ABSTRACT

An Investigation of Acute Exercise and FGF21

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Fibroblast growth factor 21 (FGF21) is a molecule that freely circulates in the blood and helps to regulate metabolism. Interest in FGF21 stems from its ability to promote weight loss and ameliorate type II diabetes in animal models. Recent findings have shown that blood levels of FGF21 increase after a single bout of aerobic exercise. Neither the mechanism behind this post-exercise increase in FGF21 nor the potential downstream effects of this increase are known. Similarly, little is known about the effect of biological sex or other types of exercise on circulating levels of FGF21. This dissertation will investigate the effects of two different types of exercise – steady state aerobic and sprint interval – on blood levels of FGF21 in healthy males and females. Related to this aim, the dissertation will also investigate the relationship between potential upstream promoters of FGF21 production and circulating levels of FGF21 as well as the potential downstream effects that arise from a post-exercise increase in FGF21. A secondary aim is to investigate the relationship between baseline levels of FGF21 and physiological and lifestyle factors.

An Investigation of Acute Exercise and FGF21

by

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DEDICATION

To God who created FGF21, metabolism, and makes all things possible, thank you for the love you show me every day and for creating the marvelous mystery of human life. To my parents – without the two of you I would literally and, most importantly, figuratively not be the person I am today. To my sisters, thank you for inspiring me.

CHAPTER ONE

Introduction

When the body's natural metabolic regulators cease to function properly, metabolic diseases such as type II diabetes and obesity can result. A novel metabolic regulator that has come to prominence over the past decade and a half is Fibroblast Growth Factor 21 (FGF21). FGF21 was originally discovered for its ability to increase cellular glucose uptake (1). FGF21 is a member of the FGF19 sub-family of FGF molecules. The FGF19 subfamily is atypical in that they have endocrine-like functions beyond the normal functions of growth and development. FGF21 production is most commonly associated with the liver; however, it is also expressed in the skeletal muscle, pancreas, and adipose tissue (2). FGF21 can cross the blood-brain barrier where it may affect the central nervous system (3). FGF21 likely has a broad role affecting multiple metabolic targets.

Specific actions that have been associated with FGF21 are the promotion of glucose homeostasis and weight balance (4,5). Exogenously administered FGF21 has been shown to improve body weight, blood glucose levels, and lipid profiles in obese animals and humans (6). Interestingly, these changes mirror many of the changes that are witnessed with exercise training. An acute bout of aerobic exercise has been shown to increase circulating FGF21 concentrations (2,7–9). As a result, it is hypothesized that FGF21 may be a mediator of exercise adaptations. Despite the promise that FGF21 holds for endogenously benefiting health and as a therapeutic target, the precise mechanisms by

which exercise regulates FGF21 levels and the extent to which the post-exercise increase in FGF21 mediates beneficial effects in the body are not completely understood.

Complicating the study of FGF21 and its regulation is the fact that FGF21 participates in many different cellular pathways and can be induced by a wide array of signaling factors. Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that is strongly associated with the *FGF21* gene ((10)). Since PPARα is associated with the fasting response and can be induced by free fatty acids (11,12) energy deprivation, fasting, and increases in free fatty acids are believed to regulate the transcription of *FGF21*. Interestingly, the *FGF21* gene also has a carbohydrate response element binding protein (ChREBP) site (10,13). This results in *FGF21* being regulated by carbohydrate consumption in addition to fasting signals. Activating transcription factor 4 (ATF4) has been shown to promote increased FGF21 protein, suggesting that an upregulation in FGF21 might also be promoted by cellular stress ((14,15)). Finally, sympathetic nervous system activity also appears to increase FGF21 production (3,16). FGF21 is regulated by a wide variety of stimuli, many of which are also activated in response to exercise.

An acute bout of aerobic exercise is a sufficient stimulus to increase FGF21 concentrations in serum. Utilizing a system of intravascular catheters in the hepatic vein, antecubital vein, and brachial artery, Hansen et al. demonstrated that the post-exercise increase in FGF21 primarily results from hepatic excretion (8). Similar results have been found in animal models, with increased FGF21 mRNA and protein being found in the livers of mice following an acute bout of exercise (2,9). Overall, the evidence indicates that the liver is the primary source of exercise induced increases to FGF21 in circulation.

The time course of this increase in FGF21 following exercise is relatively transient, with researchers showing that FGF21 peaks approximately one hour after exercise and returns to baseline levels within three hours post-exercise (7). Furthermore, the half-life of FGF21 *in vivo* is approximately two hours (17) suggesting that any hepatic excretion of FGF21 will be short lived if the stimulus is not maintained. The mechanisms behind exercise-induced increases in FGF21 are incompletely understood. Hormonal signals associated with fasting have been implicated as signaling factors for the post-exercise increase in FGF21. One study observed that an increase in glucagon closely preceded an increase in serum FGF21 (7). Another study noted that both peak glucagon level and peak glucagon to insulin ratio were positively correlated to peak FGF21 level (8). Thus, the fasting signals induced by exercise appear to be strong factors predicting the post-exercise rise in FGF21; however, it is unknown whether other fasting signals, such as free fatty acids are also associated with the post-exercise rise in FGF21. Other whole-body signaling factors that increase with exercise, such as catecholamines and oxidative stress signals remain an area of future investigation.

While an acute bout of aerobic exercise has been shown to increase circulating FGF21 post-exercise, the effect of other types of exercise, such as sprint interval exercise (SIE), remains understudied. SIE is characterized by brief bouts of supramaximal activity, followed by a longer period of low-intensity activity recovery. SIE has gained popularity for its ability to stimulate similar physiological benefits to longer duration aerobic exercise training. For example, a single bout of SIE was shown to improve insulin sensitivity to a greater extent than a longer bout of moderate-intensity aerobic

exercise (18). The role that FGF21 plays in increased insulin sensitization or in other metabolic adaptations to SIE remains an outstanding question.

Sex differences in FGF21 and the response of FGF21 to exercise have not been fully investigated in humans. Sex specific functions of FGF21 have been identified in mice, with high levels of FGF21 inhibiting female fertility (19). If high levels of FGF21 also inhibit female fertility in humans, then it would make sense for FGF21 to be differentially regulated in females than in males. In humans, resting values of FGF21 do not appear to exhibit sex differences (11); however, sex-specific changes to the post-exercise FGF21 response have not been fully evaluated. Since exercise appears to be a strong stimulant for a rise in serum FGF21, it is possible that the high demands of exercise may lead to differences that are not present during resting conditions. In humans, prior investigation has revealed sex differences in some of the factors that regulate FGF21 expression. It is generally established that circulating catecholamines do not rise to the same degree in women as they do in men (20–23); however, females may be more sensitive to the catecholaminergic effects on metabolism. In addition to stimulating FGF21 production directly, circulating catecholamines might also affect fatty acid mobilization and utilization which in turn could stimulate FGF21 production (21). Evidence indicates that during exercise glycerol appearance in the blood is greater in women than in men and that women generally rely on fatty acid metabolism to a greater degree than men (23–25). The sex-specific nature of upstream signaling factors indicates that a sex-specific FGF21 response to exercise may be present. The differential hormonal and metabolic stimuli that rise from SIE or continuous aerobic exercise may further flush out this difference.

FGF21 has the potential to regulate a wide array of metabolic activity in the body and its production is promoted by a variety of different signaling factors. Many of the signaling factors that appear to regulate FGF21 are also elevated in response to aerobic exercise, indicating that exercise may be an effective stimulus for increasing the concentration of FGF21 in circulation.

Significance

FGF21 is a potent signaling molecule with the potential to serve as a therapeutic target. Establishing a greater understanding of the mechanisms by which endogenous FGF21 is regulated, can help future investigators better target FGF21 based therapeutic interventions.

Sex differences in the FGF21 response to exercise remain understudied. Of the ten studies we identified that examined the FGF21 response to aerobic exercise, seven were exclusively done in males (2,7–9,26–28), two exclusively in females (29,30), and one with a combined sample of males and females (31). Contrary to the results of studies done on fasting men, the two exclusively examining females did not observe any changes to FGF21 with acute exercise (29,30). When comparing the FGF21 exercise response in males and females, Slusher et al. did not notice any statistically significant sex differences in the FGF21 response (31). In the investigation performed by Slusher et al., sex differences were a secondary variable and the study was likely not sufficiently powered to investigate sex differences. Given that many of the signaling factors that regulate FGF21 expression also exhibit sex-specific changes during exercise, it is fair to speculate that FGF21 also exhibits sex-specific regulation. Since FGF21 has been shown to have sex specific actions in animal models (19) a finding that FGF21 is regulated in a

sex-specific manner in humans would further shed light on the unique aspects of metabolism between males and females. Such findings could also help in determining the potential of FGF21 to serve as a therapeutic target.

By investigating two different modalities of exercise – continuous aerobic and sprint interval – in both males and females, this dissertation hopes to perturb the post-exercise FGF21 response by two different routes, which will allow us to better understand the humoral signaling factors that regulate the FGF21 exercise response. The purpose of this dissertation is to quantify the blood increase in FGF21 that occurs with acute aerobic exercise and to better characterize the signaling factors that may regulate the FGF21 response to exercise through experimental intervention.

CHAPTER TWO

Literature Review

FGF21 as a Metabolic Regulator

Interest in FGF21 has been generated as result of its potential pharmacological effects to treat metabolic diseases. One of the first studies to demonstrate the potential pharmacological value of FGF21 in animals was published in 2008 by Coskun et al. In their study, Coskun et al. used Alzet pumps to continuously raise the circulating levels of FGF21 in animals. With artificially increased FGF21 levels, the researchers witnessed improvements in glucose homeostasis starting at the lowest dosage of FGF21 utilized and saw a dose-response relationship between FGF21 and weight loss (5). In subsequent experiments, Coskun et al. investigated full body metabolism when FGF21 levels were artificially elevated compared to a placebo. In these experiments, the researchers noted that oxygen consumption and body temperature were greater in the FGF21 group while the respiratory exchange ratio (RER) was smaller suggesting that both full body metabolism and fat oxidation are increased in response to FGF21 administration (5).

A similar study to that of Coskun et al., was published in 2012 by Ding et al. In their study, Ding et al. used transgenic overexpression to increase FGF21 levels in mice, additionally the researchers investigated the effects of knocking out the FGF21 co-receptor β-klotho both with and without the overexpression of FGF21. Ding et al. noted that plasma glucose levels were only reduced when both β-klotho and FGF21 were presented at the same time (4). Similarly the researchers observed a healthier body

composition (lower percent body fat) in mice where β-klotho was present concomitant with an overexpression of FGF21, while the mice consumed normal chow (4). In order to test the robustness of this effect, the researchers next subjected mice to a high-fat diet. While all groups gained weight after consuming a high-fat diet for ninety days, the β-klotho, FGF21 overexpressing group gained the least amount of weight (4). Even in the presence of a known metabolic detriment (high fat diet), FGF21 in combination with its co-receptor is able to prevent or at the very least slow down the rate at which negative health effects occur.

The prior studies reviewed indicate a robust pharmacological effect of FGF21 in rodents, early clinical trials in humans have indicated that some amount of metabolic dysregulation may also be abrogated in humans with FGF21 administration. An FGF21 analog administered to overweight/obese people with type 2 diabetes was shown to dose-dependently decrease body weight and improve blood lipid profiles (decreased triglycerides, increased high-density lipoprotein (HDL)) (14,32). Although improvements in glucose homeostasis are one of the primary positive benefits of exogenous FGF21 administration in animals, no statistically significant changes to blood glucose occurred in the humans in either study (14,32). While no changes in glucose homeostasis were witnessed in humans taking a FGF21 analog, the positive changes in body weight and blood lipid profiles suggest that FGF21 may still have beneficial effects in humans.

The powerful potential that FGF21 displays as a metabolic regulator has established a need to better understand the molecule's endogenous regulation. Many of the apparent health benefits of FGF21 administration appear to overlap with the health

benefits of exercise. Maximizing FGF21 effectiveness either through pharmacological intervention or through regular exercise may promote beneficial health effects.

Regulation of FGF21 Production

Given the beneficial biological effects of FGF21 described above, there has been an interest in determining how FGF21 is regulated. Several signaling factors have been proposed, many of which are also increased in response to aerobic exercise (33).

Sympathetic Nervous System

The sympathetic nervous system (SNS) has been proposed as a potential regulator of FGF21 due to an overlap in some of their downstream pathways SNS and FGF21 such as the browning of adipose tissue (15,34).

During exercise epinephrine and norepinephrine, hormones of the sympathetic nervous system, are increased. To elucidate the effects of SNS activity on circulating FGF21 levels, Scalzo et al. utilized hypoxia as a tool for inducing SNS activity. When hypoxia was induced in healthy volunteers, circulating FGF21 concentrations were increased (16). When this experiment was repeated with participants being given a drug to block SNS activity, there was a blunting of the FGF21 response (16). The increase in FGF21 as a result of acute hypoxia and blunting of this response with a SNS blocker indicates that SNS may increase the circulating levels of FGF21. In line with these results, one study examining the FGF21 response to an acute bout of aerobic exercise witnessed a positive correlation between FGF21 and epinephrine (29).

Fasting

Fasting is a multifactorial process involving both hormonal and metabolite changes as the body works to preserve energy in times of deprivation. FGF21 has been hypothesized to be one hormonal component to the fasting response. Studies have been published that examine fasting both as a whole process through nutrient deprivation and by specifically examining some of the hormonal and metabolic changes that occur during the fasting response individually.

Both humans and mice experience increases in circulating FGF21 as a result of nutrient deprivation. In response to nutrient deprivation, the FGF21 levels in mice have been shown to increase quickly and dramatically (35). While humans also show a similar pattern, changes may not occur quite as quickly. In humans, it was found that a longer term fast (1 week) was needed in order to increase FGF21 levels in humans (11). Although not strictly fasting, a consistent hypocaloric diet has also been noted to increase blood FGF21 in humans (36). Similarly, in children undergoing a surgery induced weight loss procedure, FGF21 levels were found to be elevated one-month post-surgical procedure (37). However, the researchers did not measure time points earlier than one month, so it is not known if these changes occurred before the one-month time period. Regardless of species, the act of caloric restriction, if not complete fasting, has been shown to regulate FGF21 levels.

Hormonal

One of the primary hormones associated with the fasting response is glucagon. There is strong evidence to suggest that there is a relationship between glucagon and FGF21, first and foremost being that both proteins act on similar tissues (liver, pancreas,

adipose) (38,39). In a robust study, Habegger et al. used a glucagon mimetic and mouse models to demonstrate a strong link between glucagon and FGF21. The researchers first sought to establish a temporal precedence between glucagon and FGF21 and found that acute administration of the glucagon mimetic increased circulating FGF21 in the mice and that this increase returned to baseline as the glucagon levels returned to baseline (38). Next, the researchers examined a longer term administration of glucagon, finding that sixteen days of administration increased FGF21 plasma concentrations and hepatic expression (38). Additionally, the researchers found that this chronic increase in FGF21 protected mice from the detrimental effects of a high fat diet, a protection that was not present in FGF21 knockout animals undergoing similar treatments (38). Although the purpose of the study by Habegger et al. was to demonstrate the link between glucagon and FGF21, this finding further demonstrates the beneficial effects of FGF21 on metabolism. Finally, to demonstrate the translatability of their findings, the researchers injected glucagon into human volunteers and observed an increase in plasma FGF21 levels. Although a molecular mechanism was not established, this study indicates that a rise in blood glucagon levels also creates a rise in blood FGF21 levels.

Free Fatty Acids

In addition to hormonal changes during fasting, there are also metabolic changes that occur, one such change is an increase in circulating free fatty acids (FFAs). FFAs have been demonstrated to promote FGF21 production through peroxisome proliferator-activated receptor alpha (PPARα) mediated transcription. The FFA inducement of FGF21 is consistent with the role of FGF21 as marker and/or mediator of the starvation response.

