising potential in treating impaired glucose homeostasis and insulin action with incretin-related drug therapy, however the long term effects of this therapy (in relation to cancer risk specifically) are unknown. Not only will it be significant to understand the regulators of insulin itself, but also it is essential to comprehend the effects of different conditions on the release GIP and GLP-1. If we can understand these effects, it would help us to further realize their implications with insulin and treating disease.

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CHAPTER 2. THE BLUNTED INSULIN RELEASE AFTER EXERCISE AND THE RELATIONSHIP WITH GASTRIC INHIBITORY POLYPEPTIDE AND GLUCAGON-LIKE PEPTIDE-1

Abstract

Introduction: During exercise training, an increase in insulin sensitivity is accompanied by a decrease in plasma insulin concentrations during an oral glucose tolerance test (OGTT). The purpose of this study was to investigate the role incretin hormones play in the blunted insulin release after exercise. Gastric inhibitory polypeptide (GIP) and Glucagon-like peptide (GLP-1) are incretin hormones that cause insulin release after glucose ingestion. We hypothesized that insulin, GLP-1, and GIP concentrations during an OGTT would be lower after exercise compared to after 7 days of inactivity.

Methods: Nine healthy men (n = 5) and women (n = 4) currently engaged in endurance exercise (23 ± 2 y) underwent 5 d of exercise at ~75% VO_2peak for 60 min. An OGTT was performed immediately post-exercise (IPE), one day after exercise (Day 1) and one week later (Day 7). Subjects were inactive between Day 1 and Day 7 (no exercise exceeding intensity of activities of daily living).

Results: No change in body mass or body composition occurred during the study period. Glucose area under the curve (mmol/L x min) was significantly lower ($P < 0.05$) on Day 1 (160 ± 65) compared with IPE (233 ± 65) and was similar on Day 7 (186 ± 94). Although insulin sensitivity, as calculated by insulin-glucose index (pmol/L · min x mmol/L · min x 10^4), did not reach significance between Day 1 (569 ± 328) and Day 7 (1,023 ± 810), the plasma insulin response curve was significantly higher during the Day 7 OGTT compared with IPE ($P < 0.05$). Similarly, plasma GIP concentrations during the Day 7 OGTT were

significantly higher than Day 1 ($P < 0.05$). No differences occurred within GLP-1 areas or OGTT responses.

Discussion: These data suggest that GIP and GLP-1 do not play significant roles in the blunted insulin release after exercise. The mechanism for the blunted insulin response may be important for furthering treatment of Type 2 diabetes and should continue to be studied, perhaps by focusing on other factors from the gut that influence insulin secretion.

Introduction

Immediately after exercise, higher plasma glucose and insulin responses during a 75-g oral glucose tolerance test suggest impairment in insulin sensitivity (14). Within 10-16 h, the relative insulin resistance is followed by improved insulin sensitivity and glucose tolerance. This improved insulin action appears to last for three, but not 5 days (14).

Although the mechanism for a lower insulin secretion in response to glucose after exercise is unclear, there are factors in addition to glucose that stimulate the release of insulin from the pancreas. Increases in plasma amino acid concentrations, fat ingestion, and the release of gut hormones all stimulate insulin release (15, 27, 28). Two gut hormones that elicit the incretin effect are glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (also referred to as glucose-dependent insulinotropic hormone, or GIP).

The importance of gut hormones in modifying insulin release became clear after it was noted that oral glucose administration causes a higher insulin response compared to an intravenous glucose infusion (18, 23). Shuster and others (23) found that in normal subjects with the same basal plasma glucose, insulin secretion, C-peptide, and GIP were all significantly higher in response to oral glucose ingestion (cephalic response) compared to

intravenous infusion. The lower insulin response is also more pronounced with oral glucose administration.

The insulin responses to glucose, arginine, and fat ingestion are all blunted in endurance trained people (15). Although the plasma insulin concentrations continued to rise when intake of fat is combined with glucose and amino acid infusion, and fat ingestion, the plasma concentration of GIP decreased suggests other factors are regulating the release of insulin. Perhaps other gut hormones such as GLP-1 are responsible for the reduced release of insulin in endurance trained people.

Few studies have investigated the gut hormones response to exercise. An early study on GIP demonstrated lower levels of GIP after exercise compared to non-exercised individuals in response to glucose ingestion (3). O'Connor and colleagues (19) detected increases in GLP-1 during and after a 120 minute running bout. De Luis et al. (6) found resting GLP-1 levels to decrease after weight loss induced by physical activity and hypocaloric diet. Previous literature on GIP indicates that significant changes in GIP levels do not occur during and after exercise (10, 11, 19). Because of the limited research on GLP-1 and GIP related to exercise training and the blunted response of insulin, it was the purpose of this study to investigate the role of GIP and GLP-1 in the blunted insulin response after exercise. We hypothesized that plasma gut hormone concentrations will be significantly higher seven days after cessation of exercise compared to immediately after exercise and one day after exercise similar to the trend of insulin concentrations.

Materials and Methods

Subjects. Participants recruited for this study were 10 healthy, trained 18-27 y old adults. The data of one female subject was excluded from analysis due to an anomalous decrease in plasma glucose after ingestion of 75-g glucose resulting in 5 males and 4 females for final data presentation. All subjects recruited into the study were engaged in endurance exercise at a minimum level of 30 min continuous, 3 times per week, and an intensity of 60% of their maximum heart rate reserve. All subjects gave written informed consent as approved by the Iowa State Human Subjects Committee before starting the study. Subjects provided a medical history and underwent body composition analysis and a graded exercise test. Subject characteristics are given in Table 2.

Table 2. Subject Characteristics.

	Exercising	Inactive
Age, y	23 ± 2	
Height, cm	173.4 ± 9.5	
Women	166.2 ± 3.2	
Men	180.7 ± 7.7	
Body Mass, kg	67.0 ± 12.8	67.1 ± 12.7
Women	59.3 ± 5.6	59.5 ± 5.3
Men	74.6 ± 13.8	74.6 ± 13.9
Body Mass Index, kg/m^2	22.1 ± 2.5	
Body Fat %, Skinfolds	14.4 ± 5.3	14.5 ± 5.5
Women	18.5 ± 2.5	
Men	10.2 ± 3.9	
Body Fat %, Hydrostatic		
Women	19.9 ± 3.9	
Men	14.9 ± 5.0	
VO_2peak		
L/min	3.58 ± 0.7	
Women	3.09 ± 0.2	
Men	4.08 ± 0.7	
ml/kg/min	53.5 ± 2.4	
Women	52.2 ± 2.3	
Men	54.8 ± 1.9	

Values are means ± SD; n = 4 women and 5 men.VO_2peak, peak O_2 uptake.

Experimental Protocol. Subjects performed 60 min of continuous exercise at a work rate calculated to elicit 75% of VO_2peak for five consecutive d. Two subjects exercised on the treadmill and eight subjects exercised on the cycle ergometer. Heart rate and oxygen uptake (VO_2) were measured for 5 min every 20 min interval to determine exercise intensity (Max II Metabolic Systems, Physiodyne Metabolic Cart). Immediately post exercise (IPE) on the fifth d, subjects underwent an OGTT and blood sampling as well as on d 1 and 7 after the last exercise d. During this time subjects did not undergo vigorous physical activity. The experimental protocol is detailed in Figure 1.

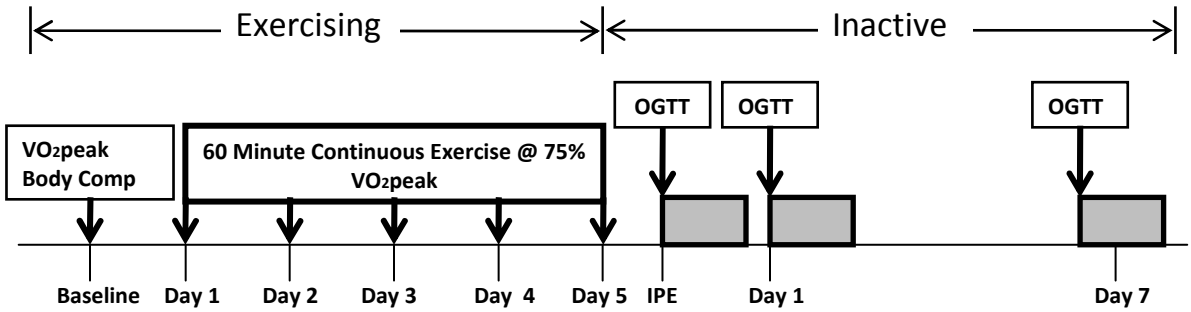

Figure 1. Experimental protocol. Body Comp, body composition via hydrostatic weighing and skinfolds; OGTT, oral glucose tolerance test; IPE, immediately post-exercise.

