RELATIONSHIPS OF FIBROBLAST GROWTH FACTOR 21 WITH INFLAMMATION AND INSULIN RESISTANCE IN RESPONSE TO ACUTE EXERCISE IN OBESE INDIVIDUALS

by

Aaron L. Slusher, B.S., C.S.C.S.

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Chun-Jung (Phil) Huang, Department of Exercise Science and Health Promotion, and has been approved by the members of his supervisory committee. It was submitted to the faculty of the College of Education and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

SUPERVISORY COMMITTEE:

Chun-Jung (Phil) Huang, Ph.D.
Thesis Advisor

Michael Whitehurst, Ed.D.

Robert F. Zoeller, Ph.D.

Deena Wener, Ph.D.
Interim Chair, Department of Exercise Science and Health Promotion

Valerie J. Bristor, Ph.D.
Dean, College of Education

Deborah L. Floyd, Ed.D.
Interim Dean, Graduate College

07/14/2014
Date

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ABSTRACT

Author: Aaron L. Slusher

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Obesity is associated with elevated levels of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), contributing to systemic insulin resistance. Fibroblast growth factor 21 (FGF21) is a vital metabolic and inflammatory regulator, however circulating FGF21 concentrations are elevated in obese individuals. Acute aerobic exercise increases systemic FGF21 in normal-weight individuals, however the effect of acute aerobic exercise on plasma FGF21 response and the relationships with inflammation (IL-6 and TNF-α), insulin resistance, and energy expenditure in obese individuals is unknown. Following 30 minutes of treadmill running at 75% VO_{2max}, plasma FGF21 response, as indicated by area-under-the-curve "with respect to increase" (AUCi) analyses, was attenuated in 12 obese compared to 12 normal-weight subjects. Additionally, FGF21 AUCi positively correlated with glucose AUCi, total relative energy expenditure, and relative VO_{2max}, suggesting that cardiorespiratory

fitness levels may predict FGF21 response, contributing to the enhanced regulation of glucose and energy metabolism.

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I. INTRODUCTION

In the United States, obesity is reaching epidemic proportions as current prevalence rates are 34.9% in adults (Ogden et al. 2014). Obesity is a low grade, chronic inflammatory state contributing to the increased risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (Kahn et al. 2006; Van Gaal et al. 2006). These obesity-associated illnesses are marked by elevated levels of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (Galic et al. 2010; Shurk et al. 2007) through the migration of blood monocytes into white adipose tissue as classically activated (M1), macrophages (Weisberg et al. 2003; Xu et al. 2003). Therefore, the identification of possible mediators contributing to obesity-associated inflammation is essential for early detection, prevention, and treatment of chronic inflammatory diseases.

Fibroblast growth factor 21 (FGF21) is a member of the fibroblast growth factor superfamily (Long and Kharitonenkov 2011) preferentially expressed by hepatocytes, adipocytes, and skeletal muscle (Izumiya et al. 2008; Muise et al. 2008; Nishimura et al. 2000). Circulating FGF21 concentrations are positively associated with body mass index (BMI) (Dushay et al. 2010; Zhang et al. 2008) and elevated in patients with type 2 diabetes mellitus (Chavez et al. 2009), potentially as a compensatory mechanism to mediate obesity related inflammatory and metabolic insult (Feingold et al. 2012; Hojman et al. 2009). For example, FGF21 is increased in response to insulin (Hojman et al. 2009), whereas Feingold et al. (2012) found increased FGF21 expression in adipose

tissue and skeletal muscle as an acute phase response in mice, protecting cells from inflammatory stimuli such as lipopolysaccharide (LPS). Furthermore, FGF21 has been shown to decrease pro-inflammatory cytokine production such as TNF-α via the inhibition of NF-κB in human myotubes (Lee et al. 2012). However, the role of FGF21 in mediating an anti-inflammatory response is unclear (Coskun et al. 2008).

While aerobic exercise training has been demonstrated to decrease the risk of chronic inflammatory diseases (Bruunsgaard 2005), Steensberg et al. (2001) found an elevation in plasma IL-6 concentrations following acute intense exercise. This exercise-induced IL-6 production has been shown to be exacerbated in obese compared to normal-weight individuals (Christiansen et al. 2013), suggesting impaired FGF21 response. However, it is also important to note that elevated IL-6 following exercise may act as an anti-inflammatory mediator. Specifically, Steensberg et al. (2003) infused IL-6 to attain intense exercise-induced concentrations in human subjects and found an elevation in anti-inflammatory cytokines, such as IL-10, after one hour of IL-6 infusion. These findings indicate that an elevation in circulating IL-6 following exercise may initiate a reciprocal anti-inflammatory response that can promote recovery.

Interestingly, exercise training has been shown to lower circulating FGF21 concentrations in overweight and obese individuals (Scalzo et al. 2014; Yang et al. 2011). In response to acute exercise, elevations in circulating FGF21 concentrations have been demonstrated in an intensity-dependent manner up to 4 hours into recovery in normal-weight subjects (Kim et al. 2013; Kondo et al. 2011). Although FGF21 may act as a metabolic regulator controlling glucose uptake (Kharitonenkov et al. 2005; Lee et al. 2012), obese individuals have been shown to be more resistant to FGF21 fluctuations

postprandially as well as during periods of negative energy balance compared to normal-weight individuals (Yu et al. 2011). Given these findings, it remains unclear whether acute exercise would elicit an elevation in circulating FGF21, thus contributing to the regulation of inflammatory responses, insulin resistance, and substrate utilization in obese individuals compared to those of normal-weight.

Therefore, the primary objective of this study was to examine the effect of acute intense exercise on plasma FGF21 response and its relationships with inflammatory cytokines (IL-6, and TNF-α), insulin resistance, and energy utilization in obese and normal-weight subjects. We hypothesized that following acute exercise, plasma FGF21 response in obese subjects would be attenuated compared to normal-weight subjects. Furthermore, we anticipate that the blunted plasma FGF21 response in obese individuals would be accompanied by an exacerbated elevation in plasma IL-6, impaired improvement in insulin resistance, as well as substrate utilization.

II. LITERATURE REVIEW

Introduction

Adult obesity prevalence rates in the United States are 34.9%according to the most recent National Health and Nutritional Examination Survey (Ogden et al. 2014). This is worrisome as obesity is a chronic inflammatory state involved in the accelerated pathogenesis of numerous inflammatory disorders including type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Kahn et al. 2006; Van Gaal et al. 2006) as well as excess mortality relative to normal-weight individuals (Flegal et al. 2007). These obesity-associated illnesses have been linked to elevations in plasma pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) (Galic et al. 2010; Shurk et al. 2007).

Numerous mechanisms are known to contribute to increased pro-inflammatory cytokine profiles including the enlargement of adipocytes and increased accumulation of adipose tissue-specific macrophages (Weisberg et al. 2003; Winkler et al. 2003; Xu et al. 2003). Additionally, elevated IL-6 and TNF-α levels have been demonstrated to play a direct role in the onset of insulin resistance (IR) by inhibiting insulin-stimulated tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1) and the deregulation of glucose transporters (Hotamisligil et al. 1993, 1994b; Rotter et al. 2003).

Fibroblast growth factor 21 (FGF21) is released by adipocytes and skeletal muscle and acts as an endocrine manner regulating insulin sensitivity and inhibiting TNF-α production (Izumiya et al. 2008; Kharitonenkov et al. 2005; Lee et al. 2012; Muise et al. 2008). Elevated plasma FGF21 has been observed in obese populations (Dushay et al. 2010; Zhang et al. 2008). Aerobic exercise training has been shown to decrease the risk of chronic inflammatory diseases and to mediate FGF21 response (Bruunsgaard 2005; Cuevas-Ramos et al. 2010).

Therefore, the purpose of this review is to focus on the mechanisms involved in the pathogenesis of obesity-related inflammatory diseases, specifically IR, and to further understand the role of FGF21 in the mediation of inflammation following exercise.