Given the overlap between FGF21 and FFA concentrations during starvation, Mai et al. sought to determine the role of FFAs in signaling FGF21 production. The ability of FFAs to stimulate FGF21 has been demonstrated in a variety of models. When HepG2 cells were incubated with a mixture of FFAs, an increase in FGF21 mRNA and protein was observed (40). After establishing the role of FFAs in promoting FGF21 from cell culture, the researchers then went on to test the hypothesis that PPARα was the mediating factor by using silencing RNA to decrease protein levels of PPARα. When PPARα was knocked down, there was a blunting in the FGF21 response to incubation with FFAs (40). While results from cell culture studies provide a safe and effective method for establishing biological plausibility, it is important that findings be compared in whole organisms. The researchers next investigated a lipid infusion into people, finding that FGF21 levels increased with a lipid infusion as compared to saline (40). FFAs appear to increase FGF21 production both in cell culture and in intact humans, with these increases likely resulting from PPARα mediated transcription of the FGF21 gene.

The link between FFAs and FGF21 has been further established by researchers, albeit less directly than indicated above. FFAs and FGF21 have been noted to be closely linked when investigating their fluctuations throughout the course of the day in healthy humans (41). It has also been hypothesized that FGF21 response to an oral glucose tolerance test (OGTT), may be more closely related to changes in FFAs than to the glucose itself (42).

Carbohydrates

Although hormonal and metabolic changes that occur during fasting increase FGF21 levels, carbohydrates also appear able to induce increases to FGF21 levels. The

FGF21 gene has a carbohydrate response element binding protein (ChREBP) (10). While there is evidence to suggest that carbohydrates can regulate FGF21 levels, this link appears to be more tenuous than for fasting signals and FGF21.

While molecular studies indicate an important role of FGF21 in maintaining glucose homeostasis, observational studies have not shown a relationship between chronic carbohydrate consumption and FGF21 levels (43). Although typical dietary carbohydrate consumption may not be related to FGF21 levels, a short term high carbohydrate diet (three days, 80% kcal from carbohydrates) has been demonstrated to increase FGF21 levels (44). Further research is needed to determine if these disparate findings are the result of the extremely high carbohydrate intake during the short-term diet or if the FGF21 levels would normalize if the high carbohydrate diet was consumed over a longer duration of time.

Some of the best evidence for carbohydrate consumption increasing FGF21 levels comes from Dushay et al. in 2015. Utilizing a randomized cross over design, Dushay et al. had participants consume either 75g of glucose, 75g of fructose, or a mixture of 37.5g glucose and 37.5g fructose. The researchers reported that FGF21 levels peaked one to two hours after consuming the fructose only beverage, while FGF21 levels increased three to four hours after consuming the glucose beverage; however the glucose induced increase was to a much lesser extent than the fructose induced increase (13). The results of the mixed monosaccharide condition were similar to the fructose only condition (13). Interestingly, in an earlier study, the same research group found that an OGTT did not influence FGF levels (43). The evidence indicates that fructose, specifically, may be a regulator of FGF21 production, but FGF21 may not be as responsive to glucose. The link

between FGF21 and glucose is further called into question by the results of Yu et al., 2011 who observed opposite fluctuations in glucose and FGF21 over the course of a day (41).

Although the FGF21 has a ChREBP domain, the extent to which its transcription can be promoted by all carbohydrates or just specific ones such as fructose remains a question for future investigation. Fructose is handled in a unique way by the body, experiencing first pass in the liver and does not raise insulin levels (unlike glucose) which may help explain some of the paradox whereby both fasting signals and carbohydrate consumption both increase FGF21 levels.

Stress Response

Various models have been utilized to show that cellular stress responses, particularly under pathological conditions, are able to increase circulating FGF21. While FGF21 is generally viewed as a hepatokine, these pathological conditions may lead to a contribution to circulating levels from the skeletal muscle.

Mitochondrial myopathy, an extreme dysfunction of the mitochondria, is considered to be one of the primary circumstances that can lead to increased skeletal muscle expression and secretion of FGF21 (45–47). One of the hallmark studies demonstrating that mitochondrial stress can induce FGF21 production in and secretion from the skeletal muscle was performed by Keipert et al. Keipert et al. overexpressed UCP1 in mice to increase mitochondrial uncoupling. Their results showed that isolated muscle cells from the UCP1 transgenes secreted FGF21 into the culture media, a feature that was not present in wild type mice (15). To further confirm these findings the researchers used myoblast cell culture to show that chemical uncouplers and

mitochondrial stress directly lead to an increase in FGF21 expression and secretion into the cell culture media (15). Mitochondrial damage and/or uncoupling appears to be one circumstance in which skeletal muscle can secrete FGF21.

While mitochondrial stress is one of the primary circumstances in which FGF21 secretion can be increased from skeletal muscle, other cellular stresses have also been shown to increase skeletal muscle production of FGF21. Other cell stress response pathways such as the eIF2α/ATF4 integrated cell stress response (15,45) and endoplasmic reticulum (ER) stress (48) have also been implicated in increased FGF21 production from skeletal muscle. The extent to which cellular stress can regulate FGF21 outside of pathological conditions is still a topic of further investigation. However, from studies investigating pathological conditions and utilizing genetic manipulations of the cellular stress response, it can be concluded that it is possible for cellular stress to play a role in whole body FGF21 levels.

Sex Differences

FGF21

One striking phenomenon of human biology is the existence of bimodal distributions for many body characteristics that appear to segregate based on biological sex. These differences can have important implications for disease prevalence treatment. Therefore, an important question of interest is the extent to which FGF21, a metabolic regulator, is differentially regulated between men and women.

The extent to which sex differences in FGF21 exist is still a topic of debate in the literature. One study in mice noted that FGF21 had a negative impact on female fertility,

through actions in the hypothalamus (19). The researchers verified their initial findings by demonstrating that this effect was negated when β-klotho, the FGF21 co-receptor, was knocked out in the hypothalamus (19). Since FGF21 promoted infertility in wild type mice, and mice that were unresponsive to FGF21 (due to β-klotho knockout in the hypothalamus) did not experience this decrease in fertility, there is evidence that FGF21 can promote infertility. Findings by Singhal et al., contradicted this finding to a certain degree. Singhal et al. observed that a ketogenic diet induced an increase in FGF21 with no disruption of fertility (49). Singhal et al. did note that a transgenic overexpression of FGF21 disrupted fertility; however, normal fertility was restored when the mice were placed on a high fat diet (49). The fact that elevated FGF21 in a ketogenic diet did not cause infertility and that infertility induced by transgenic overexpression of FGF21 could be rescued through consuming a high fat diet suggests that FGF21 may play a role in promoting infertility in mice but may not act alone. Regardless of whether or not FGF21 is capable of inducing infertility in mice on its own, the results from animal studies imply that sex-specific functions for FGF21 may exist.

If FGF21 does exhibit sex-specific functions, then it would seem probable that FGF21 levels may be different and differently regulated based on sex. The largest human study comparing FGF21 concentrations between males and females concluded that sex differences in FGF21 were not present at rest (11). While sex differences in FGF21 may not be present at rest, exercise places a large metabolic demand on the body which may lead to differences being observed that were not present at rest. To date, only one study has compared sex-differences in the post-exercise response of FGF21, they concluded that no difference exists; however, this study was likely underpowered to detect such

differences (31). Therefore, one important question in the literature is to what extent the post-exercise rise in FGF21 exhibits sex differences.

One notable study that directly investigated the role of estrogens on FGF21 in humans was conducted by Persson et al. In their study, Persson et al. induced a hyper-release of endogenous estrogens in women scheduled for in vitro fertilization, but found no changes to serum levels of FGF21 in response to this estrogen treatment (50). These results indicate that estrogens may not affect serum FGF21 levels; however, they do not rule out the effects of other sex hormones.

If sex differences in FGF21 are identified they may help lead to a more personalized approach for FGF21 treatments should they become commercialized.

Regulators of FGF21

While the extent to which sex differences in FGF21 exist is still up for debate, some of the regulators of FGF21 show sex-specific response to exercise. A greater use of fat metabolism and lower levels of circulating catecholamines appear to be present in females relative to males.

When comparing exercise metabolism between males and females, one of the most common findings is that females have a greater utilization of fatty acids. This has been demonstrated to be present in fully body metabolism with females having a lower RER value during submaximal exercise (23,24). While findings are less robust, results also demonstrate that females tend to have greater levels of circulating metabolites associated with fatty acid oxidation and lesser levels of metabolites associated with glucose metabolism in comparison to males (21,24). FFAs are one regulator of FGF21

production, thus a differential reliance on fat metabolism could lead to a differential production.

The catecholamines, epinephrine and norepinephrine, markers of SNS activity have been demonstrated to respond differentially between males and females during exercise. It has generally been established that epinephrine and norepinephrine rise to a greater extent in males than in females (21,22). Since SNS activity is believed to regulate FGF21, differences in circulating epinephrine and norepinephrine may promote differences in FGF21.

FGF21 and Acute Steady State Exercise

Acute aerobic exercise has been demonstrated to increase circulating levels of FGF21. Studies examining the effect of aerobic exercise on FGF21 are discussed in chronological order of publication date.

The first study to demonstrate an increase in circulating levels of FGF21 post-exercise was performed by Kondo et al. in 2011 as part of a larger study investigating left ventricle remodeling. In their study, nine healthy men exercised at 85% of their maximum heart rate for 30 minutes. FGF21 levels rose slightly during exercise and continued to rise post-exercise with the peak coming 60 minutes post-exercise and FGF21 levels remaining elevated for at least two hours (28). The overall conclusion to this study is that an acute bout of exercise can increase FGF21post-exercise in a sample of healthy males.

In 2012, Cuevas-Ramos et al. investigated the effects of exercise training in a sample of sixty healthy women but isolated the first exercise bout (treadmill test following the Bruce protocol) to investigate the acute effects of exercise on FGF21.

Contrary to the findings of Kondo et al., Cuevas-Ramos et al. found a non-statistically significant decrease in FGF21 one hour post-exercise (29). Whether the lack of increase in FGF21 post-exercise was the result of a short duration of time spent on the treadmill test, a biological difference in females, or some other factor remains unknown.

The first study to investigate the potential sources of FGF21 during exercise was conducted by Kim et al. In this study, Kim et al. had both mice and humans complete an acute bout of exercise. After acute exercise, their mouse sample had a significantly elevated serum concentration of FGF21 (2). The researchers also looked at FGF21 expression in skeletal muscles, white adipose tissue, and liver finding that FGF21 expression was only elevated in the liver (2). From this animal work, it was concluded that acute exercise can increase circulating FGF21 levels and that this increase is the result of increased liver expression of *FGF21*. To test the translatability of their animal findings, Kim et al. had human male participants exercise for thirty minutes at 50% and 80% of their VO_{2max}. The researchers saw an elevated blood concentration of FGF21 one hour post-exercise with both exercise intensities; however, the post-exercise increase was greater following the 80% bout (2). Thus, the post-exercise increase in FGF21 may be related to both intensity and energy expenditure of the exercise bout.

Obese individuals have greater levels of circulating FGF21 at rest when compared to their lean counterparts (31). Therefore, Slusher et al. sought to investigate if obese and normal weight people experienced a differential response to the post-exercise increase in FGF21. Slusher et al. combined men and women such that they had one group of twelve obese participants (five males, seven females) and one group of twelve normal weight participants (six males and six females). After thirty minutes of exercise at 75% of

VO_{2max}, the researchers found an elevated concentration of FGF21 in both groups at one hour post-exercise, with levels in the normal weight group remaining elevated two hours post-exercise, but levels in the obese group returning to baseline levels two hours post-exercise (31). When the area under the curve was compared between the two groups, the normal weight group had a significantly elevated FGF21 response when compared to the obese group (31). The researchers also investigated the relationship between total relative energy expenditure and FGF21 area under the curve, finding a positive correlation between the two (31) which indicates that the post-exercise rise in FGF21 may be dependent on energy expenditure. It should be noted that Slusher et al., did not observe a difference in baseline FGF21 levels between the groups as one might expect. This study did include both men and women, but sex differences in the FGF21 response to exercise were not identified. It is possible that the sample size was too small for such differences to be observed.

Similar to obese individuals having greater levels of FGF21 than their lean counterparts, older individuals tend to have higher FGF21 levels than younger individuals. Therefore, Taniguchi et al. sought to determine if older and younger men had different FGF21 responses to acute aerobic exercise. Taniguchi et al. fed the participants in their sample an energy gel before having them perform thirty minutes of cycling at 70% of VO_{2max}. The researchers saw a non-statistically significant increase in FGF21 one hour post-exercise, and overall noted that the younger individuals had lower FGF21 levels than the older individuals throughout the observed time course (26). The lack of significant increase in FGF21 following the exercise bout could be due to the pre-feeding with an energy gel which might mitigate some of the fasting signals produced by

exercise that might help regulate FGF21 production. It appears that age may affect one's circulating FGF21 levels, but this does not seem to impact the exercise response to FGF21 as both groups had similar relative changes in FGF21.

One of the most robust exercise and FGF21 studies performed in humans was published in 2015 by Hansen et al. Hansen et al. had ten healthy males exercise at 60% of their VO_{2max} for two hours, while drawing blood samples from the hepatic vein and brachial artery. Through this study, the researchers discovered that there was a net hepatic excretion of FGF21 during exercise (8). The post-exercise FGF21 peak occurred at thirty minutes post-exercise (8). The researchers also investigated the relationship between glucagon, insulin, and FGF21 finding a positive correlation between glucagon and the glucagon to insulin ratio and FGF21 (8). These results indicate that in humans, the liver is the primary organ responsible for the exercise induced increase in circulating FGF21 and that fasting signals help promote a rise in FGF21 with exercise.

To look at the role of exercise on tissue level production of FGF21 and circulating FGF21 levels, Tanimura et al. conducted experiments using both animals and humans. When mice were exercised, the researchers observed an increase in serum FGF21 levels (9). Additionally, the researchers noted that FGF21 expression and protein content was increased in both the skeletal muscles and livers of mice post-exercise (9). These results contrast with previous findings that FGF21 expression was not elevated in mouse skeletal muscle post-exercise (2). Since Tanimura et al. did witness increased expression and protein content in the livers, the fact that FGF21 was elevated in skeletal muscles post-exercise does not preclude a conclusion that the liver is the primary exporter of FGF21 following an acute bout of exercise. In the human portion of their study, Tanimura et al.

had nineteen apparently healthy but inactive men exercise for sixty minutes at 75% of their VO_{2max}. The researchers found FGF21 levels to be elevated immediately post-exercise, but did not investigate any time points during the duration of recovery (9). Overall, this study provides further evidence that FGF21 increases in response to acute exercise in humans and that increased expression and production of FGF21 occurs in the liver as a result of exercise.

With a desire to better understand the time course of the FGF21 response to exercise and to compare different types of exercise, Morville et al. had participants undergo an acute bout of aerobic exercise and an acute bout of resistance exercise. Their sample utilized ten recreationally active males and their aerobic exercise consisted of sixty minutes at 70% of VO_{2peak}. In their time course analysis, Morville et al. found that peak FGF21 levels occurred sixty minutes post-aerobic exercise with no change in FGF21 levels witnessed with resistance exercise (7). The researchers also observed that there was a peak in circulating glucagon concentration that immediately preceded the peak in FGF21 resulting from aerobic exercise (7). There was no significant increase in glucagon as a result of resistance exercise leading the researchers to conclude that glucagon is a major signaling factor for the whole body rise in FGF21 that occurs in response to acute exercise (7).