Body Composition. Body composition was measured with hydrostatic weighing and skinfold calipers with skinfold assessment done both before and after 7 d of physical inactivity (Lange Skinfold Calipers, Beta Technology Inc., Cambridge, MA). For hydrostatic weighing, subjects performed a minimum of four trials and the mean of the two highest trials was used for the underwater weight. Residual lung volume was estimated as the proportion of vital capacity (0.24 for men, 0.28 for women) after measurement with a spirometer (30). Body density for skinfold measurements was calculated for males and

females using the Jackson and Pollock equations (12, 13). Body fat percentage was calculated using the equation of Siri (4.95/density-4.50 x 100) (24).

Estimation of VO_2peak. VO_2peak was estimated during a maximal graded exercise test on an electromagnetically braked cycle ergometer or a treadmill. Tests on the ergometer consisted of 2 min stages beginning at 50 W with an increase in power output of 50 w for each stage. Tests on the treadmill started at 5 mph for females and 6 mph for males and consisted of 2 min stages with an increase in workload of 1 mph after each stage. Tests were terminated when the subjects reached maximal fatigue. Expired gases were collected during each min of exercise.

OGTT and Blood Analyses. Subjects reported to the lab after an overnight fast and blood samples were taken before, and 30, 60, 90, and 120 min after ingestion of 75 g of glucose dissolved in 300 mL of water. Blood samples were collected without stasis into EDTA tubes and kept on ice before centrifugation and storage (-80 C) until analysis. Blood samples for GIP and GLP-1 analysis were immediately combined with 200 KIU aprotinin per 1 mL whole blood. Plasma glucose concentrations were measured by the glucose oxidase method (YSI 2300 GL, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentrations were analyzed by radioimmunoassay (RIA) according to previously described methods (8, 31); porcine insulin was used as standards. The intra-assay and inter-assay coefficient of variation (CV) for the insulin assay were 8% and 12%, respectively.

GIP. Plasma GIP concentrations were measured by RIA technique using rabbit anti-human GIP antibody (Phoenix Pharmaceuticals), ^{125}I-labeled human GIP, and human GIP standards (American Peptide). Radioiodination of human GIP was done using a modified Chloramine-T method (4). The iodination mixture was applied to a G-50 Sephadex column at 4˚C and eluted with elution buffer (0.1 M acetic acid, 0.5 M NaCl, 0.1% BSA, 3 mM NaN_3, pH 5.0) while 1 ml fractions were collected at the rate of 6 ml/hr. Assay was performed in polypropylene tubes with incubations at 4˚C using a phosphate dilution buffer containing BSA, Na_2EDTA, and NaN_3 (pH 7.4). The dilution buffer containing 500 KIU aprotinin/ml was used for dilution of GIP antibody (final dilution 1:10,000), ^{125}I-GIP (15,000 cpm/0.1 ml), and GIP standards containing 100 to 12,800 pg/ml. Standards or plasma samples (0.1 ml) were added to the tubes followed by the first antibody (rabbit anti-human GIP). After 24 hr incubation, 0.1 ml of ^{125}I-GIP was added to each tube followed by 24 hr incubation again. Normal rabbit plasma (0.1 ml; 1:80) and second antibody (0.1 ml; goat antiserum vs rabbit IgG; 1:40) was added followed by 10 minute incubation at room temperature. Finally 0.1 ml of human serum was added to each standard and 1 ml of polyethylene glycol solution was added to each tube before centrifugation at 3,000 rpm for 30 min at 4˚ C. The supernatant was aspirated before the tubes were counted with a gamma counter. The interassay and intraassay CV for GIP RIA was 3% and 7%, respectively.

GLP-1. Plasma samples for GLP-1 were extracted with ethanol before radioimmunoassay procedure using mouse anti-human GLP-1 (Bachem), ^{125}I-labeled GLP-1, and human GLP-1 (American Peptide). Radioiodination of human GLP-1 was done similarly to the methods described above for GIP. Assays were performed in glass tubes with incubations at 4˚C

using a phosphate dilution buffer containing BSA, Na_2EDTA, and NaN_3 (pH 7.4). Dilution buffer containing 500 KIU aprotinin/ml was used for dilutions of ^{125}I-GLP-1 (~20,000 cpm/0.1 ml) and GLP-1 standards containing 3.9 to 500 pmol/L. Standards or samples (0.1 ml) were added to the tubes following reconstitution in assay buffer and followed by addition of the first antibody. After 24 hr incubation, 0.1 ml of ^{125}I-GLP-1 was added to each tube followed by 24 hr incubation again. Normal rabbit plasma (0.1 ml; 1:80) and second antibody (0.1 ml; goat antiserum vs rabbit IgG; 1:20) was added followed by 20 minute incubation at room temperature. Finally 0.1 ml of bovine serum and 1 ml of polyethylene glycol solution was added to each tube before centrifugation at 3,000 rpm for 30 min at 4˚ C. The supernatant was aspirated before the tubes were counted with a gamma counter.

Dietary control. Diet was controlled by instructing the subjects to consume at least 200 g of carbohydrates total 7 d before the OGTT and during the 7 d of physical inactivity. Subjects recorded a 3-d diet record for the 3 d before the first OGTT and were instructed to eat exactly the same diet for the 3 d before the d 7 OGTT therefore being the same days of the week before each test. The diet was analyzed using the United States Department of Agriculture (USDA) Center for Nutrition Policy and Promotion online computer program.

Statistical Analysis. Glucose, insulin, GIP, and GLP-1 areas during the OGTTs were calculated by using a trapezoidal model that summates the area above baseline. To estimate insulin sensitivity, the product of insulin and glucose curves was calculated (IG index). The IG index is the product of the areas under the glucose and insulin curves and is inversely

related to insulin sensitivity (16). The data was analyzed with commercially available software (SPSS, Chicago, IL). Plasma glucose, insulin, GIP, and GLP-1 responses during the OGTTs were analyzed with two-way analysis of variance for repeated-measures designs. Post-hoc tests were performed where appropriate using a multiple comparisons test with a Bonferroni adjustment. Effect sizes (ES) were calculated for areas above baseline where appropriate using Cohen's d equation and effect sizes for the two-way ANOVA OGTT response curves were calculated using a partial eta squared ($^{\eta}p^2$) equation. Incremental glucose, insulin, GIP, and GLP-1 areas and IG index were analyzed with one-way analysis of variance.

Results

Exercise, diet, and body composition. During the 5 days of exercise, subjects exercised for 60 min at an exercise intensity that elicited a VO_2 of 39.8 ± 3.4 ml/kg/min or 72 ± 5% of estimated VO_2peak. Although the subjects were not given a controlled diet, the dietary intake reported by the subjects maintained a minimum amount of total carbohydrate (200 g) intake while subjects also reported that their 3 day diet before the first OGTT was replicated before the last OGTT (Table 3). Seven days of inactivity did not result in any significant changes in body composition (Table 2).

Table 3. The dietary intake before the IPE and Day 7 oral glucose tolerance tests.

	kcal	Carbohydrate, g	Fat, g	Protein, g
Day 1	2,634 ± 522	341 ± 85	84 ± 42	113 ± 52
Day 2	2,654 ± 829	342 ± 72	88 ± 40	105 ± 43
Day 3	2,609 ± 866	326 ± 66	94 ± 33	118 ± 70

Values are means ± SD; n = 9 subjects. IPE, immediately post exercise.