Inflammation and Macrophage Accumulation

Adipose tissue is an endocrine organ contributing to the regulation of plasma cytokines (Galic et al. 2010). Adipocyte hypertrophy, a characteristic of obesity, is positively correlated with BMI and contributes to the increased production of IL-6 and TNF-α in both mRNA and plasma (Galic et al. 2010; Shurk et al. 2007). This is further exacerbated in obese compared to normal-weight populations (Winkler et al. 2003). Weight loss, however, is associated with decreased TNF-α mRNA in adipose tissue (Kern et al. 1995).

Increased adiposity is also linked to elevated monocyte chemoattractant protein 1 (MCP-1) and colony stimulating factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kim et al. 2008; Shurk et al. 2007). MCP-1 is a chemoattractant secreted by adipose tissue specific for monocytes and macrophages, contributing to the migration of blood monocytes to white adipose tissue (WAT)

(Matsushima et al. 1989; Weisberg et al. 2003; Yoshimura et al. 1989). In chronic inflammatory states, adipocyte MCP-1 mRNA and secretion levels are elevated (Sartipy and Loskutoff 2003), as well as macrophage infiltration and accumulation (Weisberg et al. 2003; Xu et al. 2003). Furthermore, GM-CSF is released by activated macrophages into circulation where they rescue monocytes from apoptosis through the induction of autophagy, an essential process for monocyte differentiation into tissue bound macrophages (Zhang et al. 2012). However, MCP-1 and GM-CSF knockout mice display blunted migration and decreased macrophage infiltration and accumulation in adipose tissue, respectively (Kanda et al. 2006; Kim et al. 2008; Lu et al. 1998).

Adipose tissue macrophages also undergo a phenotypic switch from the anti-inflammatory M2, *alternatively activated*, macrophages to pro-inflammatory M1, *classically activated*, macrophages (Lumeng et al. 2007; Weisberg et al. 2003). Weisberg et al. (2003) showed that the percentage of the M1 macrophages surface marker CD68 in human adipose tissue increases with BMI, ranging from 10% in lean subjects to 50% in obese. M1 macrophages are the predominant source of systemic IL-6 and TNF-α and enhance GM-CSF release (Hamilton 2002; Weisberg et al. 2003). These findings support the association between adipocyte hypertrophy and increased M1 macrophage accumulation, with the exacerbation of IL-6 and TNF-α levels.

Obesity, Inflammation, and Exercise

Acute aerobic exercise has been shown to elevate pro-inflammatory cytokine production. More specifically, Louis et al. (2007) examined the effect of a 30-minute acute exercise at 75% $VO_{2\ max}$ on inflammatory cytokine expression in skeletal muscle and found that there was a 187-fold increase in IL-6 mRNA 8 hours post exercise while

TNF-α mRNA levels increased 4.5-fold 12 hours post exercise. Furthermore, these exercise-induced cytokine levels have been shown to associate with BMI (Harris et al. 2008).

However, aerobic exercise training has been shown to reduce low-grade inflammation in obese individuals (Bruun et al. 2006; Bruunsgaard 2005). For example, plasma IL-6, MCP-1, and TNF- α decreased in response to aerobic exercise training greater than 12 weeks (Bruun et al. 2006; Strączkowski et al. 2001), while adipose tissue IL-6 and TNF-α mRNA levels also decreased after 15 weeks (Bruun et al. 2006). Furthermore, M1 macrophage marker CD68 mRNA levels decreased by 40% in adipose tissue following 15 weeks of aerobic exercise training (Bruun et al. 2006). Previous studies also show that at least a 10% weight reduction is needed to reduce these obese inflammatory parameters (Forsythe et al. 2008). These findings support that weight loss and exercise training act as strong intervention methods for decreasing pro-inflammation.

Inflammation and Insulin Resistance

Obesity-induced inflammation is closely related with IR. During normal homeostatic states, insulin binds to the tyrosine kinase receptor's α subunit on the cell membrane of adipose tissue and skeletal muscle, resulting in the phosphorylation of the β subunit. This triggers the phosphorylation of IRS-1 and the subsequent downstream translocation of the glucose transporter, primarily GLUT-4, to the cell membrane through the mediation of numerous proteins. Elevated plasma levels of IL-6 and TNF-α are correlated with IR and directly inhibit insulin-stimulated signaling and GLUT-4 translocation (Hotamisligil et al. 1994b; Shepherd and Kahn 1999).

In obese and insulin resistant rats, insulin-stimulated tyrosine autophosphorylation of IRS-1 by the insulin receptor is decreased in adipose tissue compared lean rats (Hotamisligil et al. 1996). Mechanistically, Hotamisligil et al. (1996) demonstrated that TNF-α treatment mediated phosphorylation of IRS-1 serine residue interfering with insulin-stimulated tyrosine phosphorylation of IRS-1. However, TNF-α knockout mice were protected against such reductions and demonstrated decreased fasting glucose and insulin levels (Uysal et al. 1997).

In skeletal muscle, TNF-α decreases insulin sensitivity by stimulating c-Jun NH_2-terminal kinase (JNK) phosphorylation and reducing the phosphorylation of Akt (Plomgaard et al. 2005; Yuasa et al. 1998). JNK phosphorylates serine reside of IRS-1 impairing insulin-stimulated autophosphorylation of IRS-1 by the insulin receptor (Aguirre et al. 2000). Akt is an important downstream kinase which regulates glucose uptake by triggering GLUT-4 translocation to the cell membrane. Reduced Akt phosphorylation decreases GLUT-4 translocation, highlighting the multi-faceted mechanistic role of TNF-α-induced IR (Plomgaard et al. 2005).

To simulate chronic low-grade inflammation characteristic of obesity, chronic treatment with TNF-α reduced insulin-stimulated insulin receptor tyrosine autophosphorylation and IRS-1 phosphorylation in a dose dependent manner (Hotamisligil et al. 1994a). In obese mice, TNF-α treatment also reduced GLUT-4 mRNA levels in adipose tissue compared to lean mice (Hotamisligil et al. 1993). IL-6 infusion displayed similar results, however the effects were neither as potent nor rapid (Hotamisligil et al. 1993; Rotter et al. 2003). Interestingly, TNF-α has also been shown to stimulate the release of IL-6 from adipocytes *in vitro* (Rotter et al. 2003), demonstrating

that TNF-α mediates IR both directly and indirectly. Therefore, the need to identify possible mediators involved in the control of obesity associated inflammation and IR is an important step towards the early detection, prevention, and treatment of chronic inflammatory diseases.

Exercise and the IL-6 Paradox

Aerobic exercise training also improves IR in both normal-weight and obese populations (Boulé et al. 2001; Goodpaster et al. 2003), even in the absence of weight loss (Duncan et al. 2003). Acute aerobic exercise also increases insulin sensitivity in normal-weight and obese subjects immediately following exercise and throughout recovery (Jamurtas et al. 2006; Magkos et al. 2010; Richter et al. 1982).

Numerous studies indicate that mRNA expression and plasma IL-6 increase following acute exercise (Bruunsgaard 2005; Louis et al. 2007; Steensberg et al. 2001, 2002). Increases in IL-6 are dependent upon the duration, intensity, and the number of skeletal muscle fibers recruited during exercise (Pedersen and Febbraio 2005). For example, Nielsen et al. (1996) showed a twofold increase in IL-6 mRNA after just six minutes of maximal rowing exercise, while Steensberg et al. (2002) demonstrated a 100-fold increase in response to three hours of strenuous exercise. Additionally, exercise induces increases in skeletal muscle JNK mRNA independent of TNFα, which has been shown to mediate IL-6 production in skeletal muscle (Boppart et al. 2000; Frost et al. 2004).

This highlights an IL-6 paradox. While IL-6 is commonly referenced to as a pro-inflammatory cytokine in association with IR, evidence indicates that IL-6 promotes

glucose uptake by stimulating GLUT-4 translocation in skeletal muscle at rest and in response to exercise independent of insulin (Carey et al. 2006; Steensberg et al. 2002).