In an examination of the effect of exercise intensity on circulating FGF21 concentrations, Willis et al. utilized a sample of ten healthy young men (27). Participants completed three conditions in a randomized, cross-over design: resting control, moderate-intensity exercise at 55% of VO_{2peak}, and high-intensity exercise at 75% of VO_{2peak}, exercise condition were matched for a caloric expenditure of 600 kcals (27). The authors

found higher blood concentrations of FGF21 after exercise than at rest and that FGF21 levels were greater after the high-intensity exercise session (27). This adds to the work done by Kim et al. (2) by indicating that the post-exercise rise in circulating FGF21 might be regulated by intensity even when conditions are matched for energy expenditure.

One of the more unique studies was performed by Hutchinson et al. who compared the effect of exercise on FGF21 levels between pregnant and non-pregnant women (30). In their study, Hutchinson et al. had thirteen pregnant women with a pre-pregnancy BMI of 18.5-29.9 and seventeen non-pregnant women with a current BMI of 18.5-29.9 exercise for thirty minutes at an intensity of 40-60% heart rate reserve (30). The authors found that FGF21 was increased post-exercise in the pregnant group, but not in the non-pregnant group (30). This study is not without criticism as they had a fairly wide range for exercise intensity, stage of the menstrual cycle or the use of hormonal contraceptives in the non-pregnant group is not mentioned, and blood was only taken immediately post-exercise, while FGF21 levels appear to peak one-hour post-exercise. In spite of these limitations, this article suggests that there might be some differential effects of FGF21 regulation in females.

Sprint Interval Exercise

Sprint interval exercise (SIE), defined as brief periods of supramaximal activities followed by very low intensity periods has gained popularity for its potential to create similar physiological benefits to endurance exercise with an overall lesser amount of work. One study indicated that SIE may improve insulin sensitivity to a greater extent than endurance exercise, despite a reduced amount of caloric expenditure in the sprint interval exercise condition (18). The high-intensity, low overall energy expenditure

nature of SIE may lead to a differential hormonal profile in circulation compared to aerobic exercise. The effect of SIE on circulating FGF21 levels remains understudied. The lone study identified in this area found that FGF21 levels did not increase following a single bout of SIE (51); however, the authors did not compare the SIE to an aerobic exercise condition. By comparing the different signaling pathways of sprint interval and aerobic exercise on FGF21 production, we may net greater insights into how FGF21 is regulated as a result of exercise.

CHAPTER THREE

Methods

Research Questions

1. To what extent is sprint interval exercise able to stimulate increased serum concentrations of FGF21 relative to endurance exercise?

   H_0: The post-exercise increase in serum FGF21 concentration will not be different between the two exercise conditions.

   H_A: Serum concentrations of FGF21 will be greater following endurance exercise.

   Rationale: If glucagon is the primary signaling factor for FGF21 in response to exercise (7), then the exercise condition that stimulates the greatest fasting response would be expected have the greatest FGF21 response.

2. To what extent are sex differences present in the FGF21 response to exercise?

   H_0: The post-exercise FGF21 response will not be different based on sex.

   H_A: Males will exhibit a greater post-exercise FGF21 response than females.

   Rationale: Anecdotally, prior research indicates males might have a greater FGF21 response to exercise than females. Additionally, if FGF21 does in fact play a role in regulating fertility in females (19) then one would expect females to exhibit a tighter control over FGF21 than males.

3. Is there a relationship between post-exercise FGF21 levels and post-exercise metabolism as measured through indirect calorimetry?

H_0: There will be no relationship between post-exercise FGF21 and post-exercise metabolism.

H_A: Greater levels of circulating FGF21 post-exercise will be positively related to increased oxygen consumption and lower RER.

Rationale: Exogenous FGF21 administration has been shown to increase oxygen consumption and lower RER in rodents (5). If FGF21 promotes weight loss and fat loss, then it is expected to promote increased energy expenditure and fat oxidation.

4. To what extent can the variance in post-exercise FGF21 be explained by the variance in circulating signaling factors that change as a result of exercise and are believed to influence FGF21 production?

   H_0: None of the variance in the post-exercise FGF21 response will be explained by circulating signaling factors.

   H_A: Some of the variance in the post-exercise FGF21 response will be explained by circulating signaling factors.

   Rationale: Circulating signaling factors such as glucagon, catecholamines, carbohydrates, and FFAs increase with exercise and are believed to regulate FGF21 production.

5. To what extent are circulating FGF21 levels related to lifestyle and physiological factors involved in the FGF21 pathway?

   H_0: FGF21 will not be related to any lifestyle or physiological factor.

   H_A: There will be a strong relationship between circulating FGF21 and lifestyle and physiological factors.

Research Design

This study will be a randomized cross-over design consisting of three study visits.

During the first study visit, participants will provide informed consent and undergo pre-exercise screening (a copy of the informed consent document is presented as an appendix). After providing informed consent and passing the pre-exercise screening, we will measure participants' height, weight, and waist circumference. Additionally, body composition will be measured by using DEXA. Following the DEXA scan, participants will undergo a peak oxygen consumption test (VO_{2peak}). For the VO_{2peak} test participants will wear comfortable exercise attire. We will fit participants with a Polar H7 Bluetooth heart rate monitor and verify that the monitor syncs with our FITIV Pulse® heart rate tracking app. Participants will also be fitted with a face mask and connected by air tube to the respiratory gas analysis instrumentation (ParvoMedics True One™) to measure ventilation and respiratory gasses (oxygen consumed and carbon dioxide produced) for a brief rest interval prior to exercise and throughout the exercise bout. The VO_{2peak} test will begin at a workload of 50W and will be increased by 50W every two minutes until volitional fatigue. The participant will be asked to maintain a cadence of sixty revolutions per minute (RPM) throughout the test. If the cadence falls below 60 RPM for more than thirty seconds, the test will be terminated.

Following the VO_{2peak} test, the two experimental conditions will be conducted using a randomized, cross-over design. Participants will be asked to refrain from vigorous activity the day before an experimental condition and to maintain a similar diet the day before experimental conditions. Female participants will be asked to complete the experimental conditions during the early to mid-follicular phase of their menstrual

cycle (days 3 through 10). Both conditions will be identical, with the exception of the exercise bout. Following an overnight fast, the participant will arrive to the laboratory during the morning of the experimental condition. We will record the participant's body weight. After recording the body weight, we will fit the participant with a Polar H7 Bluetooth heart rate monitor and verify that it syncs with our respiratory analysis instrumentation. We will then fit the participant with a face mask connected by air tube to the respiratory analysis instrumentation. The participant will then lie in a supine position for 15 minutes while we record resting heart rate and respiratory gas data. During this time, a pre-exercise blood sample will be collected (~30mL, for this blood draw and all subsequent blood draws). After the fifteen minutes of quiet rest have passed, the exercise session will begin (described below). Following the exercise session, the participant will return to the supine resting position and a post-exercise blood sample will immediately be collected. After the participant has been in the supine position for one hour, the face mask will be withdrawn. One-hour post-exercise, a final blood sample will also be collected.

One exercise session will consist of continuous moderate intensity exercise. This exercise session will begin with a five-minute warm up at 40% of VO_{2peak} followed by thirty minutes of cycling at 70% of VO_{2peak}. Participants will be asked to maintain a cycling cadence of 60 RPMs throughout the exercise session.

The other exercise session will consist of a sprint interval exercise session. This exercise session will begin with a five-minute warm up at 40% of VO_{2peak}. Following the warm-up, the workout will consist of six bouts of thirty second "all out" sprints followed by four and half minutes of cycling at a recovery pace. The sprints will be performed

against a resistance of 7.5% of the participant's body weight and recovery intervals will be unloaded.

Participant Selection and Recruitment

Inclusion Criteria

Volunteers will be recruited by word of mouth and an institutionally approved flyer. We will recruit a sample of thirty apparently healthy adults aged 18 to 45. Half of the participants will be female and half will be male. All participants will be physically active defined as participating in at least 30 minutes of physical activity three times per week for a period of at least three months. Additionally, all participants will be classified as normal weight, defined as having a body mass index (BMI) of 18.5 to 24.9 kg/m^2. Recruitment will continue until thirty eligible participants have completed this study.

Prospective participants will be invited to the Baylor Laboratories for Exercise Science and Technology located in Marrs McLean Gymnasium room 127, to be informed of all aspects of the study. After acknowledging that they meet all inclusion criteria, none of the exclusion criteria, agreeing to participate, and signing an institutionally-approved consent form, volunteers will complete the pre-exercise screening protocol outlined in American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, 10^{th} Edition (ACSM's GETP10) (52). As part of the screening we will measure height, weight, waist circumference, and body composition via Dual-Energy X-Ray Absorptiometry (DEXA). Following enrollment into the study, the order of experimental conditions will be randomly assigned.

Volunteers will be eligible to participate in this study if they: 1) are between 18 and 45 years of age; 2) participate in at least 30 minutes of moderate intensity physical activity three days/week over the last three months; 3) are of normal weight (BMI 18.5 to 24.9), 4) are free of cardiovascular, metabolic or renal disease AND signs or symptoms suggestive of cardiovascular, metabolic, or renal disease; 5) free from orthopedic conditions that could be exacerbated by exercise or would preclude completion of the study. Female participants must be eumenorrheic and in the follicular phase of their menstrual cycle during experimental conditions.

Exclusion Criteria

Volunteers that do not meet all of the conditions listed above will be excluded from participation. In addition, female volunteers who are pregnant, suspect that they may be pregnant, or take hormonal contraceptives will not be allowed to participate in this study.

CHAPTER FOUR

Manuscript One: The Acute Effects of Sprint Interval and Steady State Exercise on FGF21 Levels in Healthy Males and Females

Introduction

Fibroblast growth factor 21 (FGF21) is a circulating signaling factor that has broad actions in metabolism. Originally identified for its ability to promote glucose uptake (1) FGF21 has also been shown to promote weight loss and improve dyslipidemia when given as a pharmacological agent (14). The observation that exogenously administered FGF21 can reverse these common scourges of health has created a significant amount of research interest in FGF21 over the past decade.

Humans have a bimodal distribution when it comes to metabolic characteristics, based on biological sex. As a metabolic regulator FGF21 may play a role in these sex differences. Previous work done in mice suggests that FGF21 might disrupt female fertility (19). In humans sex based differences in FGF21 have been observed in response to hyperinsulinemia (53) and fructose overfeeding (54). Resting FGF21 concentrations are similar between females and males (11); however, exercise presents a metabolic demand that may expose sex differences not present at rest.

One major research finding is that serum FGF21 levels are increased following an acute bout of aerobic exercise (2,7–9,27) and that this rise is largely a result of hepatic excretion (8). Time course studies have shown that this increase peaks at one hour post-exercise (7) with circulating FGF21 having a half-life of around two hours (17). A variety of exercise intensities have been demonstrated to bring about this post-exercise

increase in FGF21, but the changes appear to be more robust with a greater exercise intensity (2,27). While aerobic exercise has consistently been shown to increase serum FGF21 levels post-exercise, the effect of other types of exercise such as sprint interval exercise has not been fully evaluated. Sprint interval exercise consists of short bouts of anaerobic sprints followed by low-intensity recovery periods and can have similar metabolic benefits to aerobic exercise (18,55).

The regulation of FGF21 is a complex process, with many paradoxical signaling pathways (10). Many of the signaling factors that stimulate FGF21 production are also upregulated during exercise. Prior work has shown that a spike in blood glucagon levels precedes a rise in FGF21 levels following exercise (7) and that post-exercise FGF21 levels are positively correlated with the peak glucagon:insulin ratio (8). The effect of other upstream signaling pathways such as epinephrine and glucose is not as established. Changes in epinephrine and glucose flux during exercise have been demonstrated to exhibit sex based differences (20,24) which might contribute to sex based differences in exercise induced FGF21 concentrations.

The purpose of this study was to investigate the effect of aerobic and sprint interval exercise on serum levels of FGF21 humans. Additionally, we sought to examine if these differences are mediated by sex, determine if other circulating molecules related to the FGF21 pathway are related to the FGF21 response, and investigate potential metabolic benefits to a post-exercise increase in FGF21.

Methods

Participants

Thirty people (fifteen females and fifteen males) participated in this study. Inclusion criteria included: age between 18 and 45 years old, regular exercisers achieving at least thirty minutes of exercise three days a week, normal weight determined by a BMI between 18.5 and 24.9, and free from any cardiovascular or metabolic diseases. Female participants also had to be eumenorrheic and not using any hormonal contraceptives.

All procedures complied with the Declaration of Helsinki and were approved by the Baylor Institutional Review Board. Before starting any study procedure, all participants were informed of the study procedures and provided written informed consent.

Experimental Design

We utilized a randomized cross-over design. Participants made three separate visits to the laboratory. During visit 1 we measured body composition using dual energy x-ray absorptiometry (DEXA) (Hologic Discovery Series W; Waltham, MA) and a peak oxygen consumption (VO_{2peak}) test. The VO_{2peak} test was conducted using a mechanically braked bike (Lode; Groningen, Netherlands) and respiratory gas analysis system (TrueOne 2400, Parvo Medics; Salt Lake City, UT). The VO_{2peak} test started at a resistance of 50W and the resistance was increased by 50W every two minutes, participants maintained a cadence of 60rpm for the entirety of the test. The test was terminated when the participant experienced volitional fatigue or the pedaling cadence fell below 60rpm for thirty consecutive seconds (whichever came first). VO_{2peak} was

confirmed by a respiratory exchange ratio (RER) above 1.1 and plateau in VO_2. Thirty second averages were obtained from the VO_{2peak} test and the highest thirty second average was used to determine subsequent workloads. During visits 2 and 3 the participants underwent the experimental procedures – described in more detail below.

Experimental Procedures

Participants completed visits 2 and 3 in a randomized order. The day prior to visits 2 and 3, participants were asked to keep a similar diet, exercise, and sleep pattern that was free of ethanol and vigorous activity. These procedures were confirmed by having participants complete a 24-hour dietary recall (ASA24) prior to each visit and by wearing an activity and sleep monitor (SenseWear by Bodymedia; Pittsburgh, PA). Respiratory gasses and heart rate (HR) (H7, Polar; Kempele, Finland) were continuously monitored during visits 2 and 3, and the two visits were identical except for the exercise session. Both visits started with the participant resting in a supine position for fifteen minutes while we measured their respiratory gasses. A blood sample was obtained during the first five minutes of this resting period and the final five minutes of respiratory gas analysis were averaged to obtain baseline values. Following the resting measures, both trials started with the same warm-up where participants cycled for five-minutes at 40% of their VO_{2peak}. After the warm-up the exercise session began: The exercise sessions were matched for time at 30 minutes to meet the current exercise recommendations (18). During the steady state exercise (SS) condition, participants cycled at 70% of their VO_{2peak} for 30 minutes. For the sprint interval exercise (IE) condition, participants completed six bouts of 30 second "all out" sprints against a resistance equal to 7.5% of their body weight, a 4.5 minute active recovery period of

unloaded cycling followed each sprint for a total of thirty minutes. Immediately following the exercise session, participants returned to a supine position and an immediately post-exercise (IPE) blood sample was obtained. Participants continued to rest in a supine position for 1-hour after exercise. At 1-hour post-exercise a third and final blood sample was obtained.

Visits 2 and 3 were separated by a minimum of 24 hours. Female participants completed visits 2 and 3 during the mid-follicular stage of their menstrual cycle (days 3 to 10).

Blood Sampling

Blood samples were procured from the most prominent vein of the antecubital space at baseline, IPE, and 1-hour post-exercise for each experimental trial. Both serum and plasma (KEDTA) were collected. Blood was allowed to sit at room temperature for thirty minutes and then centrifuged at 3,000 rpm for fifteen minutes. Serum and plasma were separated and stored at -80 ℃ until analysis.