Glucose Tolerance. The plasma glucose response tended to be elevated during the OGTT's performed IPE and day 7 compared with day 1 although not significantly ($P > 0.05$; Fig. 2). The largest difference occurred when comparing IPE and day 1 ($P = 0.09$; ES = 0.33). The incremental area above fasting plasma glucose concentration was calculated (Fig. 3) and the fasting glucose area under the curve was significantly elevated IPE (233 ± 65 mmol/L x min) compared to Day 1 (160 ± 65 mmol/L x min; $P \leq 0.05$; ES = 1.13). No significant change in the glucose area was observed from day 1 to day 7 (186 ± 94 mmol/L x min; ES = 0.59) nor was there a difference between IPE and day 7 (ES = 0.32).

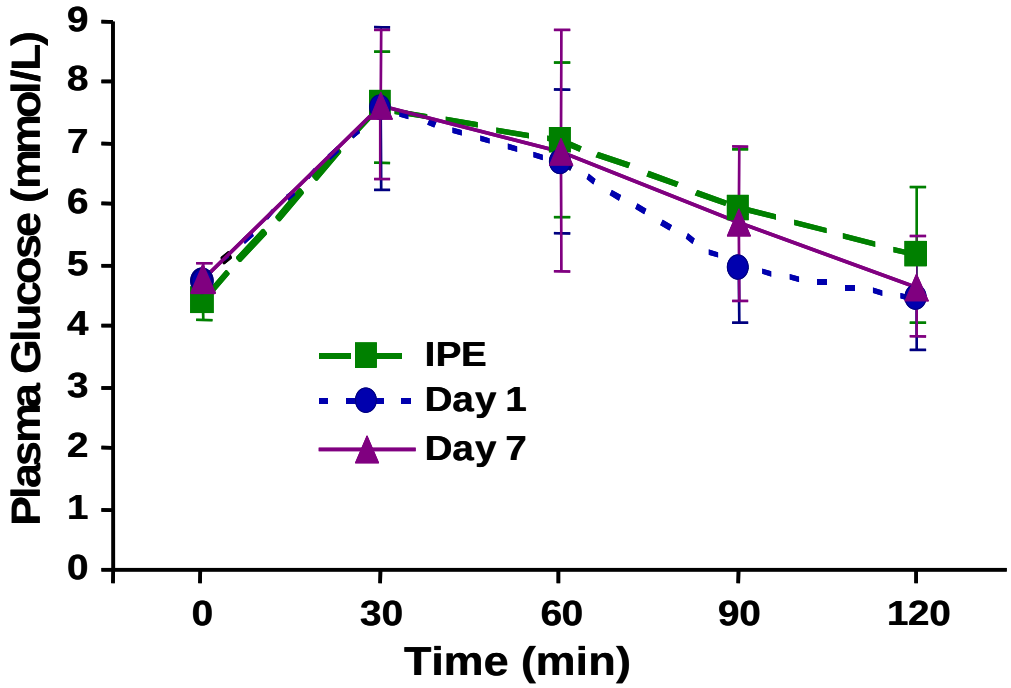

Figure 2. Plasma glucose response during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately post exercise.

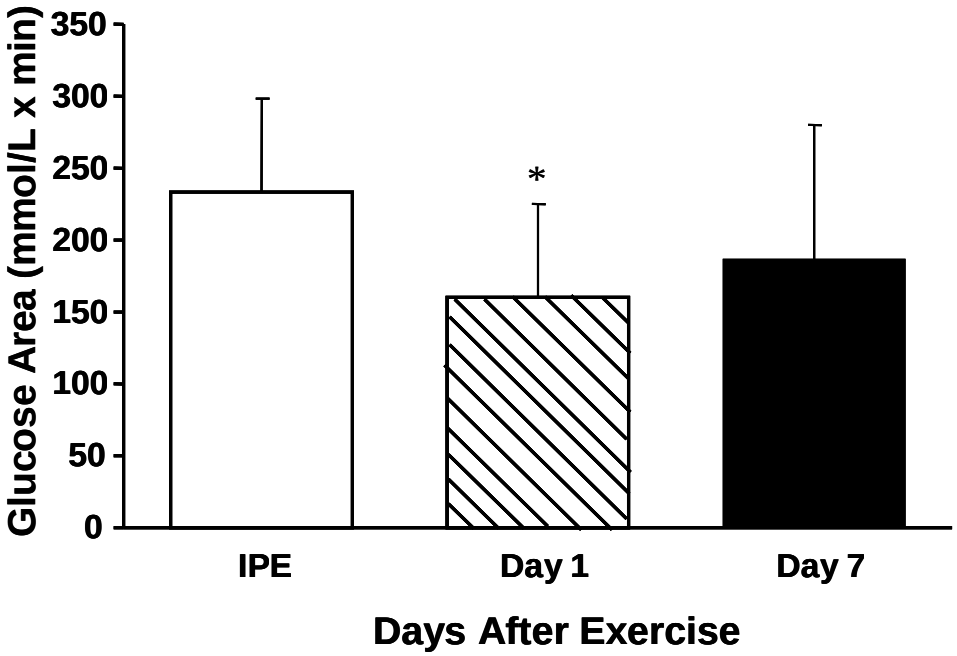

Figure 3. Area above baseline under plasma glucose response curve during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. * Significantly different from IPE, $P \leq 0.05$.

Insulin Response. The insulin response to oral glucose was significantly higher on Day 7 compared to immediately after exercise ($P < 0.05$; Fig. 4). No difference occurred between IPE and Day 1 and although the insulin response was not statistically significantly different between Day 1 and Day 7, there was a tendency for Day 7 to be greater than Day 1 also ($P = 0.08$; ES = 0.53). The incremental insulin area on Day 7 (59,041 ± 25,778 pmol/L x min) was significantly higher ($P < 0.05$) compared with immediately post exercise (35,395 ± 12,445 pmol/L x min; ES = 1.10) and Day 1 (41,568 ± 13,505 pmol/L x min; ES = 0.96). After seven days of inactivity, the insulin area was 41% higher compared with that on Day 1 (Fig. 5). No difference occurred between IPE and Day 1 (ES = 0.24).

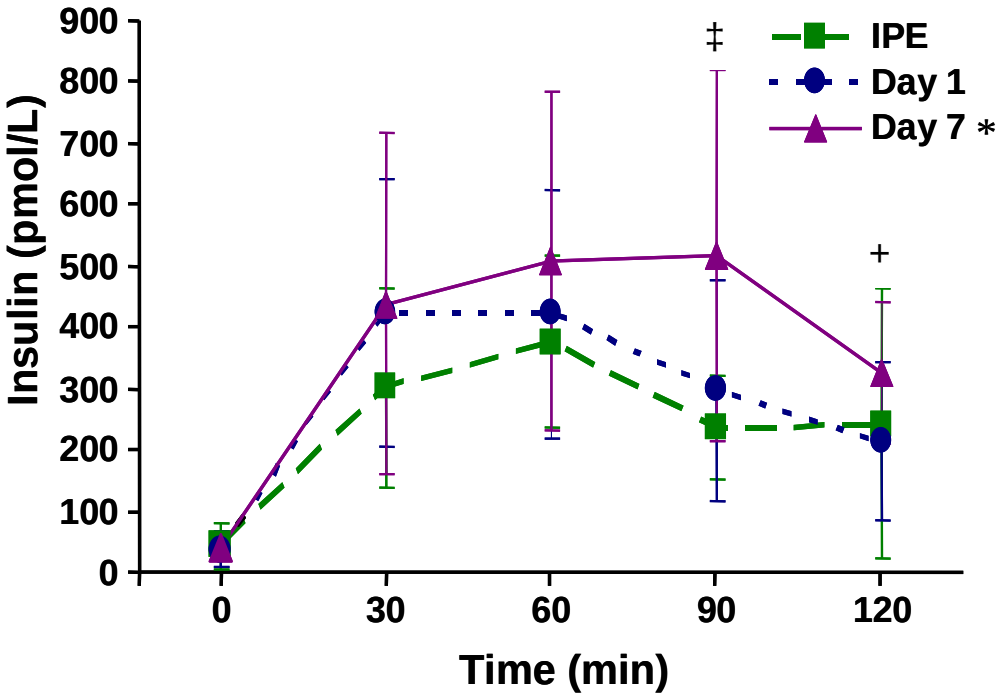

Figure 4. Plasma insulin response during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately post exercise. * Significantly different from IPE, P < 0.05; **+** Day 7 time point significantly higher than Day 1, P < 0.01; ‡ Day 7 time point significantly higher than IPE, P < 0.01.