IL-6 also works as an anti-inflammation mediator. Specially, Steensberg et al. (2003) infused IL-6 to attain intense exercise-induced concentrations in human subjects and observed an elevation in anti-inflammatory cytokines, such as interleukin-10 (IL-10), after 1 h of IL-6 infusion. Furthermore, IL-6 has been shown to antagonize the pro-inflammatory effects of TNF-α (Pedersen et al. 2003; Starkie et al. 2003). These findings indicate that an elevation in circulating IL-6 following exercise may initiate a reciprocal anti-inflammatory response promoting recovery.

Fibroblast Growth Factor 21

FGF21 is a member of the fibroblast growth factor superfamily (Long and Kharitonenkov 2011). Numerous tissues express and release FGF21 into plasma where it acts in an endocrine manner. In normal homeostatic conditions, FGF21 signals through FGF receptors (FGFR), forming a complex with the βKlotho protein required for high-affinity FGF21 binding (Kuro-o 2008). This FGFR-βKlotho complex triggers tyrosine kinase activity to regulate insulin sensitivity, lipid metabolism, as well as preserve pancreatic β-cell function by rescuing cells from death by apoptosis in diabetic animal models (Hotta et al. 2009; Kharitonenkov et al. 2007; Wente et al. 2006). Specifically, FGF21 mRNA is mediated by peroxisome proliferator-activated receptor gamma in adipose tissue and Akt activation in skeletal muscle (Izumiya et al. 2008; Muise et al. 2008). Furthermore, FGF21 promotes glucose uptake in adipose tissue and skeletal muscle through the activation of Akt and the subsequent translocation of glucose

transporters to the cell membrane (Cuevas-Ramos et al. 2009; Kharitonenkov et al. 2005; Lee et al. 2012).

FGF21, Inflammation, and Insulin Resistance

Studies have shown that obesity is associated with elevated plasma FGF21 levels (Dushay et al. 2010; Zhang et al. 2008). Fisher et al. (2010) compared diet-induced obese (DIO) FGF21 knockout mice with DIO wild-types (WT), demonstrated that DIO WT mice displayed elevated FGF21 in both plasma and adipose tissue mRNA levels after the onset of obesity while possessing a similar phenotype to FGF21 KO mice, one which was not present prior to feeding. Additionally, adipose tissue FGFR and βKlotho mRNA expression were also reduced following feeding in DIO WT mice (Fisher et al. 2010). Furthermore, Hale et al. (2012) supported that FGF21 sensitivity decreases in obese mice however, mechanisms contributing to the resistant state of FGF21 still remain uncertain.

Interestingly, a recent study in humans demonstrates FGF21 reduced the production of TNF-α via the inhibition of NF-κB (Lee et al. 2012). FGF21 also demonstrated to inhibit expression of TNF-α-induced JNK phosphorylation, subsequently increasing βKlotho expression of in skeletal muscle (Lee et al. 2012), supporting the anti-inflammatory effects of FGF21 in humans.

Therapeutically, FGF21 increased energy expenditure while enhancing the utilization of fat as energy, resulting in a 20% reduction of total body weight in diet-induced obese mice (Coskun et al. 2008). Specifically, FGF21 treatment targets whole-body and liver fat mass, while preserving fat free mass. Furthermore, Kharitonenkov et al. (2005) demonstrated that FGF21 treatment corrected IR in both obese and diabetic mice models. After 7 days of treatment, resting glucose concentrations decreased by 25-

35% due to the increased phosphorylation of the tyrosine kinase membrane receptor and downstream activation of the GLUT-1 glucose transporter. These studies indicate that FGF21 treatment increases FGF21 sensitivity in obese and IR states.

FGF21and Exercise

Exercise may serve as an effective intervention towards increasing FGF21 sensitivity in obese populations. However, studies on the response of FGF21 as result to exercise are limited. Cuevas-Ramos et al. (2010) reported that daily physical activity has a positive influence on plasma FGF21 levels. However, recent studies have shown inconsistent results in the effects of exercise training on plasma FGF21 levels. For example, Cuevas-Ramos et al. (2012) observed a significant increase in FGF21 levels in normal-weight females in response to two weeks of submaximal training. Alternatively, three weeks sprint interval training reduced circulating FGF21 levels in overweight individuals (Scalzo et al. 2014), while FGF21 concentrations were normalized in overweight and obese women after three months of submaximal aerobic training and resistance training (Yang et al. 2011), correlating with decreases in BMI as well as increases in insulin sensitivity. Furthermore, daily exercise in obese rats normalized FGF21 concentrations and increased FGF21 sensitivity by enhancing β-Klotho expression in the liver (Fletcher et al. 2012). In response to acute exercise, Kondo et al. (2011) reported an elevation in plasma FGF21 correlating with IL-6 elevation up to 4 hours recovery in healthy male subjects in response to the performance of the Bruce protocol followed by 30-minutes of cycling at 85% $VO_{2\ Peak}$, while Kim et al. (2013) demonstrated that exercise-induced FGF21 increases may be intensity dependent.

However, no studies have examined whether FGF 21 is associated with inflammatory responses and/or insulin sensitivity following acute intense exercise in obese individuals.

Summary

Obesity-associated inflammatory parameters, such as TNF-α, have demonstrated on numerous occasions to increase the risk of IR and released diseases. Furthermore, evidence has demonstrated that increased TNF-α plays a strong role to increase adipocyte hypertrophy and macrophage accumulation. Although obese individuals have higher plasma FGF21 levels compared to normal-weight individuals, FGF21 has been demonstrated to counter the effects of TNF-α-mediated conditions such as IR. Aerobic exercise training has been shown to reduce pro-inflammatory states in obese populations; however, no studies have examined whether FGF21 may mediate inflammatory responses and/or insulin sensitivity following acute exercise in obese individuals.

III. METHODS

Subjects

Twenty-four healthy subjects (12 obese [5 males; 7 female] and 12 normal-weight [6 male; 6 female]) ages 18-35 were recruited to participate in the study. Subjects with a BMI above 30 kg/m^2 were classified as obese, and those with a BMI between 18.5-24.9 kg/m^2 were classified as normal-weight. The study was approved by Florida Atlantic University's Institutional Review Board and all subjects provided informed written consent prior to participation in the study. Participants also completed a medical history questionnaire and 7-day physical activity record prior to data collection.

Subjects were excluded from the study if they possessed any known inflammatory diseases/conditions (e.g., cardiovascular disease, chronic kidney or liver disease, diabetes) or were under current administration of medication that may influence inflammatory or metabolic processes. Subjects were also excluded from the study if they were users of tobacco products (cigarettes, cigars, chewing tobacco), or if they consumed an average of ten or more alcoholic beverages per week. Subjects were instructed to undergo an overnight fast for at least eight hours and to abstain from alcohol, caffeine intake, and intense physical activity for at least 24 hours prior to each laboratory visit. Finally, women who were pregnant or nursing also were excluded from the study because of the potential effects on immune responses (Mor and Cardenas 2010).

Procedures

A schedule of subject visits is demonstrated in Table 1. Subjects were asked to arrive at the laboratory between 7:00 and 9:00 on the morning of the testing sessions. The first session consisted of completion of informed consent, familiarization with all instruments and procedures, and anthropometric measures. A maximal graded exercise test on a treadmill to assess maximal oxygen consumption (VO_{2max}) was then administered beginning with a 3 minute warm-up at 3 mph with 0% grade. Speed was subsequently increased to elicit 80% ± 5 bpm of the subject's age predicted maximal heart rate (HR). After 4 minutes, grade was increased 2% every 2 minutes while speed remained constant until voluntary exhaustion resulted within 12 to 15 minutes. Resting HR was measured prior to the test, continuously throughout exercise, and upon recovery with a HR monitor (Polar T31, Polar Electro, Kempele, Finland). Blood pressure was measured at rest and during recovery with a stethoscope and sphygmomanometer (752M-Mobile Series, American Diagnostic Corporation, Hauppauge, NY). Ratings of perceived exertion (RPE) were obtained using the Borg 15 point scale and recorded every exercise stage. Pulmonary ventilation (V_E) and respiratory gases (carbon dioxide (VCO_2) and oxygen (VO_2)) were measured continuously by open-circuit spirometry (ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT, USA)). VCO_2 and VO_2 were averaged every 15 seconds to calculate respiratory exchange ratio (RER: VCO_2/VO_2). Criteria for attaining $VO_{2\ max}$ included attainment of two of the following criteria: a plateau in O_2 consumption (defined as a failure to increase oxygen uptake by 150 ml/min with increased workload), RER ≥ 1.15, HR within 10 bpm of subject's age-predicted maximum heart rate (220-age), and an RPE ≥ 19.