FGF21, glucagon, and glucose were measured from serum while epinephrine was measured from plasma. FGF21 (DF2100, R&D Systems), glucagon (ELH-Glucagon, RayBio), and epinephrine (NBP2-62867, Novus Biologicals) were all measured using commercially available ELISAs. Glucose measurements were completed and reported by Clinical Pathology Laboratories (Waco, TX) using the Roche COBAS automated methodology. Three males and two females had baseline FGF21 levels below the limit of the detection for both SS and IE conditions, the same was present in one male for the IE condition only. One female had epinephrine levels below the limit of detection for

both SS and IE conditions. One male had significantly elevated glucagon levels for both conditions.

Statistics

Samples size was determined by evaluating typical changes to blood FGF21 concentrations following exercise. Sample size estimates indicated that 10 females and 10 males would be sufficient to examine sex differences. Accounting for dropouts and the potential for serum FGF21 levels to be undetectable in some individuals, we included 15 females and 15 males in our study. Sample size calculations were done using G*Power (Düsseldorf, Germany).

Blood variables were checked for normality using the Shapiro-Wilk test and those that violated normality were natural log transformed prior to analysis. We used a 2 by 3 (condition by time) mixed model ANOVA with repeated measures to investigate changes to blood variables across time and condition, partial eta squared was used as the effect size. Significant ANOVA findings were followed up using post-hoc tests with a Bonferroni adjustment for multiple comparisons. Dependent *t*-tests and independent *t*-tests were used to compare baseline values between conditions and between females and males, respectively. Pearson correlations were used to investigate the relationships between biomarkers and FGF21. FGF21 incremental area under the curve (iAUC) and total area under the curve (tAUC) were calculated using the trapezoidal rule. The AUC values contained negative numbers and could not be log transformed. Therefore, they were analyzed using appropriate non-parametric tests. All analyses were conducted using SPSS version 25 (SPSS, Chicago, Illinois, USA). An alpha level of $p < 0.05$ was adopted throughout.

Results

Participants

Fifteen females and fifteen males completed this study for a total of thirty participants. The participants were compliant with study procedures as dietary recalls, activity, and sleep patterns were all similar between experimental trials ($p > 0.05$, data not shown). Demographic characteristics are presented in table 4.1.

Table 4.1

Demographic Characteristics

Characteristic	Female	Male
Age	23.5 ± 4.3	26.9 ± 6.5
VO_{2peak}	30.8 ± 5.5	45.1 ± 5.3
BMI	21.4 ± 1.9	23.4 ± 1.3
Percent Body Fat	25.7 ± 3.0	15.4 ± 4.8

Data are presented as mean ± SD. Age (years); VO_{2peak}, peak oxygen consumption (mL/kg/min); BMI, body mass index (kg/m^2)

Exercise Sessions

During the SS session, the average intensity for females was 67 ± 0.01% of VO_{2peak}, for males the average intensity was 68 ± 0.02% of VO_{2peak}, these differences were not statistically significant ($t = -0.48$, $p = 0.64$). Due to feelings of nausea, two males only completed five of the six sprints during the IE condition. As expected, males had higher exercising levels for oxygen consumption, carbon dioxide production, ventilation, kcal expenditure, and cycling distance during both exercise sessions ($p < 0.05$). RER ($t = -0.69$, $p = 0.50$) and HR ($t = -1.29$, $p = 0.21$) were similar between males and females during the SS condition. During the IE condition, HR was similar between sexes ($t = -0.68$, $p = 0.50$), but RER was significantly elevated in males ($t = -3.53$, $p < 0.01$). Average values for the exercise variables are presented in table 4.2.

Table 4.2

Exercise Variables

Variable	SS		IE	
	Females	Males	Females	Males
VO_2R	20.6 ± 4.1 *#	30.5 ± 4.6 #	14.1 ± 3.0 *	18.1 ± 3.2
VO_2A	1.2 ± 0.3 *#	2.3 ± 0.4 #	0.8 ± 0.2 *	1.4 ± 0.3
VE	30.8 ± 8.3 *	54.2 ± 10.4 #	27.8 ± 7.0 *	47.6 ± 13.2
RER	0.96 ± 0.03 #	0.96 ± 0.03 #	1.10 ± 0.05 *	1.17 ± 0.05
HR	149.2 ± 18.4 #	157.0 ± 13.6 #	132.9 ± 22.7	138.4 ± 21.4
Energy Expenditure	179.2 ± 43.5 *#	338.9 ± 57.8 #	124.3 ± 26.5 *	204.9 ± 49.7
Distance cycled	9.2 ± 2.2 *#	16.8 ± 3.2 #	3.5 ± 0.6*	5.6 ± 1.0

Data are presented as mean ± SD, * = statistically significant difference between males and females, # = statistically significant difference between SS and IE. VO_2R, relative oxygen consumption (mL/kg/min); VO_2A, absolute oxygen consumption; (L/min), VE, minute ventilation (L/min); RER, respiratory exchange ratio (VCO_2/VO_2); HR, heart rate (BPM); Energy Expenditure (kcal); Distance cycled (km)

FGF21

Fasting concentrations of FGF21 were not different between females and males at baseline ($p < 0.05$). For females, fasting concentrations of FGF21 were 119 ± 21 pg/mL and 153 ± 31 pg/mL before SS and IE exercise, respectively and were not significantly different between conditions ($p > 0.05$). Immediately post-exercise, FGF21 concentrations were decreased relative to baseline. In the SS condition, FGF21 levels rose above baseline to 125 ± 23 pg/mL 1-hour post exercise, while in the IE condition, FGF21 levels remained depressed relative to baseline. ANOVA results indicated that there were no statistically significant main effects for time ($F_{(2,24)} = 0.59$, $p = 0.48$, $\eta_p^2 = 0.05$ [90% CI = 0.00 – 0.17]) or condition ($F_{(1,12)} = 0.40$, $p = 0.54$, $\eta_p^2 = 0.03$ [90% CI = 0.00 – 0.26]).

For males, fasting concentrations of FGF21 were 108 ± 27 pg/mL and 128 ± 39 pg/mL prior to SS and IE exercise, respectively and were not different between conditions ($p > 0.05$). Serum FGF21 concentrations decreased slightly immediately after

SS exercise and were elevated immediately post-exercise during the IE condition. An hour after exercise, FGF21 levels were elevated in both conditions, rising to 143 ± 34 pg/mL after SS and 158.3 ± 52 after IE. There was a statistically significant effect for time ($F_{(2,20)} = 10.64$, $p < 0.01$, $\eta_p^2 = 0.52$ [90% CI = 0.20 – 0.65]). Neither the main effect for condition ($F_{(1,10)} = 0.12$, $p = 0.74$, $\eta_p^2 = 0.01$ [90% CI = 0.00 – 0.22]) nor the condition by time interaction effect ($F_{(2,20)} = 1.45$, $p = 0.26$, $\eta_p^2 = 0.13$ [90% CI = 0.00 – 0.30]) were statistically significant. Post-hoc tests revealed that 1-hour post-exercise was significantly elevated relative to the baseline and immediately post-exercise timepoints ($p < 0.05$). FGF21 responses to SS and IE are presented in figures 4.1 and 4.2, respectively.

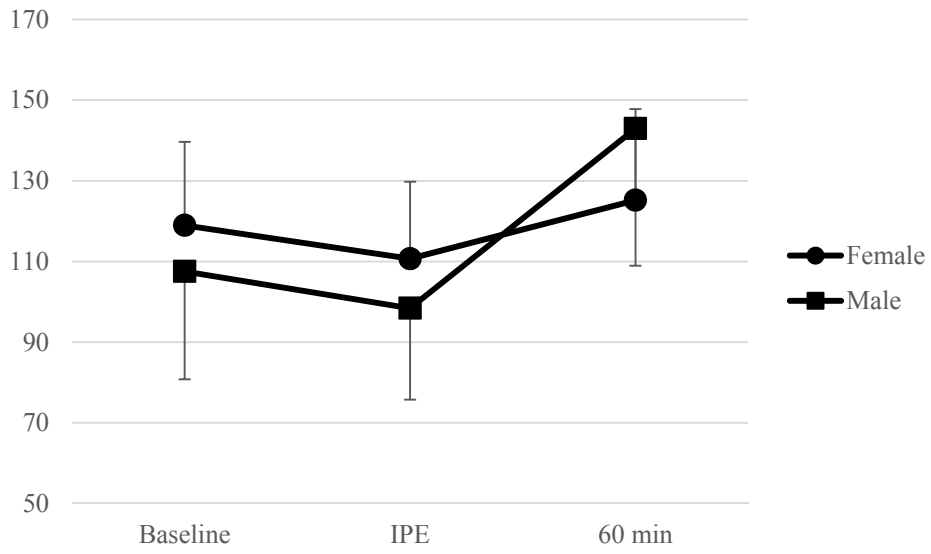

Figure 4.1 FGF21 responses to SS, data are presented as mean ± SEM pg/mL

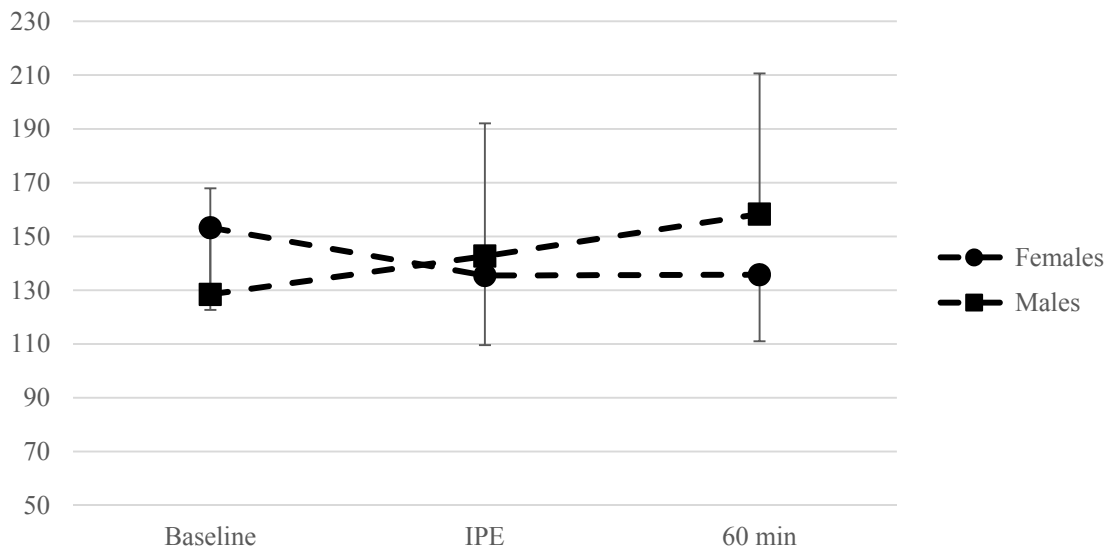

Figure 4.2 FGF21 responses to IE, data are presented as mean ± SEM pg/mL

A Mann Whitney U test was used to compare the post-exercise FGF21 response between females and males. The post-exercise iAUC in the SS condition was significantly greater in males than females (U = 46.0, p = 0.04). While males had a greater post-exercise iAUC than females during the IE condition, this elevation was not statistically significant (U = 53.0, p = 0.14) (Figure 1D). A Wilcoxon signed-rank test was used to compare post-exercise FGF21 iAUC in both sexes separately. For females, there was not a significant difference between conditions (Z = -1.22, p = 0.22). For males, the SS condition produced a significantly greater post-exercise FGF21 than the IE condition (Z = -2.3, p = 0.02) (Figure 4.3).

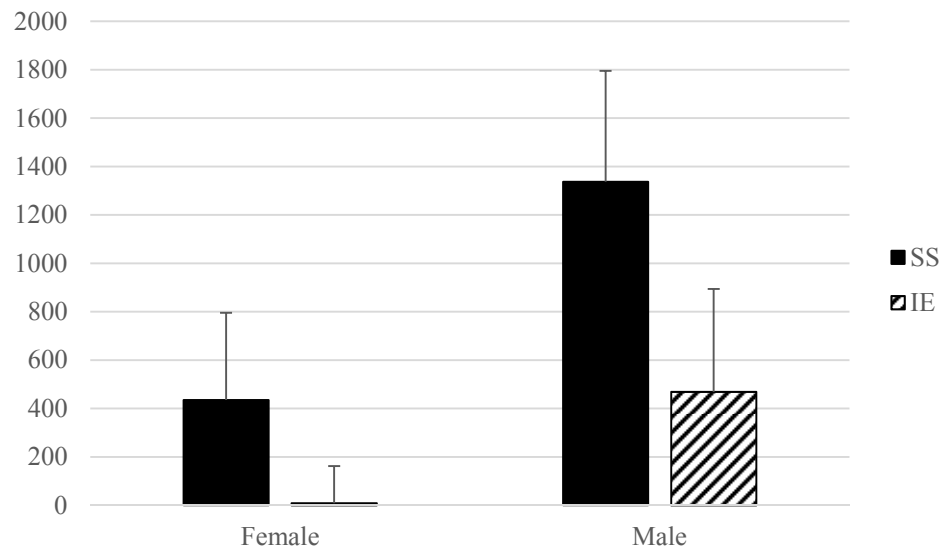

Figure 4.3 FGF21 iAUC responses to SS and IE. Data are presented as mean ± SEM pg/mL, * = statistically significant difference between males and females, # = statistically significant difference between SS and IE

Glucagon, Epinephrine, Glucose

Glucagon, epinephrine, and glucose are all mechanistically linked to FGF21 production, so we assessed their concentrations next. Baseline values for glucagon and epinephrine, were not significantly different at baseline nor at any other time point between males and females ($p > 0.05$). Fasting blood glucose was significantly higher in males before the SS condition ($p < 0.05$), but not significantly different between sexes before the IE condition or at any other time point ($p > 0.05$).