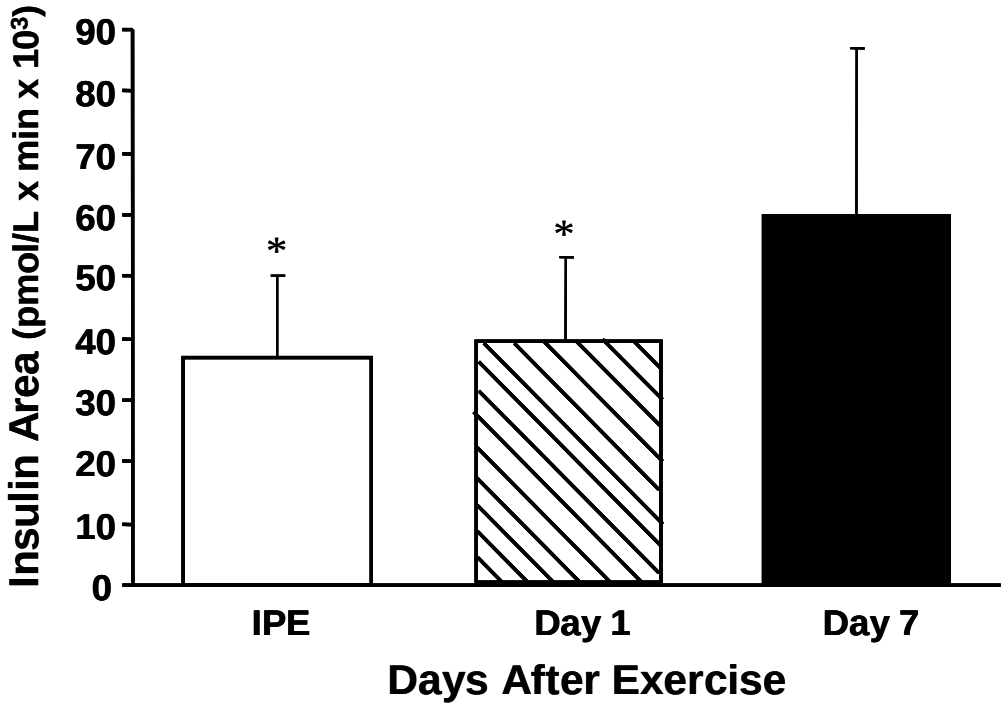

Figure 5. Area above baseline under plasma insulin repsonse curve during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately postexercise. * Significantly different from Day 7, P < 0.05.

Insulin Sensitivity. The product of the incremental glucose and insulin areas (IG index) was calculated (Fig. 6) to assess the effect of exercise on insulin sensitivity. The IG index (mmol/L · min × pmol/L · min × 10^4) was elevated (P = 0.061) on Day 7 (1,023 ± 810) compared to Day 1 (569 ± 328; ES = 0.81) although not significant. The IG index immediately after exercise (745 ± 397) was not significantly different than Day 1 (ES = 0.69) or Day 7 (ES = 0.45).

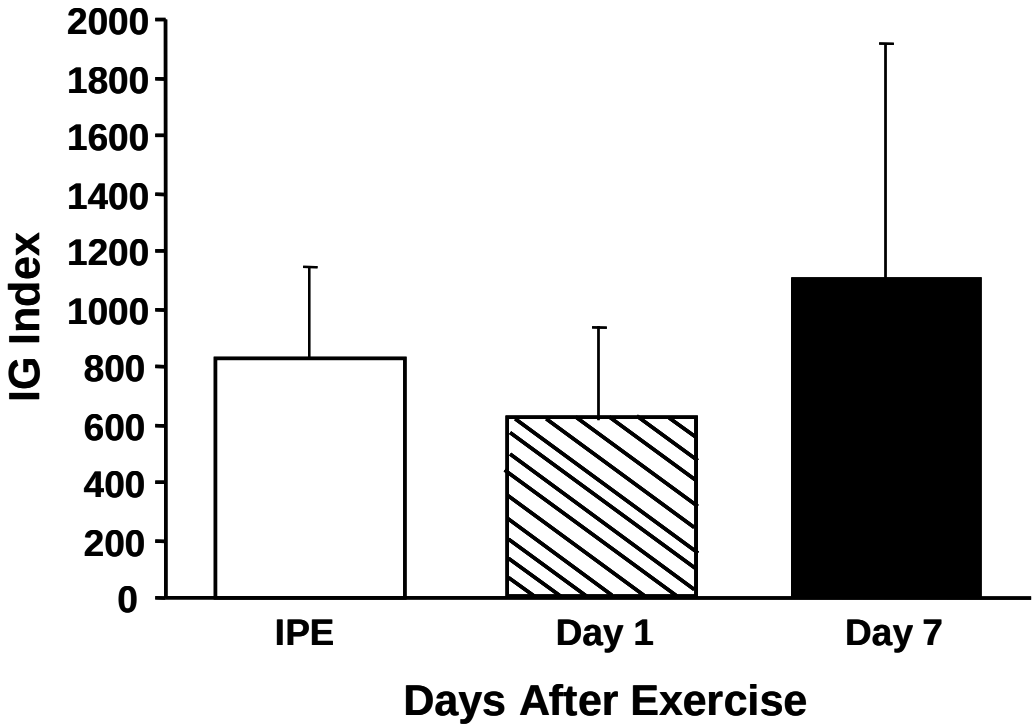

Figure 6. Insulin-glucose (IG) index [product of insulin and glucose areas (pmol/L · min x mmol/L · min x 10^4) during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately postexercise.

Plasma GIP. Although the plasma GIP response to the oral glucose tended to be higher on Day 7 compared to IPE (similar to the insulin response), the plasma GIP response was only significantly different on Day 7 compared with Day 1 (P < 0.05; ES = 0.60; Fig 7). The plasma GIP area above baseline was not different (Fig. 8) between IPE (3311 ± 1141

pmol/L x min), Day 1 (3626 ± 1707 pmol/L x min), or Day 7 (3483 ± 1963 pmol/L x min). Effect sizes were considered small for all 3 comparisons (IPE vs Day 1 ES = 0.22; IPE vs Day 7 ES = 0.11; Day 1 vs Day 7 ES = 0.08).

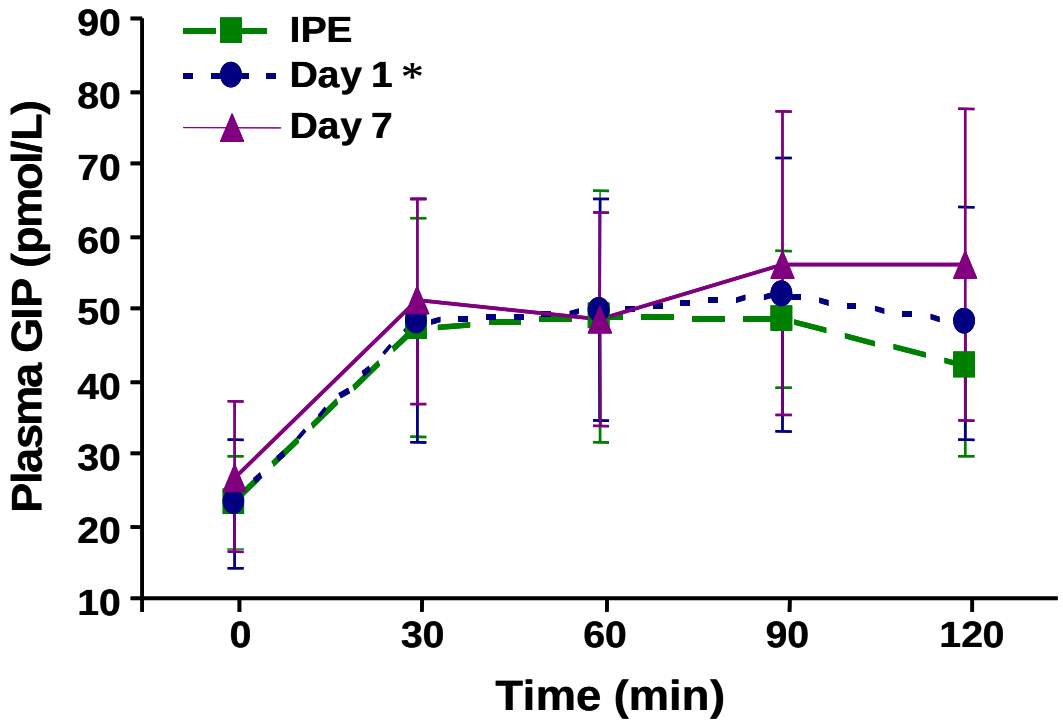

Figure 7. Plasma GIP response during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately post exercise. * Significantly different from Day 7, $P < 0.05$.