The second exercise testing session consisted of 30 minutes of continuous exercise at 75% VO_{2max} as determined during session one. HR and BP as well as metabolic/respiratory data were measured as for session one.

A 10 ml blood sample was drawn from each subject's anticubital vein prior to, immediately post, and 1 and 2 hours into recovery using a 21G butterfly needle into a tube containing K_2 ethylenediaminetetraacetic acid (K_2EDTA) (BD Vacutainer, Franklin Lakes, NJ). Blood samples were immediately centrifuged at 3000 RPM for 20 minutes at room temperature. Plasma was collected and immediately stored at -80°C in cryogenic tubes in 500µL aliquots for subsequent analysis of FGF21 (R&D Systems, Minneapolis, MN), insulin (ALPCO, Salem, NH, USA), and glucose (Cayman Chemical Company, Ann Arbor, MI, USA) using enzyme-linked immunosorbent assay (ELISA) according to manufacture instructions. High sensitivity assay kits were utilized for the quantification of the plasma inflammatory cytokines IL-6 and TNF- α (R&D Systems, Minneapolis, MN). Due to cost restraints, plasma cytokine concentrations were only assessed in 10 obese [4 male; 6 female] and 10 normal-weight [4 male; 6 female] subjects.

Measures of HOMA-IR, Substrate Utilization, and Total REE

Insulin resistance (IR) was determined by the homeostasis model assessment estimate of insulin resistance (HOMA-IR), calculated from plasma insulin and glucose values according to Matthews et al. (1985):

Fasting insulin (µU/ml) x fasting glucose (mg/dL)/405.

Upon reaching metabolic steady-state during the submaximal testing session, values for VCO_2 and VO_2 were identified for the duration of the testing and averaged for each subject for the determination of substrate utilization and total REE using

stoichiometric equations according to Frayn (1983), with the assumption that urinary nitrogen excretion rate was negligible (Achten and Jeukendrup 2003):

i) FATOxi (g/min^{-1}) = $1.67 \cdot VO_2 - 1.67 \cdot VCO_2$

ii) CHOoxi (g/min^{-1}) = $4.55 \cdot VCO_2 - 3.21 \cdot VO_2$

iii) Total REE (kcal/kg) = ([Fat Oxidation · 9.1 kcal/g · 30 min] + [CHO Oxidation · 4.1 kcal/g ·30 min]) / Body Weight (kg)

As indicated by Achten and Jeukendrup (2003), the stoichiometric calculations obtained through indirect calorimetry are based on the assumption that CO_2 originates from the oxidation of protein, fat, and carbohydrates. VO_2 will only be a reliable estimate of tissue CO_2 production in the presence of a stable bicarbonate pool, however the utilization of indirect calorimetry can be used to validly determine fuel oxidation at high intensities up to 80-85% VO_{2max} (Romijn et al. 1992).

Statistical Analyses

Data analysis was performed using the SPSS version 21.0. Independent t-tests were conducted to compare baseline levels of all variables between obese and normal-weight subjects. A two group (obese and normal-weight) by four time point (pre, immediately post-exercise, and recovery one and two hour) repeated measures analysis of variance (ANOVAs) was used to evaluate the effect of acute aerobic exercise on FGF21, IL-6, TNFα, insulin, glucose, and HOMA-IR response. If the Mauchly's test indicated violation of the sphericity assumption, the degrees of freedom were corrected by using Greenhouse-Geisser estimates. To assess intensity of FGF21 response to exercise, integrated trapezoidal area-under-the-curves with “respect to increase” from baseline (AUCi) was calculated according to the previously published formula by Pruessner et al.

(2003). AUCi was also calculated for IL-6, TNF-α, insulin, and glucose. The differences between groups were evaluated for each variable's AUCi using independent t-tests. Finally, Pearson's correlation was utilized to examine the relationships of FGF21 with IL-6, TNF-α, indices of IR at baseline and in response to exercise (AUCi) as well as with substrate utilization (CHOoxi and FAToxi) and total REE. All data are presented as means ±S.E.M. with statistical significance being defined as $p \leq 0.05$.

IV. RESULTS

Participant Anthropometric and Cardiovascular Characteristics

Baseline characteristics of obese and normal-weight subjects are reported in Table 2. Obese subjects exhibited significantly greater measures of body weight (t [22] = 5.7, p < 0.001), BMI (t [14.47] = 10.59, p < 0.001), waist and hip circumferences (t [22] = 7.64; t [15.16] = 7.61, p < 0.001, respectively), waist-to-hip ratio (t [22] = 3.54, p < 0.001), resting heart rate (t [22] = 2.26, p = 0.034), and resting systolic and diastolic blood pressures (t [22] = 3.88; (t [22] = 3.76, p = 0.001, respectively). Additionally, relative cardiorespiratory fitness levels (relative VO_{2max}) were significantly less compared to normal-weight subjects (t [19.97] = -6.0, p < 0.001), whereas no differences in absolute VO_{2max} was observed.

Comparison of Plasma FGF21, Inflammatory Cytokines, and HOMA-IR

Table 3 indicates a comparison of plasma FGF21, IL-6, TNF-α, and indices of IR at rest and in response to exercise. Our analyses did not show any differences in baseline FGF21, IL-6, or TNF-α plasma concentrations between obese and normal-weight subjects. Resting plasma insulin and glucose concentrations were elevated in obese compared to normal-weight subjects (t [22] = 2.23, p = 0.036; t [22] = 3.44, p = 0.002, respectively) as well as HOMA-IR index (t [22] = 2.33, p = 0.029). Finally, baseline FGF21 was negatively correlated with IL-6 (r = -0.462, p = 0.04), even after controlling

for BMI, whereas no relationships were observed between FGF21 with TNF-α and indices of IR.

In response to acute exercise, repeated measures ANOVA demonstrated a significantly attenuated FGF21 response (F [2.02, 44.56] = 6.33, p = 0.004) and glucose (F [1.23, 27.1] = 5.7, p = 0.019) in obese compared to normal-weight subjects while we only observed significant changes across time for IL-6 (F [3, 54] = 3.05, p = 0.036), insulin (F [1.99, 43.78] = 8.71, p = 0.001), and HOMA-IR (F [1.85, 40.62] = 14.04, p < 0.001) in both obese and normal-weight groups. However no time effect was observed in TNF-α.

Furthermore, our results also showed that FGF21 AUCi was less in obese subjects compared to normal-weight subjects (t [17.28] = -3.2, p = 0.005; Figure 1A); however no differences in IL-6 AUCi, TNF-α AUCi, insulin AUCi, and glucose AUCi were observed between groups. In addition, FGF21 AUCi was negatively correlated with BMI (r = -0.471, p = 0.02) and positively correlated with relative VO_{2max} (r = 0.646, p = 0.001; Figure 1B) and glucose AUCi (r = 0.495, p = 0.014; Figure 1C), whereas no other correlations were observed. However the relationship between FGF21 AUCi and glucose AUCi were not observed when controlling for BMI.