Glucagon levels remained fairly unchanged in females, increasing slightly during the SS trial and decreasing slightly during the IE trial. These responses were not significantly affected by time ($F_{(2,28)} = 0.46$, $p = 0.64$, $\eta_p^2 = 0.03$ [90% CI = 0.00 – 0.14]) or condition ($F_{(1,14)} = 0.67$, $p = 0.8$, $\eta_p^2 = 0.00$ [90% CI = 0.00 – 0.15]). In males, glucagon levels increased as a result of exercise in the SS condition and decreased slightly in the IE condition. These changes were not significant ($F_{(2,26)} = 0.95$, $p = 0.37$,

η_p^2 = 0.07 [90% CI = 0.00 – 0.21]) and were unaffected by exercise modality ($F_{(1,13)}$ = 1.66, p = 0.22, η_p^2 = 0.11 [90% CI = 0.00 – 0.37]); however post-hoc contrasts revealed a near significant difference for the change from baseline to 1-hour post exercise between conditions ($F_{(1,13)}$ = 4.69, p = 0.05, η_p^2 = 0.27 [90% CI = 0.00 – 0.50]). (Figure 4.4)

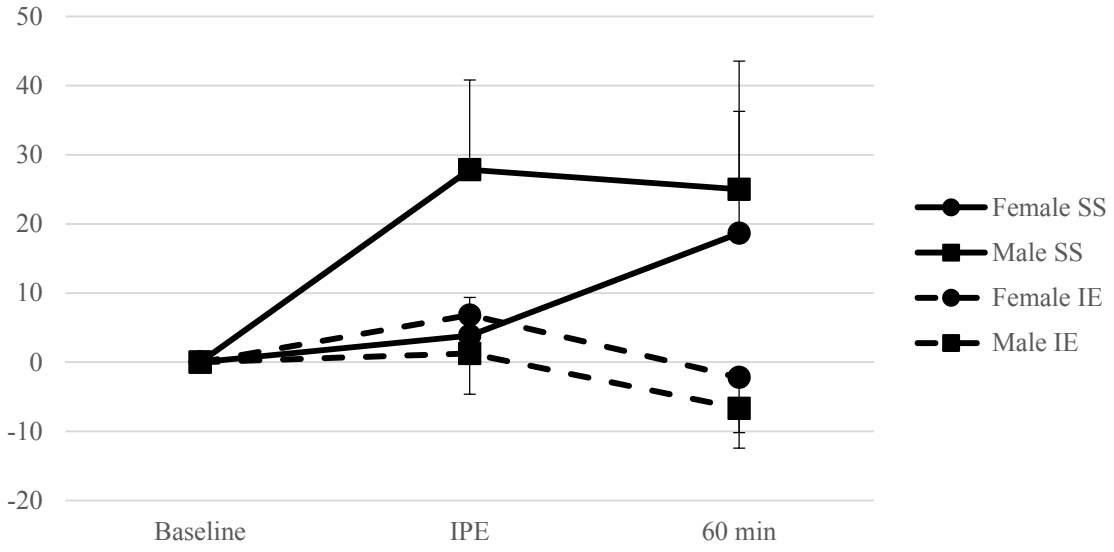

Figure 4.4 Percent change in glucagon relative to baseline. Data are presented as mean ± SEM

In females, epinephrine was elevated immediately post-exercise in both conditions and decreased from its peak concentrations 1-hour post-exercise but remained elevated relative to baseline values. These changes were not statistically significant for either time ($F_{(2,26)}$ = 1.70, p = 0.22, η_p^2 = 0.12 [90% CI = 0.00 – 0.28]) or condition ($F_{(1,13)}$ = 0.00, p = 0.97, η_p^2 = 0.00 [90% CI = 0.00 – 0.01]). In males, epinephrine increased immediately post-exercise in the SS condition and returned to baseline 1-hour later. During the IE condition, epinephrine levels decreased slightly post-exercise and were elevated relative to baseline 1-hour post-exercise. Neither time ($F_{(2,28)}$ = 0.30, p =

0.74, $\eta_p^2 = 0.02$ [90% CI = 0.00 – 0.11]) nor condition ($F_{(1,14)} = 0.12$, $p = 0.73$, $\eta_p^2 = 0.01$ [90% CI = 0.00 – 0.18]) affected this response. (Figure 4.5)

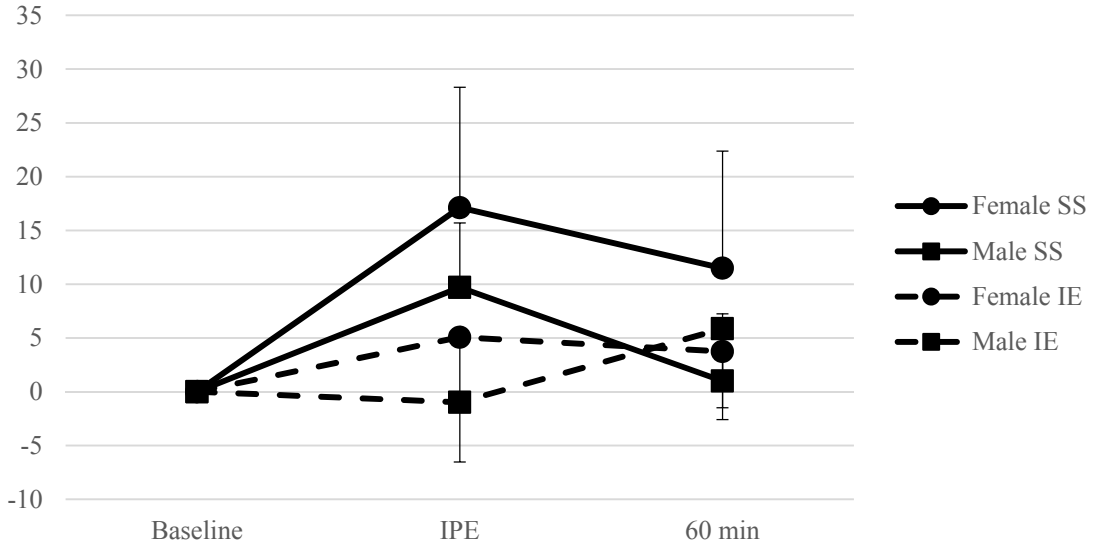

Figure 4.5 Percent change in epinephrine relative to baseline. Data are presented as mean ± SEM

For both females and males, blood glucose levels were elevated immediately post-exercise and returned to baseline levels or below by 1-hour post exercise in both conditions. In females, there was a significant main effect for time ($F_{(2,28)} = 16.90$, $p < 0.001$, $\eta_p^2 = 0.55$ [90% CI = 0.29 – 0.66]). There was not a significant main effect for condition ($F_{(1,14)} = 3.52$, $p = 0.08$, $\eta_p^2 = 0.20$ [90% CI = 0.00 – 0.45]), but there was a significant time by condition interaction effect ($F_{(2,28)} = 4.46$, $p = 0.04$, $\eta_p^2 = 0.24$ [90% CI = 0.02 – 0.41]). Post-hoc tests showed that the immediately post-exercise levels were elevated relative to baseline ($F_{(1,14)} = 13.83$, $p < 0.01$, $\eta_p^2 = 0.50$ [90% CI = 0.15 – 0.67]), that 1-hour post exercise levels were depressed relative to baseline ($F_{(1,14)} = 11.14$, $p < 0.01$, $\eta_p^2 = 0.44$ [90% CI = 0.10 – 0.63]), and that the change from baseline to 1-hour post-exercise was different between conditions ($F_{(1,14)} = 10.68$, $p < 0.01$, $\eta_p^2 = 0.43$ [90%

CI = 0.09 – 0.62]). In males, similar results were witnessed with a significant main effect for time ($F_{(2,28)}$ = 28.50, p < 0.001, η_p^2 = 0.67 [90% CI = 0.45 – 0.76]) and significant time by condition interaction ($F_{(2,28)}$ = 14.19, p < 0.001, η_p^2 = 0.50 [90% CI = 0.24 – 0.63]), but no main effect for condition ($F_{(1,14)}$ = 3.74, p = 0.07, η_p^2 = 0.21 [90% CI = 0.00 – 0.45]). Post-hoc analysis indicated that the immediately post-exercise blood glucose levels were greater than baseline values ($F_{(1,14)}$ = 28.90, p < 0.001, η_p^2 = 0.67 [90% CI = 0.36 – 0.79]). In contrast to the results witnessed in females the change from baseline to immediately post-exercise was significantly different between conditions ($F_{(1,14)}$ = 15.39, p < 0.01, η_p^2 = 0.52 [90% CI = 0.17 – 0.69]). In men blood glucose levels 1-hour after exercise were not significantly different from pre-exercise values ($F_{(1,14)}$ = 4.28, p = 0.06, η_p^2 = 0.23 [90% CI = 0.00 – 0.47]). (Figure 4.6)

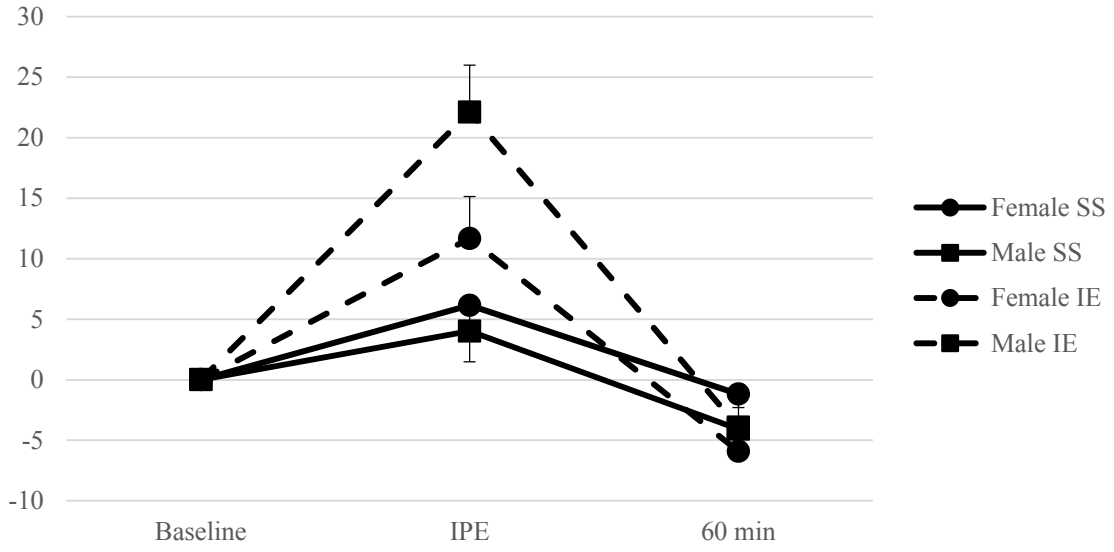

Figure 4.6 Percent change in glucose relative to baseline. Data are presented as mean ± SEM

Relationship between FGF21 and Other Blood Markers

To examine the relationship between FGF21 and other blood markers, we conducted correlations between the IPE concentration of glucagon, epinephrine, and glucose and the post-exercise FGF21 iAUC. Glucose was significantly correlated with a strong effect size to the FGF21 iAUC in males during the IE protocol ($r = 0.71$, $p < 0.01$). No other statistically significant correlations were observed (table 4.3)

Table 4.3

Correlation between FGF21 post-exercise iAUC and glucagon, epinephrine, and glucose.

Condition	Correlate	Females		Males		Whole Group	
		r	*p*	*r*	*p*	*r*	*p*
SS	Glucagon IPE	-0.08	0.40	-0.19	0.28	-0.07	0.037
	Epinephrine IPE	0.06	0.43	0.32	0.16	0.15	0.24
	Glucose IPE	-0.19	0.27	0.31	0.16	0.12	0.28
IE	Glucagon IPE	0.19	0.26	-0.43	0.11	-0.17	0.23
	Epinephrine IPE	0.05	0.44	-0.13	0.35	-0.09	0.34
	Glucose IPE	0.36	0.11	0.71	<0.01*	0.63	<0.01*

* = statistically significant finding

EPOC

Following the exercise session, we continued to monitor oxygen consumption for 1-hour post-exercise. Following the SS condition, females had an average EPOC magnitude of 8.88 ± 1.74 kcals with an RER of 0.86 ± 0.01 and males had an average EPOC magnitude of 24.06 ± 1.60 kcals with an RER of 0.81 ± 0.01. Both the EPOC magnitude ($t = -6.45$, $p < 0.001$) and RER ($t = 2.49$, $p = 0.02$) were significantly different between females and males. After the IE condition, average EPOC magnitude for females was 9.59 ± 1.99 with an RER of 0.79 ± 0.01. For males average EPOC

magnitude was 24.79 ± 2.49 with an RER of 0.75 ± 0.01. EPOC magnitude (t = -4.77, p < 0.001) and RER (t = 3.30, p < 0.01) were significantly different between females and males following the IE condition. Analyzing females and males separately indicated that EPOC magnitude was not different between condition (Females: t = -0.34, p = 0.74; Males: t = -0.28, p = 0.78), but RER was significantly greater following the SS condition (Females: t = 6.45, p < 0.001; Males: t = 4.77, p < 0.001). EPOC data are presented in table 4.4.

Table 4.4

EPOC variables

Variable	SS		IE	
	Females	Males	Females	Males
EPOC magnitude	8.88 ± 1.74 *	24.06 ± 1.60 *	9.59 ± 1.99 *	24.79 ± 2.49 *
RER	0.86 ± 0.01 *#	0.81 ± 0.01 *#	0.79 ± 0.01 *	0.75 ± 0.01 *

Data are presented as mean ± SEM, * = statistically significant difference between males and females, # = statistically significant difference between SS and IE conditions. EPOC magnitude, excess post-exercise oxygen consumption (L); RER, respiratory exchange ratio (VCO_2/VO_2)

Relationship between EPOC and FGF21

We next sought to determine if the post-exercise FGF21 response was related to EPOC magnitude or RER. We compared the FGF21 post-exercise area under the curve with the average EPOC responses as well as the FGF21 at 1-hour post exercise with the last five minutes of EPOC measurements. There were no statistically significant correlations between EPOC variables and FGF21 during the IE condition. During the SS condition, the post-exercise FGF21 tAUC was negatively correlated with RER (r = -0.69, p < 0.01) in females, indicating FGF21 was associated with greater fatty acid oxidation. FGF21 1-hour post exercise was positively correlated with VO_2 in males (r = 0.58, p = 0.02) and negatively correlated with RER in females (r = -0.49, p = 0.04) during the final

five-minutes of EPOC measurements for the SS condition. Correlations between FGF21 and EPOC are in table 4.5.

Table 4.5

Correlations between EPOC and FGF21 variables.

Condition	Correlate	Correlate	Females		Males		Whole Group	
			r	*p*	*r*	*p*	*r*	*p*
SS	FGF21 tAUC	VO_2A	-0.09	0.39	0.38	0.11	0.11	0.30
		RER	-0.69	0.01*	0.24	0.23	-0.19	0.18
		EPOC magnitude	0.14	0.33	0.15	0.32	0.11	0.30
	FGF21 iAUC	VO_2A	-0.13	0.34	0.23	0.24	0.29	0.08
		RER	-0.17	0.29	-0.04	0.45	-0.22	0.15
		EPOC magnitude	0.28	0.17	-0.02	0.47	0.31	0.06
	FGF21 1-hour post	VO_2A	-0.21	0.25	0.58	0.02*	0.18	0.19
		RER	-0.49	0.04*	-0.36	0.13	-.40	0.02*
IE	FGF21 tAUC	VO_2A	-0.35	0.12	0.25	0.23	0.08	0.35
		RER	0.23	0.22	-0.70	0.42	0.06	0.39
		EPOC magnitude	0.11	0.37	0.28	0.20	0.16	0.23
	FGF21 iAUC	VO_2A	0.33	0.14	0.088	0.40	0.27	0.11
		RER	-0.03	0.46	0.25	0.23	-0.04	0.42
		EPOC magnitude	0.30	0.16	0.08	0.41	0.26	0.11
	FGF21 1-hour post	VO_2A	0.20	0.25	0.20	0.28	0.12	0.29
		RER	0.38	0.10	-0.36	0.14	0.09	0.34

* = statistically significant finding. FGF21 tAUC, FGF21 total area under the curve; FGF21 iAUC, FGF21 incremental area under the curve, VO_2A, absolute oxygen consumption (L/min); RER, respiratory exchange ratio (VCO_2/VO_2)

Discussion

The present study explored the effect of an acute bout of sprint interval or continuous exercise on circulating levels of FGF21 and related biomarkers as well as EPOC in healthy females and males. Our novel findings are that exercise elevates

FGF21 post-exercise to a greater degree in males than it does females, that continuous exercise produces greater levels of circulating FGF21 than sprint interval exercise, and that FGF21 is not related to the EPOC response.

Sex differences in the exercise response to FGF21 are a notable finding and might help explain some of the metabolic differences observed between males and females. Steady state endurance exercise has consistently been shown to acutely increase FGF21 in healthy men (2,7–9,27). Similar to these findings, we saw a statistically significant increase in FGF21 following the SS exercise protocol in our cohort of males, but in females this response was not significantly elevated. Furthermore, we even saw a decrease in FGF21 levels (relative to baseline) in females following the IE condition. Previous research in females has been scant, though it is notable that one female only cohort did not see a rise in FGF21 following acute exercise (29) and another investigation was unable to study FGF21 in females due to baseline levels being below the limit of assay detection (26). Slusher et al. included both men and women in their study of acute exercise and FGF21 and did not find any sex differences; however, this was not their main outcome variable and their sample size – one third of the present study – was likely too small to detect such differences (31). One interesting study found that FGF21 levels increased post-exercise in pregnant women, but not in a similar non-pregnant cohort (30).