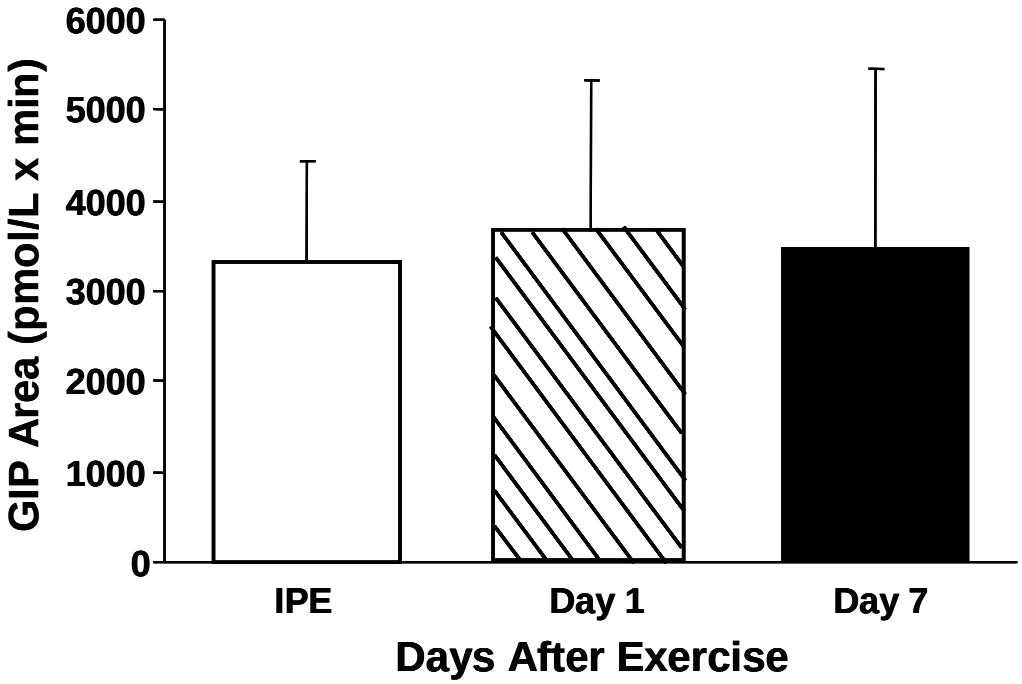

Figure 8. Area above baseline under plasma GIP repsonse curve during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately postexercise.

Plasma GLP-1. There was no main effect of day for the plasma GLP-1 responses during the oral glucose tolerance tests ($P > 0.05$; ES = 0.03; Fig 9). The plasma GLP-1 area above baseline was not different (Fig. 10) between IPE (2287 ± 2034 pmol/L x min), Day 1 (2704 ± 3201 pmol/L x min), or Day 7 (1040 ± 1199 pmol/L x min). Effect sizes were medium for IPE vs Day 7 (ES = 0.74) and Day 1 vs Day 7 (ES = 0.69) and small for comparison between IPE and Day 1(ES = 0.16).

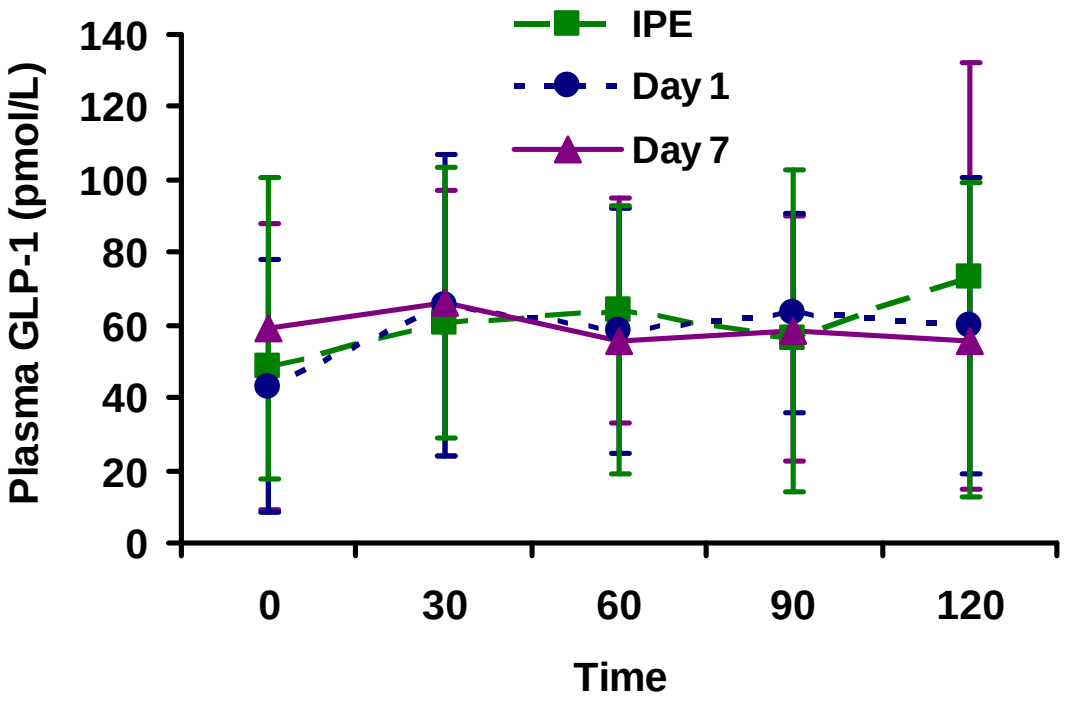

Figure 9. Plasma GLP-1 response during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately post exercise.

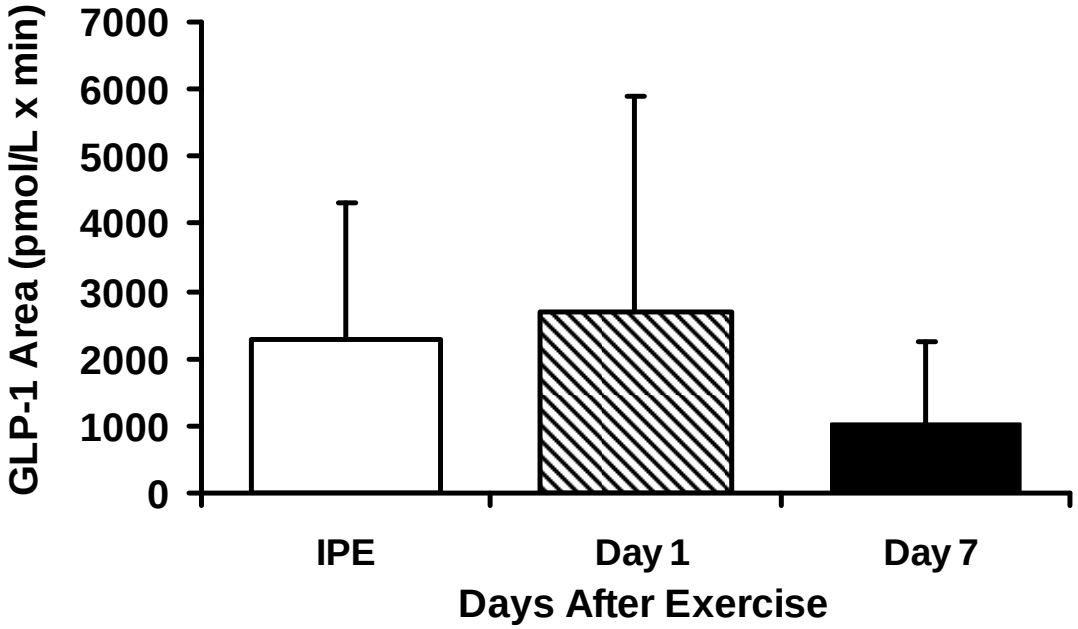

Figure 10. Area above baseline under plasma GLP-1 repsonse curve during 75 g oral glucose tolerance test. Values are means ± SD of 9 subjects. IPE, immediately postexercise.