Measures of Substrate Utilization and Total REE during Exercise

Table 4 demonstrates substrate utilization and total REE during exercise in both groups. Our analyses did not show any differences in CHOoxi and FATOxi following acute aerobic exercise between the two groups. When assessing total REE, obese subjects expended less total energy relative to body weight than normal-weight subjects (t [11.77]

= -7.73, $p < 0.001$). Additionally, FGF21 AUCi was positively correlated with total REE (r = 0.562, $p = 0.004$; Figure 1D).

V. DISCUSSION

The present study sought to examine the effect of acute intense exercise on plasma FGF21 response and the relationships with inflammatory cytokines (IL-6 and TNF-α), insulin resistance, and substrate utilization (CHOoxi, FATOxi, and total REE) in obese and normal-weight subjects. Our main findings demonstrate obese subjects had an attenuated FGF21 response to acute aerobic exercise compared to normal-weight subjects while FGF21 levels remained elevated and were greater in normal-weight compared to obese subjects following two hours recovery. However, this elevated FGF21 response was not associated with any inflammatory cytokine responses following exercise. Despite no difference in substrate utilization between the two groups, total REE was lower in obese compared to normal-weight subjects. Furthermore, FGF21 AUCi was positively correlated with glucose AUCi, total REE, and relative VO_{2max}. These findings suggest that impaired cardiorespiratory fitness level may influence the sensitivity of FGF21 response (AUCi) to acute exercise in obese individuals, potentially contributing to the attenuated metabolic response (e.g., glucose) and total exercise energy expenditure.

To date, various studies have indicated that FGF21 levels are moderately elevated in obese populations (Chavez et al. 2009; Reinehr et al. 2012; Zhang et al. 2008), serving as a compensatory response to counteract inflammatory (Feingold et al. 2012) and metabolic insult (Hojman et al. 2009), indicating that increased circulating FGF21 levels are the result, not a causation, of obesity (Fisher et al. 2010). Interestingly, our study did not observe a difference in baseline levels of plasma FGF21 between obese and

normal-weight subjects. Although fasting concentrations of circulating FGF21 in obese and normal-weight individuals have been shown to peak during nocturnal hours (05:00), daily variations are less pronounced in obese compared to normal-weight subjects (Yu et al. 2011). These patterns of flux contribute to negligible differences in circulating FGF21 concentrations during overnight fasting and peak disparities observable at midday (14:30) when both obese and normal-weight individuals reach the nadir, suggesting that circulating FGF21 similarities observed between obese and normal-weight subjects in this study may partially reflect sample timing. Additionally, HOMA-IR values were elevated in obese compared to normal-weight subjects, suggesting that the lack of FGF21 flux contributes to dysregulated glucose and insulin concentrations in obese subjects (Yu et al. 2011).

Furthermore, acute exercise induces plasma FGF21 increases, which have been positively associated with circulating free fatty acids (FFA) levels (Kim et al. 2013). This exercise-induced release of FGF21 is intensity-dependent and preferentially released from hepatocytes (Kim et al. 2013), resulting in increased levels of circulating FGF21 up to four hours post-exercise in normal-weight populations (Kim et al. 2013; Kondo et al. 2011; Lee et al. 2014). However, while circulating FGF21 concentrations are less responsive in obese individuals during periods of negative energy balance (Yu et al. 2011), and FFA flux has been shown to be attenuated during exercise in obese individuals (Kanaley et al. 1993), we are the first to report that the exercise-induced FGF21 response is attenuated in obese individuals compared to those of normal-weight. Our study also found that the FGF21 response, as measured by AUCi, was positively correlated with glucose AUCi and total REE. Thus, these findings may support our

results demonstrating more robust elevation in plasma FGF21 to acute exercise in normal-weight, not obese, subjects, potentially contributing to the regulation of glucose and energy metabolism during exercise.

While IL-6 is commonly identified as a pro-inflammatory cytokine in association with IR, evidence indicates that IL-6 promotes glucose uptake in skeletal muscle at rest and in response to exercise, independent of insulin (Carey et al. 2006; Steensberg et al. 2002). Furthermore, *in vivo* IL-6 infusion as well as exercise-induced IL-6 increases have been shown to antagonize the pro-inflammatory effects of TNF-α (Starkie et al. 2003), suggesting that IL-6 is not a catalyst of metabolic dysfunction (Pedersen et al. 2003). In this study, FGF21 was negatively correlated with IL-6 concentrations at baseline; however, like FGF21, no differences in baseline inflammatory cytokine concentrations were observed in obese and normal-weight subjects. Furthermore, our study showed no difference between groups for the increase in IL-6 following exercise. A recent study by Christiansen et al. (2013) has observed a greater production of IL-6 in obese compared to normal-weight subjects following two hours cycling exercise at 55-60% maximal heart rate, suggesting that the lack of difference in IL-6 response between both subject groups in our study may have been due to differences in exercise protocols. Since it still remains unclear whether cytokines represent a mechanism for the potential anti-obesity action of FGF21 (Coskun et al. 2008), future research is warranted to gain a better understanding of the interaction between FGF21 and obesity-associated inflammation.

Another major fining of this study was the positive correlation observed between FGF21 AUCi and relative VO_{2max}, even after controlling for BMI. Previous studies have demonstrated that FGF21 increases following cardiovascular stress, rescuing cardiac cells

from apoptosis *in vitro* and potentially contributing to tissue remodeling (Kondo et al. 2011; Kotulák et al. 2011; Lü et al. 2010). Furthermore, baseline FGF21 levels have been shown to be positively associated with daily levels of physical activity (Cuevas-Ramos et al. 2010), while exercise training-induced FGF21 increases in normal-weight individuals corresponded with increased cardiorespiratory fitness (Cuevas-Ramos et al. 2012). In obese individuals, exercise training has shown to reduce chronic inflammation and IR (Bruun et al. 2006; Goodpaster et al. 2003), potentially through the normalization of FGF21 levels (Scalzo et al. 2014; Yang et al. 2011). Additionally, like therapeutic FGF21 administration, daily exercise in obese animal models increases expression of β-Klotho (Fletcher et al. 2012; Lee et al. 2012), an FGF co-binding protein down-regulated in obese individuals contributing to an FGF21-resistant state (Adams et al. 2012; Fisher et al. 2010; Ogawa et al. 2007). These findings and the fact that our study showed that healthy controls had higher FGF21 levels during exercise than obese subjects supports the position that, enhanced cardiorespiratory fitness augments the FGF21 response. Importantly, the exercise induced FGF21 response may contribute to improved cardiac health and reduce the risk for CVD.

In summary, our study demonstrates that the FGF21 response to a bout of vigorous exercise is attenuated in obese individuals compared to those of normal weight. However, while it remains unclear whether FGF21 initiates an anti-inflammatory response, FGF21 response is positively associated with glucose response and total REE, suggesting that increased FGF21 levels as result of acute aerobic exercise may play a role in modulating glucose and energy metabolism. Furthermore, relative VO_{2max} may be a strong predictor of FGF21 response, indicating that improved cardiorespiratory fitness

may better serve to beneficially modulate obesity-associated CVD through enhanced FGF21 flux, however future studies are warranted to elucidate the possible role of FGF21 on inflammation and vascular health.