The lack of post-exercise FGF21 increase in females may offer a potential protective effect to reproductive and bone health. One study performed in mice showed that FGF21 played a role in disrupting female fertility (19). While this finding has been called into question by a more recent article (49), FGF21 still appears capable of disrupting female fertility, even if it is not robust to all situations. In humans, FGF21

may be inversely related to bone density (56,57). Since females are at a greater risk for osteoporosis than males, it is possible that a lack of FGF21 response to exercise may be an adaptive mechanism for maintaining bone mass. The precise mechanism by which FGF21 may be differentially regulated between females and males is a subject of future investigation. A hyper-release of estrogen does not appear to influence FGF21 (50) and the high levels of estrogen associated with pregnancy did not prevent pregnant women from experiencing increased FGF21 level post-exercise (30); therefore, the difference is unlikely to be due to differences in estrogen. One of the primary biological differences between females and males is in muscle mass. While FGF21 may act as a myokine in some situations (15,58), circulating levels of FGF21 are believed to be of hepatic origin (34), particularly after exercise (2,8). While liver mass differences are much closer between males and females (59) than skeletal muscle, a difference in liver size could at least be partially responsible. Future research could be directed towards finding mechanisms by which FGF21 may be differentially regulated between females and males.

The regulation of FGF21 is pluripotent and many upstream regulators of FGF21 are increased as a result of exercise. We investigated three biomarkers – glucagon, epinephrine, and glucose – to see if their changes were related to the FGF21 response. Glucagon has been directly linked with circulating FGF21 (38). Previous studies investigating acute exercise and FGF21 have shown that glucagon levels peak approximately one hour before FGF21 levels peak (7) and that there is a positive correlation between glucagon and FGF21 (8). In contrast to prior studies we did not see a significant elevation in glucagon post-exercise nor did we observe a significant

correlation between IPE glucagon levels and FGF21. The exercise duration of thirty minutes in this study was noticeably shorter than other studies that saw a significant elevation in glucagon which may explain this difference. Our exercise sessions were designed to be thirty minutes in order to match current recommendations for daily exercise (52) and to match conditions for exercise time. Although we did not see a significant correlation between glucagon IPE and the post-exercise FGF21 iAUC; both sexes did experience the highest average glucagon levels following the SS condition and this condition produced the highest FGF21 iAUCs for both sexes, which may indicate that glucagon is still an important regulator of circulating FGF21 following exercise.

Sympathetic nervous system activity, which may be mediated by the hormone epinephrine, is associated with FGF21 production. Circulating epinephrine was found to be related to blood FGF21 levels when using hypoxia to induce the response (16). A link between circulating epinephrine and circulating FGF21 in response to exercise has not been established. In one study on female participants epinephrine concentration increased post-exercise, but not FGF21 (29). In another study, epinephrine was not significantly increased post-exercise, but FGF21 levels were (9). We did not find a significant relationship between IPE epinephrine and post-exercise FGF21 iAUC. Exercise-induced epinephrine levels have previously been identified to exhibit sex differences (20,24,60); but epinephrine levels in our study did not exhibit any sex differences.

The *FGF21* gene has a carbohydrate-response element-binding protein site (10), allowing for its transcription to be upregulated by carbohydrates. Consistent with this theory, a high dose of glucose can increase circulating FGF21 levels (13). We observed

significantly elevated glucose levels IPE in all conditions. During the IE trial in males, blood glucose levels were significantly greater IPE than in the SS trial and strongly correlated with the FGF21 iAUC. The fact that IPE glucose was correlated to FGF21 in the IE condition, but not SS condition is an interesting finding. There might be a glucose threshold that must be exceeded in order for glucose to promote *FGF21* transcription. Although very different circumstances, FGF21 has been shown to be correlated with elevated blood glucose in pathological conditions (61,62). The fact that the post-exercise iAUC was greater for the SS condition than IE condition, yet only correlated to blood glucose in the IE condition might indicate that FGF21 can be produced via multiple mechanisms during exercise or regulated differently by different types of exercise.

Circulating levels of FGF21 have been shown to differ in response to different types of exercise. As noted previously, FGF21 have been shown to consistently rise in response to steady state endurance exercise (at least in males), resistance exercise has not shown the same response (7,51). Sprint interval exercise combines elements of both resistance and endurance exercise by alternating periods of high anaerobic energy demand with periods of low intensity recovery and has been shown to have similar metabolic benefits to steady state endurance activity (18,55). Work by other researchers indicated that blood FGF21 levels do not increase after sprint interval exercise in healthy men (51). However, we show that serum FGF21 concentrations are increased 1-hour after a bout of sprint interval exercise in healthy men. Previous research investigating the effect of steady state aerobic exercise in healthy men showed that exercising at a higher intensity was associated with a greater post-exercise FGF21 response (2,27). We found a greater post-exercise iAUC in response to SS compared to IE, for both males and

females, in males this difference was significant. Our results suggest that the post-exercise FGF21 response is not governed by exercise intensity alone as our participant's experienced supra-aerobic intensities in the IE condition while experiencing a lesser FGF21 response in comparison to the aerobic condition (SS). The metabolic pathways activated by a steady state aerobic protocol and a sprint interval protocol are distinctly different, the pathway activated in aerobic exercise might more favorably promote FGF21 production.

Following exercise, the body continues to experience an elevated metabolic rate as a result of EPOC. We found a similar magnitude of EPOC as measured by total kcals between the SS and IE conditions, despite the greater total volume of exercise performed in the SS condition. Intensity is generally believed to be more important than volume when it comes to EPOC magnitude (63,64) and interval exercise has been shown to produce a greater EPOC than steady state exercise when energetically balanced (65). Therefore, it is interesting, but not surprising that we saw similar EPOC magnitudes between conditions. Both females and males had greater RER values following the SS exercise bout compared to the IE bout, despite having higher blood glucose levels following IE.

In mice, exogenous administration of FGF21 is associated with increased caloric expenditure, increased fat oxidation and increased core temperature (4,5), all of which are also present during the EPOC response. To investigate the relationship between FGF21 and EPOC, we looked at both the total and incremental area under the FGF21 curve to see if total FGF21 or excess FGF21 were associated with EPOC variables. We also looked to see if FGF21 concentration 1-hour post-exercise was related to the last five

minutes of EPOC measurements. Despite observing similar magnitudes of EPOC between SS and IE conditions we saw different FGF21 iAUC during post-exercise measurements indicating that FGF21 and EPOC are not linked. Furthermore, we saw very few statistically significant correlations between FGF21 and EPOC variables, and only in the SS condition. We conclude that if FGF21 is related to the EPOC response, it is very minor. The lack of association between FGF21 and EPOC calls into question the metabolic benefits of the post-exercise rise in FGF21. Unlike most members of the FGF family, FGF21 lacks a heparin binding domain and requires the presence of its co-receptor, β-klotho, for it to properly bind to its receptor (66,67). The presence of β-klotho is important for FGF21 to exert its metabolic benefits in mice models (4). If FGF21 levels in circulation increase, but are unable to bind or bind effectively, the beneficial metabolic effects of FGF21 may not take place.

The present study is not without limitations. We used day counting as a method for determining menstrual cycle phase for female participants, which may not accurately reflect hormone levels (68). FGF21 can be cleaved by the enzyme fibroblast activating protein, which is known to circulate in human plasma (69,70). We did not investigate levels of fibroblast activating protein or distinguish between active and inactive forms of FGF21 which could alter FGF21 actions *in vivo*. Finally, we only monitored EPOC levels for one hour. While the majority of the excess energy expenditure as a result of EPOC occurs during the first hour, by not measuring EPOC until oxygen levels returned to baseline we missed some of the excess energy expenditure that may offer important insights.

Conclusion

In this study we present the novel finding that males experience a greater post-exercise FGF21 response than females. This finding may lead to a better understanding of the differences in metabolic health between sexes and it may suggest the need for different treatment pathways in the development of FGF21-analog drugs. We also show that continuous aerobic exercise is able to increase circulating levels of FGF21 to a greater extent than sprint interval exercise and that these changes are not associated with EPOC. Future research should further examine sex-based differences in FGF21 and explore the extent to which post-exercise increases in FGF21 influence metabolic health.

CHAPTER FIVE

Manuscript Two: Relationship Between Circulating FGF21 and Physiological and Lifestyle Characteristics, an Exploratory Study

Introduction

Fibroblast growth factor 21 (FGF21) is a member for the FGF19 subfamily of FGF molecules (71). The FGF19 subfamily is unique in that it lacks a heparin binding domain, allowing its members to circulate throughout the body (66). As a result, FGF21 freely circulates and has a broad range of endocrine-like effects on the body. Studies in mice have demonstrated that pharmacological administration of FGF21 is able to promote weight loss, reverse type II diabetes, and protect against the detrimental effects of a high fat diet (4). This has led some to speculate that FGF21 may serve as a potential pharmaceutical agent.

The study of FGF21 in humans has been complicated by the fact that the molecule may be regulated differently in humans versus what is seen in animal models (72). A large variance in baseline FGF21 levels has also been observed, even among the same individuals (11,73). Additionally, the upstream regulators and downstream effectors in the FGF21 pathway are multifactorial and oftentimes exhibit paradoxical relationships (10).

One of the first paradoxes witnessed in the regulation of FGF21 is with obesity. Observational studies have indicated that obese individuals have greater levels of circulating FGF21 than do normal weight individuals (74). This is paradoxically in

contrast with experimental studies in animals and humans that have shown exogenous FGF21 promotes weight loss.

Similar to weight status, lifestyle variables such as physical activity and dietary intake are associated with circulating levels of FGF21. Acute bouts of exercise have been shown to increase FGF21 levels post-exercise (2,7,8). However, this increase is generally short-lived due to the half-life of endogenous FGF21 being around two hours (17). Studies investigating the effect of habitual exercise have similarly shown a positive relationship between physical activity and FGF21 (75). Transcription of the *FGF21* gene can be promoted by both carbohydrates through the carbohydrate-response element-binding protein (ChREBP) (10) and by fatty acids through the peroxisome proliferator-activated receptor alpha (PPARα) transcription factor (10,73). This further underscores the paradox of FGF21 regulation, as it can be regulated through both feeding and fasting mechanisms. Additionally, nutrients that are metabolized in the liver have been shown to have acute effects on circulating FGF21 levels (13,76). Complicating matters, not only does FGF21 respond to nutrient intake, but there is evidence to suggest that FGF21 may also influence nutrient intake and preference through its actions in the central nervous system (32,77).

In addition to lifestyle factors, FGF21 may also be associated with other circulating factors. Prior research has demonstrated a positive correlation between blood glucose and FGF21 levels (75,78). Research in animals has shown a relationship between FGF21, fluid balance and kidney function (79), which might mean FGF21 is associated with human markers of kidney function as well as the regulation of blood pressure. While FGF21 may be expressed in a variety of tissues, the majority of evidence

in humans suggests that under normal conditions circulating FGF21 is derived from the liver (8); however, there has been minimal work examining the relationship between FGF21 and other enzymes produced by the liver. Finally, one of the major findings from a study that investigated the pharmacological dosing of an FGF21 analog was an improvement in dyslipidemia (14), indicating that FGF21 may promote the beneficial regulation of blood cholesterol (decreasing LDL and increasing HDL).

While much may be known about upstream and downstream pathways of FGF21, the extent to which these processes occur *in vivo* in healthy individuals are unknown. Therefore, the purpose of this study is to conduct an exploratory analysis on relationships between FGF21 and factors that are believed to either regulate FGF21 or serve as downstream targets of FGF21 action.

Methods

Experimental Design

This study is an observational study that was conducted as part of a larger investigation into the relationship between metabolism and FGF21. The study was approved by the Baylor University Institutional Review Board. The present study required three visits. On visit one, anthropometric measurements and the VO_{2peak} test were conducted. Visits 2 and 3 were identical: Following an overnight fast, participants reported to the lab in the morning at a time that was consistent with their normal sleep schedule. Upon arriving to the lab, the participant's body weight was recorded and their resting metabolic rate was measured for fifteen minutes. During the first five minutes of the resting measures, a blood sample was obtained and their blood pressure was

measured. Participants were asked to match their diet and physical activity as closely as possible for the days prior to visit 2 and visit 3; as well as to avoid vigorous physical activity and ethanol consumption during this time. Due to the high reported variability in FGF21 measures, the results from visits 2 and 3 were averaged before conducting the statistical analyses.

Participants

Thirty apparently healthy, physically active adults were recruited for this study. Fifteen participants were female and fifteen were male. All participants were briefed on study procedures and provided written informed consent prior to enrolling in the study. Recruitment criteria included: eighteen to forty-five years of age, participated in at least thirty minutes of moderate activity three days a week for the past three months or longer, normal weight as indicated by a BMI between 18.5 and 24.9, and free of any cardiovascular, metabolic, or renal diseases.

Blood Draws

Blood was drawn in the morning, following an overnight fast of at least ten hours. Blood was drawn from the most prominent vein in the antecubital space into serum collection tubes. Following the blood collection, the blood clotted at room temperature for thirty minutes. After clotting, the blood was centrifuged at 3,000 rpm for fifteen minutes. Serum was separated and stored at -80 ℃ until the analysis was conducted.

Blood Analyses

Serum FGF21 was measured using a commercially available ELISA (R&D Systems, DF2100). A metabolic panel and lipid panel were completed and reported by

Clinical Pathology Laboratories (Waco, TX) using the Roche COBAS automated methodology and the Roche Cobas enzymatic colorimetric generation 2, respectively.

Anthropometrics

Height was measured to the nearest 0.1 cm in using a stadiometer and weight was measured to the nearest 0.1 lbs on a calibrated digital scale (Seca; Hamburg, Germany). Both measurements were performed with the participant in exercise clothes, without shoes, but in stocking feet.

Physiological Measurements

During the first visit, a VO_{2peak} test was conducted on a mechanically braked bike (Lode; Groningen, Netherlands), oxygen consumption was measured using a Parvo Medics TrueOne 2400 (Parvo Medics; Salt Lake City, UT). The participants maintained a pedaling cadence of 60rpm throughout the test. The bike resistance started at 50W and was increased 50W every two minutes. The test terminated when the participant either chose to stop or was no longer able to maintain a cadence of 60rpm for a period of thirty consecutive seconds (whichever came first). A VO_{2peak} was confirmed by a respiratory exchange ratio (RER) above 1.1 and plateau in VO_2.

During the second and third visits, resting metabolic rate (RMR) was measured using the same Parvo Medic measuring system. RMR was measured with participants resting in a supine position for fifteen minutes, with the final five minutes used for analysis. Blood pressure was measured with the participant in the supine position using an automated blood pressure cuff (ADC E-sphyg 2; Hauppauge, NY).

Sleep and Physical Activity

Participants were given an activity monitor (SenseWear by Bodymedia; Pittsburgh, PA) that measures sleep and physical activity. The participants were asked to wear the monitor for three days (one weekend day and two weekdays) outside of the study activities that represented their normal habits. The monitor had to be worn for at least twenty-three hours for the day to be considered valid.

Diet

To assess the impact of typical diet on circulating FGF21 levels, participants were asked to fill out a food frequency questionnaire (DHQIII) that asked them about the types and quantities of food they typically consumed during the preceding month.

Statistics

FGF21 values were not normally distributed and were log-transformed prior to analysis. Correlations between FGF21 and variables related to either its downstream or upstream pathways were performed. A Pearson correlation was conducted for normally distributed data and a Spearman correlation was conducted for non-normally distributed data. There is some evidence to suggest that FGF21 may be differentially regulated based on biological sex (21), therefore we present our results for the entire group and for each sex individually. All analyses were conducted using SPSS version 26 (SPSS; Chicago, IL). An alpha level of $p<0.05$ was adopted throughout.