Gender Responses: No significant differences occurred between males and females within the areas under the curves for any of the variables measured. Within the responses to the OGTT's, the insulin concentrations were significantly higher for females on Day 7 ($P < 0.05$); the GIP responses were significantly higher for males IPE ($P < 0.05$); and the GLP-1 responses were significantly higher for females IPE, Day 1, and Day 7 ($P < 0.05$).

Discussion

The purpose of this study was to examine the possible relationship that GIP and GLP-1 has on insulin release immediately after physical activity and after a period of inactivity. In order to study this relationship, the subjects went through 5 days of exercise followed by 7 days of inactivity aimed to elicit changes in insulin sensitivity and therefore insulin release. The results of the oral glucose tolerance tests indicated that the subjects tended to have decreased insulin sensitivity after 7 days of inactivity as shown by the IG index. Although the IG index on Day 7 compared to Day 1 was not significant, the insulin area under the curve on Day 7 was significantly higher than both IPE and Day 1 supporting a decrease in insulin sensitivity during inactivity characterized by higher insulin release (as well as a large effect size comparing the IG index between Day 1 and Day 7). The glucose response however, did not elicit a significant increase from Day 1 to Day 7 as would be expected. The decreased insulin sensitivity in young, healthy, and endurance-trained subjects during a short period of inactivity agrees with several previous studies. Arciero et al. (2) observed significant increases in the area under the glucose and insulin curves after 7-10 days of inactivity relatively within the same target population, although much larger changes occurred compared to the current study.

One reason glucose and insulin responses to inactivity were not as exaggerated compared to other studies could be explained by dietary intake. The subjects in the current study recorded a dietary intake of 1,000 kcal/day less than the study by Arciero. A higher caloric intake combined with inactivity has been shown to contribute to an increase in insulin resistance in young, healthy subjects (9). Also lower dietary fat intake could support the moderate change in insulin sensitivity. The subjects in our study reported a dietary fat intake of less than 30 percent of total energy intake. Stettler et al. (26) compared a high fat diet (~45 percent of total energy intake) versus a high carbohydrate diet on insulin sensitivity illustrating a decrease in glucose tolerance with the high fat diet. Moderate calories combined with low fat intake could be contributors to the moderate changes in insulin sensitivity during 7 days of inactivity in this population.

Gender differences could also explain some of the variability within the analysis. The insulin response to glucose after 7 days of inactivity was significantly higher in females than males. Previous literature on insulin concentrations in response to glucose is contraindicating with several studies indicating no difference between genders (1, 5). The response of GLP-1 during all the OGTT's were significantly higher for females also which agrees with previous research (1) in response to a standard breakfast meal while other research indicates males having higher GLP-1 responses (5).

The plasma insulin concentrations were higher for Day 7 compared to IPE and Day 1 and were significantly different compared to IPE. This trend coincided with the plasma GIP concentrations during the oral glucose tolerance test as the plasma GIP curve for Day 7 was the highest, while only significantly higher than Day 1. These data demonstrate that as insulin sensitivity decreases over a week of inactivity, GIP may be a factor contributing to

the changes in insulin release according to the main effect of the different days of the tests. Previous work by Hansen et al. (9) demonstrated a large increase in GIP concentrations after a period of inactivity combined with a high-caloric diet and steroid treatment. The incretin effect was also calculated using the difference between β-cell secretory responses for insulin, C-peptide, or insulin secretion rate between stimulation with the oral glucose tolerance test and the isoglycemic intravenous glucose infusion. The relative incretin effect for all three comparisons was significantly worse after the 12 days of treatment. Although this evidence illustrates an effect that exercise may have on GIP to influence changes on insulin release, a closer look at the current data may highlight that the role of GIP is not so significant. When comparing the shapes of the insulin and GIP curves during the OGTT's, it is evident that the rise and fall of concentrations are not temporally related. The insulin curve has a very exaggerated increase from 0 to 30 minutes followed by a similar exaggerated decrease in concentrations starting at 30 to 60 minutes. GIP similarly increases from 0 to 30 minutes however after 30 minutes illustrates a relatively small increase or plateau. The lack of similarity between insulin and GIP release patterns during the OGTT's indicates that in fact, GIP is not the significant contributor to insulin release changes with exercise even with a similar main effect for day.

The idea that GIP may only be a small or insignificant component of the cause of the insulin release after exercise is supported by other studies. Blom (3) previously compared insulin and GIP release during exercise and for 6 hours after that exercise with glucose given every 2 hours. The results compared the exercise group to a non-exercise group and showed no difference in insulin release between the groups. Interestingly, the GIP was significantly higher in the plasma in the non-exercise group. This significant

change in GIP without a corresponding change in plasma insulin concentrations indicates GIP was not the primary factor in causing insulin release. A later study by King et al. (15) examining the insulin secretory capacity in trained versus untrained men also investigated plasma GIP and found that during a hyperglycemic clamp with arginine infusion and fat meal, insulin levels continued to rise throughout the 135 minutes of the modified hyperglycemic clamp. Plasma GIP, however actually decreased during the modified clamp procedure. Although the glucose was infused intravenously (perhaps explaining some of the decreases in GIP different to our study in which glucose was ingested orally), this data still illustrates a further aspect to significantly control insulin release with exercise, such as GLP-1.

There are several possible reasons for the changing GIP concentrations with inactivity. The lower plasma GIP released after exercise could be related the activity of α-adrenergic receptors with exercise. Salera et al. found that after infusion of epinephrine increased the activity of α-2 adrenergic receptors, this increased and significantly reduced the GIP response (22). Somatostatin also inhibits GIP release and is affected by exercise, and somatostatin concentrations are elevated after exercise (20).

It is evident that GLP-1 is also not responsible for the changes in insulin release with exercise. There were no significant differences between the different days for the GLP-1 curves or areas. Although the plasma GLP-1 concentrations tended to increase from baseline to 30 minutes, the changes were very small changes with subsequent plateaus unlike the insulin responses. The minimal increases in GLP-1 concentrations in response to 75-g of oral glucose intake is consistent with previous literature. Steiner et al. found increases in GLP-1 to be a little as 2.7 pmol/L in response to a glucose load in healthy

subjects (25). Others have observed increases of as much as 20 to 40 pmol/L of plasma GLP-1 within the first 30 minutes of OGTT's (7, 17, 29). More importantly, in a similar intervention study by Hansen et al. the area under the curve for GLP-1 was not significantly different before and after a period of physical inactivity (9). Hansen et al did find, however, that the mean GLP-1 concentration at baseline was significantly higher after 12 days of inactivity combined with a high-caloric diet and steroid treatment.

The large range in basal and peak levels of GLP-1 within the literature illustrates the difficulty in measuring GLP-1. In the current study there was also very large variability in concentrations. The rapid rate of GLP-1 degradation in the blood could be one possible reason for these differences. Another possible limitation to our study could be the conservative amount of aprotinin added to the blood samples to prevent incretin degradation. Limited aprotinin along with the absence of specific DPP IV inhibitors could combine to magnify the variation in GLP-1 concentrations. Other limitations within this study include a small sample size and subjects' accountability concerning inactivity and diet replication.