TABLES

Table 1. Schedule of Subject's Visit

Time	Session 1
7:30 a.m.	Subject Arrival; Obtainment of consent
7:45 a.m.	Anthropometric assessment; Resting values
8:00 a.m.	Begin maximal exercise test
8:30 a.m.	End maximal exercise test
	Session 2
7:30 a.m.	Subject Arrival
7:45 a.m.	Resting values
8:00 a.m.	Pre-exercise blood sample
8:05 a.m.	Begin submaximal exercise test
8:35 a.m.	End submaximal exercise test
8:35 a.m.	Immediate post-test blood sample
9:35 a.m.	Recovery 1 hour blood sample
10:35 a.m.	Recovery 2 hour blood sample

Table 2. Participant descriptive characteristics and anthropometric measures

Variable	Normal-weight (n = 12: 6M/6F)	Obese (n = 12: 5M/7F)	*P* value
Age (y)	23.17 ± 0.63	22.67 ± 1.25	0.723
Absolute VO_{2max} (L/min^{-1})	3.01 ± 0.27	2.97 ± 0.2	0.907
Relative VO_{2max} ($mL/kg/min^{-1}$)	46.95 ± 2.24	30.44 ± 1.61	*< 0.001
Weight (kg)	64.22 ± 3.51	99.08 ± 5.0	*< 0.001
Height (m)	1.7 ± 0.03	1.67 ± 0.03	0.458
BMI	22.04 ± 0.47	35.46 ± 1.18	*< 0.001
Waist (cm)	71.67 ± 2.0	98.88 ± 2.95	*< 0.001
Hip (cm)	94.96 ± 1.19	117.33 ± 2.69	*< 0.001
WH	0.75 ± 0.01	0.84 ± 0.02	*0.002
Resting HR (bpm)	67.83 ± 2.07	75.42 ± 2.64	*0.034
Resting SBP (mmHg)	110.67 ± 2.79	125.83 ± 2.75	*0.001
Resting DBP (mmHg)	72.17 ± 1.85	82.17 ±1.91	*0.001

Note: The * indicates a significant difference between normal-weight and obese groups at baseline ($p < 0.05$). Data are presented as means ± SEM. VO_{2max}, maximal oxygen uptake; BMI, body mass index; WH, waist-to-hip ratio; HR, Heart Rate; SBP, systolic blood pressure; DBP, diastolic blood pressure

Table 3. Assessment of plasma FGF21, IL-6, TNF-α, insulin, and glucose concentrations and HOMA-IR index at baseline and in response to acute exercise in normal-weight and obese subjects

Variable		Pre-Exercise	Post-Exercise	Recovery 1 hour	Recovery 2 hour	Effect
FGF21 (pg/ml)	Normal-Weight	114.21 ± 20.37	140.88 ± 19.76	187.72 ± 27.87	198.42 ± 25.74*	*time* = 0.002
	Obese	103.21 ± 12.87	109.71 ± 14.75	128.97 ± 20.14	91.79 ± 11.63	*group* = 0.004
IL-6 (pg/ml)	Normal-Weight	2.18 ± 0.4	4.64 ± 0.99	3.25 ± 0.48	3.92 ± 0.82	*time* = 0.036
	Obese	3.95 ± 1.23	4.62 ± 0.67	3.83 ± 0.35	4.88 ± 0.8	*group* = NS
TNF-α (pg/ml)	Normal-Weight	2.8 ± 0.18	2.99 ± 0.21	2.87 ± 0.16	2.75 ± 0.15	*time* = NS
	Obese	2.65 ± 0.12	2.72 ± 0.15	2.25 ± 0.14	3.86 ± 1.13	*group* = NS
Insulin (μIU/ml)	Normal-Weight	7.56 ± 1.66	11.87 ± 2.03	6.15 ± 2.37	4.19 ± 0.97	*time* = 0.001
	Obese	17.26 ± 4.02*	23.65 ± 6.69	13.6 ± 2.46*	11.96 ± 2.13*	*group* = NS
Glucose (mg/dl)	Normal-Weight	90.22 ± 1.94	134.47 ± 8.73	86.3 ± 2.6	88.59 ± 1.33	*time* < 0.001
	Obese	98.47 ± 1.41*	117.06 ± 5.84	95.24 ± 2.58*	87.7 ± 2.91*	*group* = 0.019
HOMA-IR	Normal-Weight	1.72 ± 0.41	3.81 ± 0.58	1.41 ± 0.61	0.91 ± 0.01	*time* < 0.001
	Obese	4.18 ± 0.97*	6.69 ± 1.74	3.15 ± 0.52*	2.88 ± 0.5*	*group* = NS

Note: There was a significant change across time for all variables in both the normal-weight and obese groups. The * indicates a significant difference between normal-weight and obese groups at each timepoint ($p < 0.05$). Data are presented as means ± SEM.

Table 4. Substrate Utilization and Total REE in response to acute exercise in normal-weight and obese subjects

Variable	Normal-Weight	Obese	*P* value
CHOoxi (g/min^{-1})	2.51 ± 0.22	2.36 ± 0.15	0.583
FATOxi (g/min-1)	0.22 ± 0.04	0.25 ± 0.04	0.655
Total REE (kcal/kg)	5.68 ± 0.25	3.69 ± 0.18	*< 0.001

Note: The * indicates a signficant difference between normal-weight and obese groups following exercise ($p < 0.05$). Data are presented as means ± SEM.

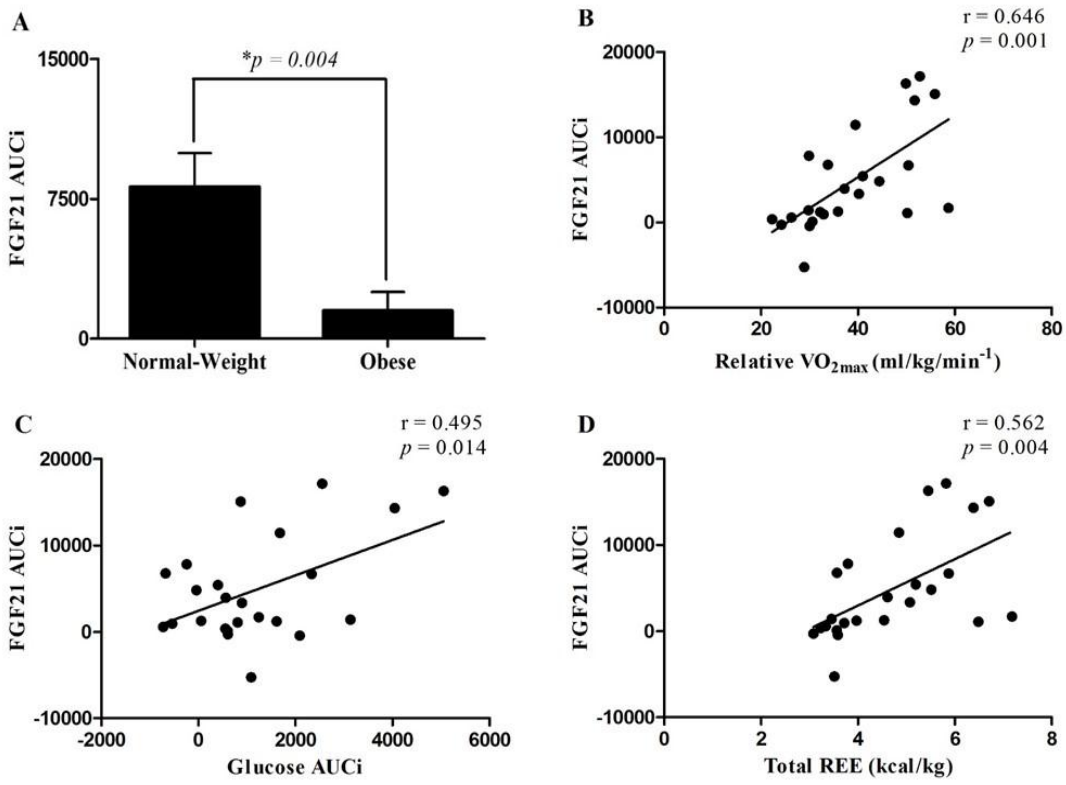

Figure 1. The intensity of FGF21 response (AUCi) to exercise between obese and normal-weight subjects and the relationship between FGF21 AUCi with VO_{2max}, glucose AUCi,and total REE. FGF21 AUCi was significantly blunted in obese compared to normal-weight subjects (panel A). Following exercise, FGF21 AUCi was and positively correlated with VO_{2max}, glucose AUCi, and total REE (panels B-D). The * indicates a significant difference between normal-weight and obese groups ($p < 0.05$). Data are presented as means ± SEM.

REFERENCES

Achten J, Jeukendrup AE (2003) Maximal fat oxidation during exercise in trained men. Int J Sports Med 24:603-608.

Adams AC, Cheng CC, Coskun T, Kharitonenkov A (2012) FGF21 requires βKlotho to act *in vivo*. PLOS ONE 7:ee49977.