Results

Participants

A total of 30 participants completed this study (n = 15 females, n = 15 males); however, 5 had FGF21 levels that were below the minimum level of detection, leaving us with a total of 25 participants (n = 13 females, n = 12 males). The average coefficient of variation for blood concentration of FGF21 between the two visits was 38.0% with a range of 1.1% to 105.0%. There were no statistically significant differences in serum FGF21 levels between males and females ($p>0.05$). Demographic characteristics of our sample are shown in table 5.1.

Table 5.1

Demographic characteristics.

Variable	Whole Group	Females	Males
Age	25.2 ± 5.6	23.5 ± 4.3	26.9 ± 6.5
BMI	22.4 ± 1.9	21.4 ± 1.9	23.4 ± 1.3
VO_{2peak}	38.0 ± 9.0	30.8 ± 5.5	45.1 ± 5.3

Data are presented as mean±SD.

Age and Anthropometrics

For the whole sample, we observed a weak, positive correlation between age and FGF21 ($r = 0.015$, $p = 0.40$). Weight ($r = -0.18$, $p = 0.19$), BMI ($r = -0.26$, $p = 0.10$), and waist circumference ($r = -0.15$, $p = 0.23$) were all negatively, associated with FGF21 levels. In females, a greater weight ($r = -0.53$, $p = 0.03$), BMI ($r = -0.49$, $p = 0.04$), and waist circumference ($r = -0.53$, $p = 0.03$) were all significantly associated with lower FGF21 levels. Results for the age and anthropometric analyses are shown in table 5.2.

Table 5.2

Correlations between FGF21 and age and anthropometric variables.

	Whole Group		Females		Males	
Correlate	*r*	*p*	*r*	*p*	*r*	*p*
Age	0.05	0.40	0.22	0.23	0.07	0.41
Weight	-0.18	0.19	-0.53	0.03*	0.43	0.08
BMI	-0.26	0.10	-0.49	0.04*	0.20	0.27
Waist Circum.	-0.15	0.23	-0.53	0.03*	0.37	0.20

* = statistically significant correlation.

Metabolism, Activity, and Sleep

VO_{2peak} was negatively correlated with FGF21 (r = -0.26, p = 0.11) and in males this relationship was statistically significant with a strong effect size (r = -0.71, p<0.01). RMR was negatively related to FGF21 (r = -0.20, p = 0.17) and in females this was statistically significant with a strong effect size (r = -0.48, p = 0.04). Greater rates of FGF21 were associated with a lower RER (r = -0.16, p = 0.22) indicating that FGF21 was associated with greater amounts of fatty acid oxidation. Daily activity levels were associated with lower FGF21 levels (r = -0.11, p = 0.30), with a trend for greater intensity of activity being associated with lower FGF21 levels, while amount of sedentary time was positively associated with FGF21 (r = 0.17, p = 0.22). Sleep was positively correlated with FGF21 for the whole group (r = 0.34, p = 0.05) and in females this relationship was statistically significant had had a large effect size (r = 0.71, p<0.01); however in males there was a negative correlation between FGF21 and sleep (r = -0.26, p = 0.21). Correlations for the metabolic, activity, and sleep data are shown in table 5.3.

Table 5.3

Correlations between FGF21 and resting metabolism and activity variables.

	Whole Group		Females		Males	
Correlate	*r*	*p*	*r*	*p*	*r*	*p*
VO_{2peak}	-0.26	0.11	0.19	0.27	-0.71	<0.01*
RMR	-0.20	0.17	-0.48	0.04*	0.30	0.17
RER	-0.16	0.22	-0.45	0.06	0.08	0.41
Average daily activity	-0.11	0.30	-0.20	0.26	-0.02	0.48
Sedentary time	0.17	0.22	0.36	0.12	-0.05	0.44
Light activity	-0.15	0.24	-0.2	0.47	-0.23	0.24
Moderate activity	-0.07	0.37	-0.24	0.21	0.11	0.37
Vigorous activity	-0.33	0.05	0.05	0.44	-0.44	0.08
Time in bed	0.36	0.04*	0.77	<0.01*	-0.27	0.20
Measured sleep	0.34	0.05	0.71	<0.01*	-0.26	0.21

* = statistically significant correlation.

Diet

Serum FGF21 were compared to the average diet each participant consumed over the month prior to starting the study. Caloric consumption was weakly associated with circulating FGF21 levels (r = -0.11, p = 0.31). Fatty acid consumption was negatively related to blood FGF21 concentration (r = -0.23, p = 0.14), with all types of lipid species having a negative relationship; this effect was strongest for both saturated fat (r = -0.37, p = 0.04) and cholesterol (r = -0.42, p = 0.02). There was a positive relationship between carbohydrates and FGF21 (r = 0.12, p = 0.28) with all carbohydrate types sharing this positive relationship, the effect was strongest for sucrose (r = 0.31, p = 0.07). In females there was a statistically significant relationship for total sugar (r = 0.58, p = 0.03) and added sugar (r = 0.52, p = 0.04). Protein was negatively related to FGF21 (r = -0.29, p = 0.08). Ethanol was positively correlated with FGF21 (r = 0.20, p = 0.18). Finally, the healthy eating index (HEI) was positively associated with FGF21 for the whole group (*r*

= 0.19, p = 0.19) and for females (r = 0.63, p = 0.01); however, in males, the HEI was negatively related to FGF21 levels (r = -0.20, p = 0.27). Dietary results are presented in table 5.4.

Table 5.4

Correlations between FGF21 and diet variables.

	Whole Group		Females		Males	
Correlate	*r*	*p*	*r*	*p*	*r*	*p*
kcal	0.11	0.31	0.04	0.45	-0.23	0.24
Fat	-0.23	0.14	0.11	0.36	-0.43	0.08
Saturated	-0.37	0.04*	-0.02	0.48	-0.44	0.08
Monounsaturated	-0.23	0.14	0.12	0.36	-0.47	0.06
Polyunsaturated	-0.09	0.35	0.28	0.19	-0.32	0.16
Cholesterol	-0.42	0.02*	-0.30	0.17	-0.51	0.04
Carbohydrates	0.12	0.28	0.42	0.09	-0.01	0.49
Total Sugar	0.21	0.16	0.58	0.03*	0.05	0.44
Added Sugar	0.28	0.09	0.52	0.04*	0.31	0.16
Glucose	0.13	0.27	0.43	0.08	0.09	0.39
Sucrose	0.31	0.07	0.46	0.07	0.16	0.31
Fructose	0.14	0.26	0.41	0.10	0.11	0.37
Fiber	0.12	0.29	0.25	0.22	0.08	0.41
Protein	-0.29	0.08	0.09	0.39	-0.32	0.15
Dairy	-0.23	0.14	0.07	0.41	-0.37	0.12
Ethanol	0.20	0.18	0.07	0.42	0.48	0.05
HEI	0.19	0.19	0.63	0.01*	-0.20	0.27

* = statistically significant correlation.

Metabolic Panel

There was a weak, positive correlation between fasting blood glucose and FGF21 (r = 0.10, p = 0.32). There was a significant, negative relationship between blood urea nitrogen (BUN) levels and FGF21 (r = -0.47, p = 0.01) as well as the BUN:Creatinine ratio (r = -0.37, p = 0.03). Overall, higher levels of FGF21 were associated with decreased glomerular filtration rates (eGFR) (r = -0.90, p = 0.33). FGF21 was positively associated with bilirubin (r = 0.13, p = 0.27) and aspartate transaminase (AST) (r = 0.22, p = 0.14) and negatively associated with the liver enzymes alkaline phosphatase (r = -

0.03, $p = 0.44$) and alanine transaminase (ALT) ($r = -0.21$, $p = 0.16$). In males, this association with ALT was statistically significant ($r = -0.53$, $p = 0.04$). Relationships between FGF21 and metabolic panel variables are shown in table 5.5.

Table 5.5

Correlations between FGF21 and metabolic panel variables.

	Whole Group		Females		Males	
Correlate	*r*	*p*	*r*	*p*	*r*	*p*
Glucose	0.10	0.32	0.24	0.21	0.10	0.37
BUN	-0.47	0.01*	-0.32	0.15	-0.58	0.03*
Creatinine	-0.06	0.39	0.12	0.34	-0.10	0.37
BUN:Creatinine Ratio	-0.37	0.03*	-0.20	0.25	-0.56	0.03*
eGFR	-0.90	0.33	-0.23	0.23	0.07	0.42
Bilirubin	0.13	0.27	0.33	0.14	0.47	0.06
Alkaline Phosphatase	-0.03	0.44	0.35	0.12	-0.01	0.49
AST	0.22	0.14	0.31	0.15	0.23	0.24
ALT	-0.21	0.16	0.09	0.38	-0.53	0.04*

* = statistically significant correlation.

Lipid Panel

Cholesterol was very weakly correlated to FGF21 for the whole group ($r = -0.04$, $p = 0.43$), but in females there was a strong, positive correlation between FGF21 and cholesterol ($r = 0.54$, $p = 0.03$). Both HDL ($r = 0.15$, $p = 0.24$) and LDL ($r = 0.14$, $p = 0.26$) cholesterol were positively related to FGF21. Correlations for the lipid panel results are in table 5.6.

Table 5.6

Correlations between FGF21 and lipid panel variables.

	Whole Group		Females		Males	
Correlate	*r*	*p*	*r*	*p*	*r*	*p*
Cholesterol	-0.04	0.43	0.54	0.03*	-0.33	0.15
Triglycerides	0.00	0.5	0.12	0.34	-0.21	0.26
HDL	0.15	0.24	0.39	0.09	0.18	0.29
LDL	0.14	0.26	0.23	0.23	-0.15	0.32

* = statistically significant correlation.

Blood Pressure and Heart Rate

Systolic blood pressure (SBP) (r = -0.33, p = 0.05) had a stronger relationship to serum FGF21 levels than diastolic blood pressure (DBP) (r = -0.08, p = 0.36). Pulse pressure had a negative correlation with FGF21 (r = -0.31, p = 0.07) and in females this relationship was statistically significant with a strong effect size (r = -0.76, p<0.01). Resting pulse (RP) had a weak, positive relationship with blood FGF21 levels (r = 0.18, p = 0.19). Results for the blood pressure and heart rate data are shown in table 5.7.

Table 5.7

Correlations between FGF21 and cardiovascular variables.

	Whole Group		Females		Males	
Correlate	*r*	*p*	*r*	*p*	*r*	*p*
SBP	-0.33	0.05	-0.49	0.10	0.00	0.50
DBP	-0.08	0.36	-0.20	0.26	-0.01	0.49
MAP	-0.24	0.13	-0.39	0.10	-0.01	0.49
PP	-0.31	0.07	-0.76	<0.01*	0.01	0.49
RP	0.18	0.19	0.21	0.24	0.14	0.34
RPP	-0.06	0.39	-0.13	0.34	0.09	0.39

* = statistically significant correlation.

Discussion

In the present study, we demonstrate a relationship between serum FGF21 levels and the protein's upstream and downstream signaling pathways *in vivo*.

We first investigated the relationship between FGF21 and anthropometric variables. In this study, we witnessed a negative relationship between FGF21 and bodyweight, BMI, and waist circumference. This is in contrast to prior studies that have witnessed a positive relationship between FGF21 and obesity both in mice (4,80) and in humans (43,74,81). The paradoxical relationship of obese individuals having greater levels of circulating FGF21 than normal weight individuals has led to the hypothesis of "FGF21 resistance" where FGF21 levels are elevated as a compensatory mechanism to obesity, but are unable to exert their normal effect. Our inclusion criteria restricted the BMI to normal weight individuals which may help explain this discrepancy.

Next, we investigated the relationship between FGF21 and daily activity and metabolic variables. Acute exercise studies have consistently shown a brief increase in FGF21 post-exercise (2,7,8), while the results of exercise training studies have not been as consistent (82). Previous observation studies have shown a negative relationship between circulating FGF21 and daily physical activity (75) along with cardiorespiratory fitness (83). Similar results are presented in this study with FGF21 being negatively correlated to VO_{2peak} and average daily activity with a trend for greater effect size as activity intensity increased. Furthermore, we saw a positive relationship between FGF21 and sedentary time providing further evidence that more active individuals have lower circulating levels of FGF21. These findings may relate back to the idea of FGF21 resistance where more active individuals have a healthier metabolism and do not experience elevated FGF21 levels. One of the strongest effect sizes in our study was in the investigation of sleep and FGF21 levels. FGF21 has been shown to oscillate in a circadian pattern (41) and sleep plays an important role in maintaining metabolic health

(84); however, little research has been done on the relationship between FGF21 and sleep.

One of the promising findings in FGF21 biology from animal models is that exogenous FGF21 administration can induce weight loss. This weight loss has been demonstrated to be the result of an increased energy expenditure coupled with an increase in fat oxidation (5). In agreement with the work done in animals we observed that circulating FGF21 levels were associated with a greater percent of fuel oxidation coming from fatty acids. In contrast, FGF21 was linked with a lower RMR in our study, particularly in females. The participants in our study were all weight stable, indicating they were at a metabolic homeostasis which may explain this finding. The relationship between metabolic rate and FGF21 in humans is a subject for further investigation, in particular to determine if sex differences observed in this study are present in other cohorts.

The transcription of the *FGF21* gene can be promoted by fatty acid and carbohydrate stimuli (10). We found a negative relationship between FGF21 and fat consumption. In HepG2 cells, fatty acids, particularly unsaturated fatty acids, promoted increased FGF21 secretion (40) and in mice, fasting and the associated rise in free fatty acids can increase circulating FGF21 levels (72). However, in humans a much longer fast is required than in mice to induce similar increases in FGF21. Ketogenic diets in humans also have a minimal effect on FGF21 levels, unlike in mice (72). Furthermore, a high fat diet did not increase human FGF21 levels (44). This provides evidence that the link between FGF21 and fatty acids may not be as strong in humans as it is mice, which may explain the negative relationship found in our study. We found a positive

relationship between carbohydrate consumption and FGF21 levels, with particularly strong effect sizes for added sugar and sucrose. This is consistent with other studies that have indicated an acute overconsumption of carbohydrates is associated with a rise in FGF21 levels (13,44). Protein does not seem to be as involved in the FGF21 as other macronutrients, except that low levels of protein consumption are associated with increased FGF21 levels as part of the fasting response (85). Our observation of a negative relationship between protein consumption and FGF21 is consistent with this idea. Finally, nutrients that are metabolized by the liver such as ethanol and fructose have been shown to dramatically increase FGF21 levels in acute feeding studies (13,76). We saw a positive relationship between both ethanol and fructose with FGF21 levels. One-third of our sample reported zero ethanol consumption over the prior month, which may limit the strength of association in our study.

In our metabolic panel results, we witnessed a smaller correlation between FGF21 and fasting glucose than has been witnessed in other studies (75,81); however, our study was limited to metabolically healthy individuals which may explain this difference. We observed a statistically significant, negative relationship between BUN levels and BUN:Creatinine ratios and FGF21. The role of FGF21 in kidney function is an emerging topic of study (86,87), that will require specific intervention studies to fully elucidate. Decreased BUN levels and a decreased BUN:Creatinine ratio can be caused by problems in the liver, while FGF21 is elevated in some liver diseases (43), which may explain the link between the two.

When exogenous FGF21 has been given to humans, researchers have noted an improvement in dyslipidemia (14). FGF21 is believed to inhibit cholesterol synthesis by

inhibiting sterol regulatory element-binding protein-2 (Srebp-2) in the liver (88). We sought to determine if there is a relationship between endogenous FGF21 and blood lipids. In contrast to pharmaceutical intervention studies, we noticed a significant correlation between cholesterol and FGF21 in females and a positive relationship between LDL and FGF21.