Conclusions

The responses of GIP and GLP-1 in this study indicate these incretins are not responsible for the reduced insulin response after exercise. The high variability with GLP-1 values specifically, sheds light on the need for further research on this peptide in general and in relation to exercise-related insulin changes. More importantly, because the blunted insulin release after exercise has been shown to be more exaggerated with an oral glucose load, other factors from the gut should be studied to determine the cause of this change with

insulin. Since cholecystokinin (CCK) has been linked to changes in insulin sensitivity, it may be one candidate (21). The value of determining the causes of the blunted insulin release after exercise may be significant in understanding the mechanisms behind insulin resistance and Type 2 diabetes and the evidence indicating the connection between the incretins and insulin sensitivity and the reduced incretin effect in diabetic patients further illustrates the importance of studying this issue. Future studies using incretin infusion or tracer techniques could be focused on to determine the mechanism(s) for the exercise effect of insulin sensitivity as well as studies investigating the role of other insulin factors from the gut.

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CHAPTER 3. GENERAL CONCLUSIONS

Insulin resistance is currently a huge problem for a large part of the population in our country, especially for the clinically obese. Becoming glucose intolerant is caused by factors including physical inactivity, high fat body composition, and poor diet to name a few. Pharmacology is becoming an important method of treatment; however it is also very important to utilize exercise as an effective method for both prevention and treatment of insulin resistance. As indicated by numerous studies, exercise can effectively benefit insulin sensitivity through aerobic endurance and resistance activities; however these benefits are diminished if the exercise is not maintained.

The effect of exercise on insulin is associated with a decrease in plasma insulin concentrations with no change or an increase in glucose disposal in response to an oral glucose load. The reasons for this blunted insulin release after exercise however is unknown. This study investigated two of the factors that cause insulin release to examine this mechanism. The incretin GIP coincided with insulin levels in response to exercise and a period of inactivity within the main effect for day only. The specific pattern of GIP release compared to insulin however, indicates that GIP probably does not play a role in the blunted insulin response after exercise. The response of GIP makes sense when examining the factors that influence GIP release, such as somatostatin and the adrenergic receptors, and connecting the idea that they too could be affected by exercise. The GLP-1 data also showed no similarities with insulin data. In general, neither of these hormones indicates a significant contribution to the insulin mechanism with exercise.

There remains a need for future research within this topic as the literature also supports that GIP and GLP-1 are not the only factors to contribute to the blunted insulin mechanism with exercise. The idea that the exercise induced insulin changes are more pronounced with an oral glucose load rather than an intraveneous load still point to factors from the gut. One factor that needs to be investigated further would be CCK. On the other hand, knowing there are some similarities with GIP and the highly inconsistent data on GLP-1 indicates that it may still be important to further study these incretins to help confirm these ideas. Using a tracer or infusion techniques may help to investigate the issue further. Future research on this mechanism is important because it could help to treat diabetic patients with a better understanding of the physiological level of the disease. Along with furthering the literature on this topic, it is also important as an exercise physiologist to stress the take-home message as using exercise as an effective way to maintain a healthy and disease-free lifestyle.

APPENDIX. RAW DATA

Table 4. Subject characteristics.

Subject	Age yr	Height cm	Body Mass kg	BMI	Body Composition %Fat UWW	PreTest Skinfolds	PostTest Skinfolds	VO_2peak L/min	VO_2peak ml/kg/min
801	23	189	73.1	24.1	11.9	9.0	8.7	4.70	55.1
802	27	184	62.2	18.4	22.5	9.0	9.1	3.38	54.4
803	23	185.5	92.7	26.9	16.4	16.7	17.0	4.86	52.4
804	22	173	70.2	23.5	9.5	6.2	6.4	3.82	54.4
805	22	172	62.5	21.1	14.4	10.4	10.3	3.62	57.8
Male Mean	**23.4**	**180.7**	**72.14**	**22.8**	**14.9**	**10.2**	**10.3**	**4.08**	**54.8**
806	25	166.5	66	23.8	21.4	21.5	20.9	3.28	49.6
807	23	169.5	59.2	20.6	21.2	15.3	14.6	3.05	51.3
808	24	162	53.7	20.5	13.6	16.6	17.5	2.99	55.9
809	21	168.9	63.7	22.3	19.4	19.5	20.5	3.32	52.3
810	21	164	54	20.1	24.0	19.6	20.5	2.81	52.1
Female Mean	**22.8**	**166.2**	**59.3**	**21.5**	**19.9**	**18.5**	**18.8**	**3.09**	**52.2**
Total Mean	**23.1**	**173.4**	**65.7**	**22.1**	**17.4**	**14.4**	**14.5**	**3.58**	**53.5**

Table 5. Plasma glucose concentrations during the oral glucose tolerance tests for each subject.

	Time	**801**	**802**	**803**	**804**	**805**	**806**	**807**	**808**	**809**	**810**	***Mean***
IPE	0	4.3	4.2	4.5	4.8	4.5	5.4	4.7	4.2	4.7	4.0	4.4
	30	7.2	7.9	8.4	9.0	8.5	4.7	7.6	6.1	7.2	6.7	7.6
	60	8.1	4.9	8.4	7.9	7.7	2.9	8.2	6.0	6.2	6.3	7.1
	90	6.7	3.9	6.3	6.0	6.9	3.7	6.9	5.1	5.8	5.9	5.9
	120	5.4	3.9	4.0	4.2	4.8	4.3	7.4	5.5	5.6	6.0	5.2
Day 1	0	4.6	4.7	4.9	5.0	5.0	4.6	4.7	4.5	4.6	4.7	4.7
	30	6.9	7.5	9.3	9.4	9.0	6.0	6.1	6.9	7.3	5.9	7.6
	60	7.3	6.2	7.5	8.2	6.6	4.4	7.7	5.1	7.1	4.7	6.7
	90	6.5	4.3	3.6	5.3	5.6	4.0	5.5	4.1	5.4	4.5	5.0
	120	5.3	4.1	2.9	5.0	5.2	4.3	4.5	3.5	5.5	4.4	4.5
Day 7	0	4.8	4.7	5.3	4.7	5.2	4.6	4.7	4.5	4.6	4.9	4.8
	30	6.6	8.0	7.3	9.5	9.4	6.0	7.8	5.8	7.6	6.8	7.6
	60	6.5	7.4	8.0	9.3	8.2	4.5	8.6	4.1	3.4	6.8	6.9
	90	6.8	5.9	4.3	6.1	7.2	5.2	7.0	3.5	4.9	5.6	5.7
	120	5.7	4.0	4.0	3.7	4.8	4.6	5.2	3.8	5.5	5.4	4.7

Units: mmol/L

Table 6. Glucose area above baseline for each subject.

	801	**802**	**803**	**804**	**805**	**806**	**807**	**808**	**809**	**810**	***Mean***
IPE	294	132	281	246	289	0	293	158	168	241	233
Day 1	220	128	200	236	184	37	155	89	194	36	160
Day 7	182	209	132	314	273	54	290	34	99	144	186

Units: mmol/L x min

Table 7. Plasma insulin concentrations during the oral glucose tolerance tests for each subject.

Time		801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	0	7.8	19.3	33.9	74.2	7.4	163	69.8	33.5	121	15.3	42.5
	30	39.4	316	163	488	230	473	216.1	284	549	426	302
	60	205	308	265	378	266	309	369.7	549	641	396	375
	90	107	210	98.5	284	220	305	296.9	315	287	315	237
	120	15.8	129	21.2	118	83.5	296	593.7	349	557	310	242
Day 1	0	9.25	24.6	19.6	58.8	17.8	59.0	51.3	26.2	97.3	33.2	37.6
	30	117	116	426	707	528	675	217	577	544	579	423
	60	338	314	340	929	211	519	450	428	350	433	421
	90	120	147	40.3	596	237	363	454	270	376	428	296
	120	99.9	121	14.2	273	154	451	427	193	317	313	213
Day 7	0	16.8	16.1	20.4	62.1	38.1	256	46.0	61.0	42.8	52.6	39.5
	30	235	285	155	628	217	1043	266	934	453	776	438.8
	60	192	420	364	489	364	266	583	1080	297	798	509.6
	90	283	368	220	451	602	787	1236	561	340	577	515.4
	120	211	284	122	329	300	417	382	495	362	457	326.9

Units: pmol/L

Table 8. Insulin area above baseline for each subject.