Aguirre V, Uchida T, Yenush L, Davis R, White MF (2000) The c-Jun NH_2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser^{307}. J Biol Chem 275:9047-9054.

Boppart MD, Asp S, Wojtaszewski JFP, Fielding RA, Mohr T, Goodyear LJ (2000) Marathon running transiently increases c-Jun NH_2-terminal kinase and p38γ activities in human skeletal muscle. J Physiol 526:663-669.

Boulé NG, Haddad E, Kenny GP, Wells GA, Sigal RJ (2001) Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: A metaanalysis of controlled clinical trials. JAMA 286:1218-1227.

Bruun JM, Helge JW, Richelsen B, Stallknecht B (2006) Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. Am J Physiol Endocrinol Metab 290:E961-E967.

Bruunsgaard H (2005). Physical activity and modulation of systemic low-level inflammation. J Leukoc Biol 78:819-835.

Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, Prelovsek O, Hohnem-Behrens C, Watt MJ, James DE, Kemp BE, Pedersen BK, Febbraio MA (2006) Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes 55:2688-2697.

Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, de Fronzo RA, Tripathy D (2009) Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care 32:1542-1546.

Christiansen T, Bruun JM, Paulsen SK, Ølholm J, Overgaard K, Pedersen SB, Richelsen B (2013) Acute exercise increases circulating inflammatory markers in overweight and obese compared with lean subjects. Eur J Appl Physiol 113:1635-1642.

Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A (2008) Fibroblast growth factor 21 corrects obesity in mice. Endocrinology 149:6018-6027.

Cuevas-Ramos D, Almeda-Valdes P, Aguilar-Salinas CA, Cuevas-Ramos G, Cuevas-Sosa AA, Gomez-Perex FJ (2009) The role of fibroblast growth factor 21 (FGF21) on energy balance, glucose and lipid metabolism. Current Diabetes Reviews 5:216-220.

Cuevas-Ramos D, Almeda-Valdes P, Gómez-Pérez FJ, Meza-Arana CE, Cruz-Bautista I, Arellano-Campos O, Navarrete-López M, Aguilar-Salinas CA (2010) Daily physical activity, fasting glucose, uric acid, and body mass index are independent

factors associated with serum fibroblast growth factor 21 levels. Eur J Endocrinol 163:469-477.

Cuevas-Ramos D, Almeda-Valdés P, Meza-Arana CE, Brito-Córdova G, Gómez-Pérez FJ, Mehta R, Oseguera-Moguel J, Aguilar-Salinas CA (2012) Exercise increases serum fibroblast growth factor 21 (FGF21) levels. PLOS ONE 7:e38022.

Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW (2003) Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. Diabetes Care 26:557-562.

Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, Badman MK, Martinez-Chantar ML, Maratos-Flier E (2010) Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterol 139:456-463.

Feingold KR, Grunfeld C, Heuer JG, Gupta A, Cramer M, Zhang T, Shigenaga JK, Patzek SM, Chan ZW, Moser A, Bina H, Kharitonenkov A (2012) FGF21 is increased by inflammatory stimuli and protects leptin-deficient *ob/ob* mice from the toxicity of sepsis. Endocrinology 153:2689-2700.

Fisher FM, Chui PC, Antonellis PJ, Bina HA, Kharitonenkov A, Flier JS, Maratos-Flier E (2010). Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 59:2781-2789.

Flegal KM, Graubard BI, Williamson DF, Gail MH (2007) Cause-specific excess deaths associated with underweight, overweight, and obesity. JAMA 298:2028-2037.

Fletcher JA, Meers GM, Laughlin MH, Ibdah JA, Thyfault JP, Rector RS (2012) Modulating fibroblast growth factor 21 in hyperphagic OLETF rats with daily exercise and caloric restriction. Appl Physiol Nutr Metab 37:1054-1062.

Forsythe LK, Wallace JMW, Livingstone MBE (2008) Obesity and inflammation: The effects of weight loss. Nutrition Research Reviews 21:117-133.

Frayn KN (1983) Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55:628-634.

Frost RA, Nystrom GJ, Lang CH (2004) Epinephrine stimulates IL-6 expression in skeletal muscle and C_2C_{12} myoblasts: role of c-Jun NH_2-terminal kinase and histone deacetylase activity. Am J Physiol Endocrinol Metab 286:E809-E817.

Galic S, Oakhill JS, Steinberg GR (2010) Adipose tissue as an endocrine organ. Mol Cell Endocrinol 316:129-139.

Goodpaster BH, Katsiaras A, Kelley DE (2003) Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabetes 52:2191-2197.

Hale C, Chen MM, Stanislaus S, Chinookoswong N, Hager T, Wang M, Véniant MM, Xu J (2012) Lack of overt FGF21 resistance in two mouse models of obesity and insulin resistance. Endocrinology 153:69-80.

Hamilton JA (2002) GM-CSF in inflammation and autoimmunity. Trends Immunol 23:403-408.

Harris RA, Padilla JN, Hanlon KP, Rink LD, Wallace JP (2008) The flow-mediated dilation response to acute exercise in overweight active and inactive men. Obesity 16:578-584.

Hojman P, Pedersen M, Nielsen AR, Krogh-Madsen R, Yfanti C, Akerstrom T, Nielsen S, Pedersen BK (2009) Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. Diabetes 58:2797-2801.

Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 259:87-91.

Hotamisligil GS, Budavari A, Murry D, Spiegelman BM (1994a) Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes: Central role of tumor necrosis factor-α. J Clin Invest 94:1543-1549.

Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM (1994b) Tumor necrosis factor α inhibits signaling from the insulin receptor. Proc Natl Acad Sci U.S.A 91:4854-4858.

Hotamisligil GS, Peraldi P, Budavari AA, Ellis R, White MF, Spiegelman BM (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha and obesity-induced insulin resistance. Science 271:665-668.

Hotta Y, Nakamura H, Konishi M, Murata Y, Takagi H, Matsumura S (2009) Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver. Endocrinology 150:4625-4633.

Izumiya Y, Bina HA, Ouchi N, Akasaki Y, Kharitonenkov A, Walsh K (2008) FGF21 is an Akt-regulated myokine. FEBS Letters 582:3805-3810.

Jamurtas AZ, Theocharis V, Koukoulis G, Stakias N, Fatouros IG, Kouretas D, Koutedakis Y (2006) The effects of acute exercise on serum adiponectin and

resistin levels and their relation to insulin sensitivity in overweight males. Eur J Appl Physiol 97:122-126.

Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840-846.

Kanaley JA, Cryer PE, Jensen MD (1993) Fatty acid kinetic responses to exercise: Effects of obesity, body fat distribution, and energy-restricted diet. J Clin Invest 92:255-261.

Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 116:1494-1505.

Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB (1995) The expression of tumor necrosis factor in human adipose tissue: Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95:2111-2119.

Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li D, Mehrbod F, Jaskunas R, Shanafelt AB (2005) FGF-21 as a novel metabolic regulator. J Clin Invest 115:1627-1635.

Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CJ, Tigno XT, Hansen BC, Shanafelt AB, Etgen GJ (2007) The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 148:774-781.

Kim DH, Sandoval D, Reed JA, Matter EK, Tolod EG, Woods SC, Seeley RJ (2008) The role of GM-CSF in adipose tissue inflammation. Am J Physiol Endocrinol Metab 295:E1038-E1046.

Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS (2013) Acute exercise induced FGF21 expression in mice and in healthy humans. PLOS ONE 8:e63517.

Kondo H, Hojo Y, Saito T, Ikemoto T, Katsuki T, Shimada K, Kario K (2011) Relationship between fibroblast growth factor 21 and extend of left ventricular remodeling after acute myocardial infarction. J Clinic Experiment Cardiol 2:139.

Kotulák T, Drápalová J, Kopecký P, Lacinová Z, Kramář P, Říha H, Netuka I, Malý J, Housa D, Bláha J, Svačina Š, Haluzík M (2011) Increased circulating and epicardial adipose tissue mRNA expression of fibroblast growth factor-21 after cardiac surgery: Possible role in postoperative inflammatory response and insulin resistance. Physiol Res 60:757-767.