Finally we explored the relationship between FGF21 and indices of blood pressure. We observed a negative relationship between FGF21 and systolic and diastolic blood pressure. We also witnessed a negative relationship between FGF21 and pulse pressure, indicating that FGF21 is associated with greater arterial compliance. This is in agreement with prior work which found that FGF21 protected against atherosclerosis (88). In one study, a decrease in FGF21 was associated with an increase in arterial compliance as measured by brachial-ankle pulse wave velocity (89); however, that finding was part of an exercise intervention study, where decreased FGF21 levels may have been caused by weight loss and where improvements of arterial compliance may have arisen from other factors. In summary, we present evidence that there may be a link between FGF21 and arterial compliance, though this is unlikely to result via direct link and may instead be the result of an intermediary such as adiponectin (88).

Our study is not without limitations. First, this was an observational study with a relatively small number of participants. Therefore, we did not find statistical significance in many variables, but by examining the effect size and direction of the relationship we are able to provide meaningful insights into the relationship between FGF21 and other physiological factors. By averaging physiological variables and blood FGF21 we hoped to overcome the common problem of inter-day variation in FGF21 within the same

individual (11,73). We also employed a fairly homogenous, metabolically healthy cohort of individuals. Endogenous FGF21 levels have been shown to be altered in metabolically unhealthy individuals, and as such our results may not be translatable to other populations.

Conclusion

In this study we present mixed results that suggest normal circulating levels of FGF21 may be related to physiologic and lifestyle factors commonly associated with its action, while other factors such as RMR and LDL cholesterol may not be as related as animal studies and pharmacological interventions indicate. We present strong correlations between FGF21 and sleep and kidney function which are areas prime for future intervention studies. Finally, we observed some sex differences that could be followed up to determine if FGF21 does exhibit sex differences in humans.

CHAPTER SIX

Conclusion

In this dissertation I present two manuscripts – an experimental intervention investigating the effect of acute exercise on serum FGF21 levels and an exploratory analysis investigating the relationship between circulating FGF21 and related physiological factors. Five primary research questions were addressed as part of this dissertation:

1. To what extent is sprint interval exercise able to stimulate increased serum concentrations of FGF21 relative to endurance exercise?

   As indicated by the post-exercise area under the curve, FGF21 concentrations were greater following endurance exercise in both males and females. This evidence supports the alternative hypothesis.

2. To what extent are sex differences present in the FGF21 response to exercise?

   Our data supports the alternative hypothesis. Post-exercise FGF21 concentrations were greater in males for both exercise conditions. This difference was statistically significant for the steady state condition.

3. Is there a relationship between post-exercise FGF21 levels and post-exercise metabolism as measured through indirect calorimetry?

   We support the null hypothesis. Although the pharmaceutical administration of FGF21 in animals leads to an increased energy expenditure and fat oxidation, similar to what is seen in humans in the hours following exercise,

we did not observe any significant relationships between post-exercise metabolism and post-exercise FGF21 levels.

4. To what extent can the variance in post-exercise FGF21 be explained by the variance in circulating signaling factors that change as a result of exercise and are believed to influence FGF21 production?

   Evidence presented here supports the null hypothesis. We did not witness any statistically significant correlations between circulating signaling factors and serum FGF21 concentrations.

5. To what extent are circulating FGF21 levels related to lifestyle and physiological factors involved in the FGF21 pathway?

   Mixed findings for this research question are presented. We found a relatively strong relationship between some factors such as sleep and carbohydrate intake, while other factors such as age and cholesterol levels were not related to FGF21 levels quite as strongly.

Overall Conclusion and Impact

The first manuscript of this dissertation presents novel findings that post-exercise FGF21 levels are greater in males than females, that steady state exercise increases FGF21 levels to a greater extent than sprint interval exercise, and that post-exercise FGF21 levels are not correlated to post-exercise metabolism. The identification of sex differences in the FGF21 response to exercise furthers animal model research that indicates FGF21 has sex specific actions (19). Further research is needed to determine the extent to which sex differences in FGF21 may contribute metabolic differences between males and females. If future studies support the finding of sex-specific roles in

FGF21, this may make a large impact on the development and therapeutic use of FGF21 analogs to treat metabolic disorders. Although sprint interval exercise and steady state exercise have been shown to have similar metabolic effects we found a greater rise in FGF21 levels following the steady state session. This could indicate that steady state exercise is able to promote beneficial metabolic effects not witnessed in sprint interval exercise or alternatively that the post-exercise rise in FGF21 does not impact metabolism. Along these lines, we did not witness a relationship between post-exercise FGF21 and post-exercise metabolism suggesting that the post-exercise increase in FGF21 may not offer a metabolic benefit, or at least not one directly associated with energy expenditure.

In the second manuscript we present an exploratory analysis of FGF21 and physiological and lifestyle factors that are believed to either regulate FGF21 or be regulated by FGF21. We identified strong relationships between FGF21 and sleep and kidney function. This manuscript provides a rationale for future investigation into the biochemical pathways that link FGF21 with sleep and kidney function.

APPENDIX

APPENDIX

Informed Consent Document

Baylor University
DEPARTMENT OF HEALTH, HUMAN PERFORMANCE & RECREATION (HHPR)

Consent Form for Research

PROTOCOL TITLE:	**Metabolic Effects of Sprint Interval and Continuous Exercise**
PRINCIPAL INVESTIGATOR:	**Matthew Peterson**
SUPPORTED BY:	**Texas American College of Sports Medicine** **Baylor University Doctoral Grant**

Invitation to be Part of a Research Study

You are invited to be part of a research study. This consent form will help you choose whether or not to participate in the study. Feel free to ask if anything is not clear in this consent form.

Important Information about this Research Study

Things you should know:

- The purpose of the study is to compare the effects of sprint interval and continuous exercise on markers of metabolism.
- In order to participate, you must be apparently healthy, aged 18 to 45, regularly physically active, have a BMI between 18.5 and 24.9, and if you are a female you must not be pregnant or on hormonal contraceptives**.**
- If you choose to participate, you will be asked to visit the lab two additional times to complete a single sprint interval exercise session and a single continuous exercise session. Each of these sessions will take two hours.
- Risks or discomforts from this research include an adverse reaction to exercise or blood draw.
- The possible benefits of this study include: detailed information about your health such as your current fitness levels, information about the number of calories you burn at rest and during exercise, and body composition (percent body fat, muscle mass, bone density).
- Taking part in this research study is voluntary. You do not have to participate, and you can stop at any time.

More detailed information may be described later in this form. Please take time to read this entire form and ask questions before deciding whether to take part in this research study.

Why is this study being done?

The purpose of this study is to compare the effects of sprint interval and continuous exercise on how your body uses energy. You will be asked to complete two experimental exercise sessions, one will be a sprint interval workout and the other will be a bout of continuous exercise. We will monitor the air that you exhale before, during, and after these exercise sessions to monitor whole body metabolism. We will also take blood samples before and after the exercise sessions (three for each session) to analyze circulating markers of metabolism.

What will happen if I take part in this research study?

If you agree to take part in this study, you will be asked to make three visits to the Baylor Laboratories for Exercise Science and Technology in Marrs McLean Gymnasium, room #127.

Visit 1- Consent, Screening, Baseline Assessment

If you agree to take part in this study, we will describe all aspects of study participation and answer any questions you have. Next, we will ask you to sign this consent form before we do any study procedures. After agreeing to participate we will ask you some questions about your health and exercise habits as part of a pre-exercise screening. We'll ask you to complete a short healthy history form and an exercise questionnaire and review your answers with you.

If you qualify for the study, we will measure your height, weight, waist circumference, and body composition using Dual Energy X-Ray Absorptiometry (DEXA). Next, we will orient you to our stationary bike and assess your maximal exercise capacity. During this process you will be fitted with a heart rate monitor and breathing mask that will allow us to collect the air that you exhale. The assessment of your maximal exercise capacity will begin with you cycling at a light intensity. The resistance will increase every two minutes. The test will continue until you can no longer maintain the desired pedaling rate or feel you are not able to continue. The first visit should take one hour to complete.

Diet and Physical Activity Assessment

As part of our baseline measures, we would like to gain an understanding of your typical dietary and physical activity patterns. To do so, we will ask you to complete the Diet History Questionnaire (DHQ) created by the National Institutes of Health. You may complete the DHQ at home. This will prompt you to indicate the typical foods and amount of those foods you have consumed over the past year. We will also ask you to

wear one of our activity monitors for a three day period consisting of two week days and one weekend day in order to get a better understanding of your usual physical activity and sleep patterns.

Visits 2 and 3 – Experimental Protocols

Visits 2 and 3 will take place in the morning, following an overnight fast, consisting of water only. If you are a female, these experimental protocols will be done while you are in the follicular phase of your menstrual cycle (days 3 through 10). We ask that you maintain a similar diet between the two visits and refrain from strenuous activity the day before each session. As part of this process, we will ask you to keep track of your diet the day before each session and wear one of our activity monitors for twenty-four hours before your session. These two visits will be identical with the exception of the exercise bout. We will start by recording your body weight, then fit you with the heart rate monitor and breathing mask. Once all of the equipment is connected, you will lie down for fifteen minutes while we record your resting heart rate and expired air. During this time, a pre-exercise blood sample will be drawn. After the fifteen minutes of rest have passed, you will begin the exercise session (described below). Following the exercise session, you will return to the supine resting position and a post-exercise blood sample will immediately be collected. One hour after returning to the supine resting position, the face mask will be withdrawn. A final blood sample will also be collected one hour post-exercise. These visits are expected to take two hours to complete.

The order of exercise sessions will be decided at random. Both workouts will be on the stationary bike and begin with a five-minute warmup at a light intensity. For the continuous exercise condition, you will pedal at a moderate intensity for 30 minutes. For the sprint interval condition, you will have six "all out" sprints lasting 30 seconds each, followed by light cycling for four and half minutes (30 minutes of total workout time).

How long will I be in this study and how many people will be in the study?

Participation in this study will require approximately five hours in the lab and we would like all experimental sessions to be completed within one month of enrollment into the study. About thirty-two subjects will take part in this research study.

What are the risks of taking part in this research study?

There are some risks you might experience from being in this study. They are:

Blood Draw Risks

Blood samples will be taken from a vein in your arm during the study. The taking of a blood sample may cause some discomfort and bruising, and there is a potential for infection. Other risks, although rare, include dizziness and fainting.

Radiation Risks

A DEXA scan will be used during this research study. The cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you. However, the effects of radiation add up over a lifetime. It is possible that having several of these tests may add to your risk of injury. When deciding to enter this study, think about your past and future contact with radiation. Examples of contact with radiation include x-rays taken for any reason or radiation therapy for cancer treatment.

Vigorous Exercise

You will be asked to partake in vigorous exercise as part of this study. The risks associated with vigorous exercises include abnormal blood pressure responses, the possibility of fainting, abnormal heart beats, heavy or difficult breathing, and in rare instances, heart attack or death. The risks of vigorous exercise in this study are no greater than they would be outside of the study.

Equipment Contamination

The primary risks associated with the breathing mask and heart rate monitoring are related to using clean equipment. All equipment used in this study will be cleaned using standard procedures (10% bleach solution and/or 70% alcohol).

Are there any benefits from being in this research study?

You might benefit from being in this study because:

- You will receive information about your health and physical capabilities including information about your body composition (body fat, muscle mass, bone density), maximal exercise capabilities, and your body's response to common exercise protocols.
- As part of this study, you will be asked to engage in three bouts of exercise. Physical activity provides well-defined health benefits.

What if you learn something about my health that I did not know?

Although the procedures you will undergo in this study are being undertaken for research purposes only, it is possible that researchers may notice something that could be important to your health. If so, we will contact you to explain what was noticed. If you so desire, we will also talk with your private physician. If you do not have a private physician, we will refer you to an appropriate clinic for follow-up. It will be your choice whether to proceed with additional tests and/or treatments to evaluate what we observed, and you or your insurer will be responsible for these costs.

How Will You Protect my Information?

A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The researcher plans to protect your confidentiality.

We will keep the records of this study confidential by assigning you a unique study ID after you choose to participate in this study. A master list with participant name and

study ID numbers will be kept in a separate locked cabinet from the data collection forms. We will make every effort to keep your records confidential. However, there are times when federal or state law requires the disclosure of your records.

The following people or groups may review your study records for purposes such as quality control or safety:

- The sponsor or funding agency for this study. The sponsor has chosen not to access study records.
- Representatives of Baylor University and the BU Institutional Review Board
- Federal and state agencies that oversee or review research (such as the HHS Office of Human Research Protection or the Food and Drug Administration)

The results of this study may also be used for teaching, publications, or presentations at professional meetings. If your individual results are discussed, your identity will be protected by using a code number or pseudonym rather than your name or other identifying information.

Will information and/or biospecimens you collect about me be used for future research studies?

Information and/or biospecimens collected from you as part of this research may be shared with the research community at large to advance science and health. We will remove or code any personal information that could identify you before the information and/or biospecimens are shared with other researchers to ensure that, by current scientific standards and known methods, no one will be able to identify you from what is shared.

Will I be compensated for being part of the study?

You will be compensated $50.00 in the form of a pre-paid debit card on the last day of your participation in this study, after completing all data collection requirements. We do not have money built into our budget for partial payments if you do not complete all study requirements. However, if you must withdraw early from the study, you will still receive a report of the data we collect.

What happens if I am hurt by participating in this research study?

If you become ill or injured as a result of your participation in the study, you should seek medical treatment from your doctor or treatment center of choice. You should promptly tell the researcher about any illness or injury.

There are no plans for Baylor University to pay you or give you other compensation for your injury or illness. You do not give up any of your legal rights to seek compensation by signing this form.

Who can profit from study results?

Your samples will not be used for commercial profit.

Is it possible that I will be asked to leave the study?

The researcher may take you out of this study without your permission. This may happen because:

- The researcher thinks it is in your best interest
- You can't make the required study visits
- Other administrative reasons

Your Participation in this Study is Voluntary

Taking part in this study is your choice. You are free not to take part or to withdraw at any time for any reason. No matter what you decide, there will be no penalty or loss of benefit to which you are entitled. If you decide to withdraw from this study, the information that you have already provided will be kept confidential. You cannot withdraw information collected prior to your withdrawal.

If you are a Baylor student or faculty/staff member, you may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your grades or job status at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.

Contact Information for the Study Team and Questions about the Research

If you have any questions about this research, you may contact:
Matthew Peterson
Phone: 720-234-0985
Email: Matthew_Peterson1@Baylor.edu

Or

Dr. LesLee Funderburk
Phone: 210-710-7318
Email: LesLee_Funderburk@Baylor.edu

Contact Information for Questions about Your Rights as a Research Participant

If you have questions about your rights as a research participant, or wish to obtain information, ask questions, or discuss any concerns about this study with someone other than the researcher(s), please contact the following:

Baylor University Institutional Review Board

Office of Research Compliance
Phone: 254-710-3708
Email: irb@baylor.edu

Your Consent

SIGNATURE OF SUBJECT:

By signing this document, you are agreeing to be in this study. We will give you a copy of this document for your records. We will keep a copy with the study records. If you have any questions about the study after you sign this document, you can contact the study team using the information provided above.

I understand what the study is about and my questions so far have been answered. I agree to take part in this study.

_____ _____

Signature of Subject Date

Signature of Person Obtaining Consent:

I have explained the research to the subject and answered all his/her questions. I will give a copy of the signed consent form to the subject.

_____ _____

Signature of Person Obtaining Consent Date

Optional Research

The following are optional and will not affect your ability to participate in this research in any way:

Consent to Use Data for Future Research
I agree that my information may be shared with other researchers for future research studies that may be similar to this study or may be completely different. The information shared with other researchers will not include any information that can directly identify me. Researchers will not contact me for additional permission to use this information.

YES_____ NO_____ Initials _____

Consent to be Contacted for Participation in Future Research

I give the researchers permission to keep my contact information and to contact me for future research projects.

YES_____ NO_____ Initials _____

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