	801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	20360	32083	17013	31059	47569	24179	35957	48098	50281	47348	36641
Day 1	24363	22685	27476	54911	35917	58702	46650	48055	40593	56329	39664
Day 7	29878	36982	30025	57713	68124	50448	89371	101034	41951	84887	59996

Units: pmol/L x min x 10^3

Table 9. Insulin-Glucose index for each subject.

	801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	599	424	478	764	1374	0	1065	761	845	1140	828
Day 1	535	290	549	1298	660	219	724	429	785	203	608
Day 7	542	774	396	1809	1860	272	2594	343	414	1222	1106

Units: pmol/L · min x mmol/L · min x 10^4

Table 10. Plasma GIP concentrations during the oral glucose tolerance tests for each subject.

	Time	801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	0	29.0	24.9	26.6	29.3	30.8	-	14.4	19.6	14.4	19.4	23.15
	30	48.2	67.0	56.0	55.0	66.1	-	40.0	27.6	38.9	26.3	47.22
	60	49.2	55.1	57.1	44.8	88.9	-	36.6	43.3	32.4	32.3	48.85
	90	49.3	59.7	43.9	43.0	66.6	-	43.3	52.8	39.6	39.5	48.62
	120	44.9	68.7	32.1	46.0	40.4	-	25.4	48.6	42.8	30.8	42.20
Day 1	0	23.7	32.4	34.2	25.4	31.8	-	7.9	18.3	20.2	14.0	23.09
	30	50.5	54.0	49.7	55.0	69.1	-	18.0	67.3	42.3	27.6	48.15
	60	54.1	56.6	56.6	59.8	59.0	-	34.3	69.2	34.4	23.3	49.70
	90	48.0	60.2	39.3	42.1	89.5	-	42.6	73.6	42.0	30.7	51.99
	120	49.1	62.7	28.9	58.0	62.6	-	39.2	69.8	32.2	28.9	47.92
Day 7	0	29.0	31.3	31.0	36.8	42.9	-	12.0	19.0	23.7	13.9	26.63
	30	39.9	63.1	43.9	63.8	58.7	-	28.0	70.5	51.8	39.2	51.00
	60	52.6	56.1	45.5	60.4	62.2	-	37.0	66.7	23.7	32.2	48.49
	90	46.6	69.8	43.7	51.1	79.7	-	41.6	94.8	48.8	28.8	56.12
	120	53.7	65.3	46.5	48.2	71.4	-	36.0	102.7	48.0	32.9	56.08

Units: pmol/L

Table 11. GIP area above baseline for each subject.

	801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	2636	4846	3120	2335	5423	-	3235	3229	3109	1865	3311
Day 1	3650	3440	1668	3671	5405	-	3516	7025	2466	1794	3626
Day 7	2546	4311	1837	2696	3425	-	3296	8354	2345	2539	3483

Units: pmol/L x min

Table 12. Plasma GLP-1 concentrations during the oral glucose tolerance tests for each subject.

	Time	801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	0	8.1	22.7	14.3	15.5	43.4	-	49.9	78.0	124.0	83.9	48.9
	30	62.1	31.1	15.5	15.5	52.6	-	88.4	63.7	95.7	119.9	60.5
	60	41.3	15.5	69.1	23.2	73.4	-	77.9	76.5	98.6	100.4	64.0
	90	20.2	32.0	15.5	27.7	62.4	-	80.5	104.0	97.5	66.6	56.3
	120	200.0	35.7	15.5	27.0	15.5	-	90.0	100.1	91.7	85.2	73.4
Day 1	0	15.5	15.5	15.5	31.2	31.0	-	15.5	88.5	100.8	73.9	43.1
	30	46.1	22.1	20.4	39.1	40.9	-	110.2	134.0	102.8	75.2	65.6
	60	15.5	15.5	51.0	47.7	30.1	-	89.2	102.9	84.4	89.4	58.4
	90	35.6	47.3	23.9	39.4	71.7	-	102.4	87.7	85.5	76.1	63.3
	120	43.8	15.5	15.5	49.5	15.5	-	81.0	103.3	93.7	119.1	59.7
Day 7	0	15.5	48.0	15.5	41.5	15.4	-	85.9	93.4	91.2	126.6	59.2
	30	42.7	51.1	29.4	17.4	40.9	-	97.5	101.7	120.5	95.5	66.3
	60	31.1	43.1	20.7	15.5	16.7	-	102.0	87.3	92.3	93.3	55.8
	90	61.9	15.5	8.4	16.6	15.5	-	102.3	113.2	91.0	100.4	58.3
	120	17.2	28.8	15.5	15.5	21.7	-	114.4	94.0	95.3	100.4	55.9

Units: pmol/L

Table 13. GLP-1 area above baseline for each subject.

	801	802	803	804	805	806	807	808	809	810	*Mean*
IPE	6537	684	2570	1067	2314	-	4396	1456	0	1564	2287
Day 1	2245	1631	2122	1621	1922	-	11050	2204	32	1509	2704
Day 7	3628	65	560	0	928	-	2241	959	976	0	1040

Units: pmol/L x min

Table 14. Oxygen consumption during 5 days of exercise for each subject.

Subject	Day 1	Day 2	Day 3	Day 4	Day 5
801	45.3	42.5	42.5	cart error	44.0
802	40.0	40.4	40.4	cart error	42.4
803	41.1	38.1	38.1	42.1	43.1
804	41.9	43.6	43.6	42.9	42.5
805	43.3	42.0	42.0	43.9	42.2
806	35.1	34.7	34.7	36.5	34.8
807	39.3	37.6	37.6	35.5	35.9
808	42.7	42.1	42.1	42.2	43.1
809	34.6	37.6	37.6	34.6	38.2
801	37.6	37.0	37.0	41.5	38.9
Mean	**40.1**	**39.6**	**39.6**	**39.9**	**40.8**

Units: ml/kg/min

Table 15. Oxygen consumption during 5 days of exercise for each subject.

	Day 1	Day 2	Day 3	Day 4	Day 5
801	80.9	75.7	76.0	cart error	78.4
802	71.7	72.5	74.3	cart error	76.5
803	76.9	70.7	74.1	79.0	80.9
804	75.4	78.9	81.6	77.4	76.6
805	73.3	70.9	68.0	74.4	71.2
806	68.4	67.7	69.7	71.6	68.0
807	74.9	71.3	61.3	67.1	67.8
808	74.7	73.6	64.1	74.0	75.7
809	63.8	69.9	60.4	63.8	50.7
801	70.2	68.9	71.4	78.1	72.9
Mean	**73.0**	**72.0**	**70.1**	**73.2**	**74.2**

Units: Percent of VO_2peak

Table 16. Three-day diet record for each subject.

	Day 1				Day 2				Day 3			
Subject	**CHO**	**Fat**	**Protein**	**kcal**	**CHO**	**Fat**	**Protein**	**kcal**	**CHO**	**Fat**	**Protein**	**kcal**
801	299	121	168	2889	376	116	136	3019	328	111	127	2746
802	432	78	76	2683	378	62	84	2369	318	96	138	2648
803	210	35	37	1597	347	115	142	3515	339	90	90	2484
804	291	160	198	3483	423	151	187	4104	444	158	290	4581
805	439	97	110	3056	380	83	107	2697	319	109	124	2747
Male	334.2	98	118	2742	381	105	131	3141	350	113	154	3041
806	-	-	-	-	-	-	-	-	-	-	-	-
807	291	62	82	2498	234	33	52	1454	209	35	57	1349
808	285	106	161	2707	241	46	62	1670	279	73	76	1972
809	452	30	88	2379	282	119	95	2568	307	96	77	2363
810	374	69	94	2417	419	62	77	2491	391	81	85	2593
Female	351	67	106	2500	294	65	72	2046	297	71	74	2069
Mean	**341**	**84**	**113**	**2634**	**342**	**88**	**105**	**2654**	**326**	**94**	**118**	**2609**

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