Kuro-o M (2008) Endocrine FGFs and Klothos: Emerging concepts. Trends Endocrin Met 19:237-245.

Lee MS, Choi S, Ha ES, An S, Kim TH, Han SJ, Kim HJ, Kim DJ, Kang Y, Lee K (2012) Fibroblast growth factor-21 protect human skeletal muscle myotubes from palmitate-induced insulin resistance by inhibiting stress kinase and NF-κB. Metabolism 61:1142-1151.

Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS (2014) Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metabolism 19:302-309.

Long YC, Kharitonenkov A (2011) Hormone-like fibroblast growth factors and metabolic regulation. Biochim Biophys Acta 1812:791-795.

Louis E, Raue U, Yang Y, Jemiolo B, Trappe S (2007) Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. J Appl Physiol 103:1744-1751.

Lu BB, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ (1998) Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattratant protein 1-deficient mice. J Exp Med 187:601-608.

Lü Y, LIU J, Zhang L, Du J, Zeng X, Hao G, Huang J, Zhao D, Wang G, Zhang Y (2010) Fibroblast growth factor 21 as a possible endogenous factor inhibits apoptosis in cardiac endothelial cells. Chin Med J 123:3417-3421.

Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117:175-184.

Magkos F, Mohammed BS, Mittendorfer B (2010) Enhanced insulin sensitivity after acute exercise in not associated with changes in high-molecular weight adiponectin concentrations in plasma. Eur J Endocrinol 162:61-66.

Matsushima K, Lrsen CG, DuBois GC, Oppenheim JJ (1989) Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med 169:1485-1490.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Turner RC (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419

Mor G, Cardenas I (2010) The immune system in pregnancy: A unique complexity. Am J Reprod Immunol 63:425-433.

Muise ES, Azzolina B, Kuo DW, El-Sherbeini M, Tan Y, Yuan X. Mu J, Thompson JR, Berger JP, Wong KK (2008) Adipose fibroblast growth factor 21 is up-regulated by peroxisome proliferator activated receptor gamma and altered metabolic states. Mol Pharmacol 74:403-412.

Nielsen HB, Secher NH, Christensen NJ, Pedersen BK (1996) Lymphocytes and NK cell activity during repeated bouts of maximal exercise. Am J Physiol Regul Integr Comp Physiol 271:R222-R227.

Nishimura T, Nakatake Y, Konishi M, Itoh N (2000) Identification of a novel FGF, FGF21, preferentially expressed in the liver. Biochim Biophys Acta 1492:203-206

Ogden CL, Margaret D, Kit BK, Flegal KM (2014) Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311:806-814.

Ogawa Y, Kurosu H, Yamamoto M, Nandi A, Rosenblatt KP, Goetz R, Eliseenkova AV, Mohammadi M, Kuro-o M (2007) βKlotho is required for metabolic activity of fibroblast growth factor 21. PNAS 104:7432-7437.

Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, van Hall G, Plomgaard P, Febbraio MA (2003) Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. Pflüger Archiv Eur J Phy 446:9-16.

Pedersen BK, Febbraio M (2005) Muscle-derived interleukin-6-A possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun 19:371-376.

Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK (2005) Tumor necrosis factor-α induces skeletal muscle insulin resistance in health human subjects via inhibition of akt substrate 160 phosphorylation. Diabetes 54:2939-2945.

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003) Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrino 28:916-931.

Reinehr T, Woelfle J, Wunsch R, Roth CL (2012) Fibroblast growth factor 21 (FGF21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: A longitudinal analysis. J Clin Endocrinol Metab 97:2143-2150.

Richter EA, Garetto LP, Goodman MN, Ruderman NB (1982) Muscle glucose metabolism following exercise in the rat. J Clin Invest 69:785-793.

Romijn J, Coyle E, Hibbert J, Wolfe R (1992) Comparison of indirect calorimetry and a new breath 13C/12C ratio method during strenuous exercise. Am J Physiol Endocrinol Metab 263:E64-E71.

Rotter V, Nagaev I, Smith U (2003) Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-α, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 278:45777-45784.

Sartipy P, Loskutoff DJ (2003) Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci 100:7265-7270.

Scalzo RL, Peltonen GL, Giordano GR, Binns SE, Klochak AL, Paris HLR, Schweder MM, Szallar SE, Wood LM, Larson DG, Luckasen GJ, Hickey MS, Bell C (2014)

Regulators of human white adipose browning: Evidence for sympathetic control and sexual dimorphic responses to sprint interval training. PLOS ONE 9:e90696.

Shepherd PR, Kahn BB (1999) Glucose transporters and insulin action: implications for insulin resistance and diabetes mellitus. N Engl J Med 341:248-257.

Shurk T, Alberti-Huber C, Herder C, Hauner H (2007) Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 92:1023-1033.

Starkie R, Ostrowski SR, Jauffred S, Fabbraio M, Pedersen BK (2003) Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. FASEB J 17:884-886.

Steensberg A, Toft, AD, Bruunsgaard, H, Sandmand M, Halkjaer-Kristensen J, Pedersen BK (2001) Strenuous exercise decreases the percentage of type 1 T cells in the circulation. J Appl Physiol 9:1708–1712.

Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Pedersen BK (2002) IL-6 and TNF-α expression in, and release from, contracting human skeletal muscle. Am J Physiol Endocrinol Metab 283:E1272-E1278.

Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK (2003) IL-6 enhances plasma Il-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab 285:E433–E437.

Strączkowski M, Kowalska I, Dzienis-Strączkowska S, Stępień A, Skibińska E, Szelachowska M, Kinalska I (2001) Changes in tumor necrosis factor-α system and insulin sensitivity during an exercise training program in obese women with normal and impaired glucose tolerance. Eur J Endocrinol 145:273-280.

Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature 389:610-614.

Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444:875-880.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr. AW (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796-1808.

Wente W, Efanov AM, Brenner M, Kharitonenkov A, Köster A, Sandusky GE, Sewing S, Treinies I, Zitzer H, Gromada J (2006) Fibroblast growth factor-21 improves pancreatic β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. Diabetes 55:2470-2478.

Winkler G, Kiss S, Keszthelyi L, Sapi Z, Öry I, Salamon F, Kovács M, Vargha P, Szekeres O, Speer G, Karádi I, Sikter M, Kaszás E, Dworak O, Gerö G, Cseh K (2003) Expression of tumor necrosis factor (TNF)-α protein in the subcutaneous and visceral adipose tissue in correlation with adipocyte cell volume, serum TNF-α, soluble serum TNF-receptor-2 concentrations and C-peptide level. Eur J Endocrinol 149:129-135.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112:1821-1830.

Yang SJ, Hong HC, Choi HY, Yoo HJ, Cho GJ, Hwang TG, Baik SH, Choi DS, Kim SM, Choi KM (2011) Effects of a three-month combined exercise programme on

fibroblast growth factor 21 and fetuin-A levels and arterial stiffness in obese women. Clin Endocrinol 75:464-469.

Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu JI, Leonard EJ (1989) Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. J Exp Med 169:1449-1459.

Yu H, Xia F, Lam KSL, Wang Y, Bao Y, Zhang J, Gu Y, Zhou P, Lu J, Jai W, Xu A (2011) Circadian rhythm of circulating fibroblast growth factor 21 is related to diurnal changes in fatty acids in humans. Clin Chem 57:691-700.

Yuasa T, Ohno S, Kehrl JH, Kyriakis JM (1998) Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH_2-terminal kinase (JNK) and p38. J Biol Chem 273,22681-22692.

Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RLC, Chow W, Tso AWK, Lam KSL, Xu A (2008) Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 57:1246-1253.

Zhang Y, Morgan MJ, Chen K, Choksi S, Liu Z (2012) Induction of autophagy is essential for monocyte-macrophage differentiation. Blood 119:2895-2905.