Effect of Acute Aerobic Exercise on the

Thioredoxin System in Lean, Healthy Subjects

BY

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B.S., Salisbury University, 2015

THESIS

Submitted as partial fulfillment of the requirements for the degree of
Master of Science in Kinesiology
Graduate College
University of Illinois at Chicago, 2017

Chicago, Illinois

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ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS

T2DM   Type II diabetes

TXNIP   Thioredoxin Interacting Protein

REDD1   Regulated in Development and DNA damage responses 1

TRX   Thioredoxin

NGT   Normal Glucose Tolerant

IGT   Impaired Glucose Tolerant

ROS   Reactive Oxygen Species

PKC   Protein Kinase C

JNK   c-jun N terminal kinases

IRS   Insulin Receptor Substrate

PI3K   Phosphatidylinositol-3-Kinase

AGEs   Advanced Glycation Endproducts

RAGE   Receptor for Advanced Glycation Endproducts

AT   Aerobic Training

RT   Resistance Training

CT   Combined Training

GSH-Px   Glutathione Peroxidase

CAT   Catalase

SOD   Superoxide Dismutase

TBARS   Thiobarbituaric Acid Reactive Substance

TRXr   Thioredoxin Reductase

ASK1	Apoptotic Signaling Kinase
PTEN	Phosphatase Tensin homolog 10
REF-1	Redox Factor-1
HIF-1	Hypoxia-Inducible Factor-1a
NF-κB	Nuclear Factor Kappa-light-chain-enhancer of activated B cells NF-κB
AP-1	Activator Protein-1
NRF-2	Nuclear Factor E2-Related Factor 2
GR	Glucocorticoid Receptor
ER	Estrogen Receptor
ETC	Electron Transport Chain
AICAR	5-aminoimidazole-4-carboxamide ribonucleotide
PBMC	Peripheral Blood Mononuclear Cells
PP2A	Protein Phosphatase 2
AKT	Protein Kinase B
P38 MAPK	P38 Mitogen-Activated Protein Kinase
FOXO1	Forkhead Box 01 Transcriptional Factor
CHREBP	Carbohydrate Response Element Binding Protein
P300	Histone Acetyltransferase p300
PA	Palmitic Acid
NAC	N-Acetyl-L-cysteine
AMPK	AMP-Activated Protein Kinase

LIST OF ABBREVIATIONS (continued)

SIRT1   Sirtuin 1

mTOR   Mammalian Target of Rapamycin

TSC1/2   Tuberous Sclerosis Complex

1. INTRODUCTION

Type II diabetes (T2DM) is a debilitating disease affecting over 29 million Americans which places significant economic burden on the healthcare system with over $245 billion in annual costs (1). T2DM is characterized by chronic hyperglycemia which promotes oxidative stress and inflammation in multiple tissues. If left untreated, this can lead to further damage increasing the risk of heart disease, kidney failure, and augmented cognitive decline (2-5). Although T2DM requires the coordinated dysfunction of several tissues, understanding the precise molecular mechanisms that promote skeletal muscle insulin resistance should be a chief concern, considering it has been shown to the be the primary defect in T2DM (6). Specifically, a protein target that has gained recent attention in this area is thioredoxin-interacting protein (TXNIP).

TXNIP is a member of the ∝-arrestin protein family capable of eliciting its cellular effects by forming complexes with other protein targets including thioredoxin (TRX) and the protein Regulated in Development and DNA damage responses 1 (REDD1) (7) . By binding to redox-sensitive cysteine residues of TRX, TXNIP is capable of reducing its activity in order to promote oxidative stress (8, 9). Additionally, TXNIP has also been shown to dimerize with REDD1 to inhibit nutrient-sensitive growth signaling pathways in skeletal muscle (10). Together, this suggests that through its protein-protein interactions, TXNIP plays a role in skeletal muscle metabolism and redox state, both of which are dysregulated in individuals with T2DM (11, 12) In agreement, skeletal muscle biopsies taken from individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and T2DM have shown that TXNIP gene expression is elevated across the glucose tolerance continuum, indicating that changes in its expression parallels with the severity

of the disease (13). Additionally, *in vitro* analysis using primary human skeletal muscle cells has revealed that overexpression of the TXNIP gene caused significant reductions in insulin-mediated glucose uptake, whereas silencing TXNIP produced an opposite, insulin-sensitizing effect (13).

Although TXNIP expression has been shown to vary across the glucose tolerance continuum, the mechanisms underlying these disparities remain largely unknown. (14). In terms of lifestyle choices, it is well-documented that a lower percentage of individuals with T2DM participate in regular aerobic exercise compared to their age-matched, non-diabetic counterparts. As an intervention, aerobic exercise is effective in improving skeletal muscle insulin sensitivity, but the molecular mechanisms have yet to be fully elucidated (15-21). Recent evidence from pre-clinical and cell culture models have indicated that exercise may be able to regulate TXNIP expression via posttranslational modifications that augment its rate of proteasome degradation (22, 23). Hence, modulation of TXNIP may be involved in the improvements in insulin sensitivity following acute aerobic exercise. In order to gain a comprehensive understanding of the exercise's effect on TXNIP, it is important to first establish a normal physiological response to acute exercise that can be juxtaposed with responses of a diseased population.

1.1 Purpose of the study

The purpose of this study was to determine the effect of high intensity (80% VO$_{2max}$) aerobic exercise in young (18-35 yrs.), lean (BMI 18-26 kg/m^2), healthy individuals on TXNIP and its interacting partners, REDD1 and TRX. Muscle biopsies were taken from the vastus lateralis before, immediately post, and 3 h post exercise to track the changes in TXNIP protein levels, post translational modifications, and interactions with protein targets that mediate its cellular effects.

1.2 Specific Aims and Hypotheses

To address the purpose of the study we developed the following specific aims:

Specific Aim 1: *Determine the effect of acute aerobic exercise on protein expression, post translational modifications, and protein-protein interactions of TXNIP.* Our working hypothesis is that exercise will increase the phosphorylation and ubiquitination status of TXNIP, marking it for degradation, leading to reduced protein expression in the post exercise state. As a result, TXNIP will have a reduced capacity to form a protein complex with REDD1. We will accomplish this specific aim by obtaining skeletal muscle biopsy samples prior to exercise, immediately post exercise, and 3h post exercise. Immunoprecipitation and western blotting will be used to quantify protein expression, posttranslational modifications of interest, and protein interactions with REDD1.

Specific Aim 2: *Determine the effect of acute aerobic exercise on TRX protein expression, and protein-protein interactions.* Our working hypothesis is that exercise will increase TRX protein expression and reduce TRX: TXNIP complex formation, resulting in a greater proportion of unbound TRX in the post-exercise state. We will accomplish this specific aim by obtaining skeletal muscle biopsy samples prior to exercise, immediately post

exercise, and 3h post exercise. TRX will be immunoprecipitated from the tissue homogenate and immunoblotted for its inhibitor, TXNIP.

1.3 Problem Statement

Understanding the normal regulation of TXNIP following acute aerobic exercise is required for the proper interpretation of responses seen in a diseased population. It is also important to understand how exercise may dependently and independently modulate its interacting partners REDD1 and TRX. Establishing the effects of exercise on TXNIP, REDD1, and TRX will further implicate exercise as medicine and may lead to the development of exercise mimetics so that individuals who are contraindicated for exercise can still obtain the potential benefits.

1.4 Significance of Problem

T2DM affects 9.3% of the worldwide population with a rate of 1.7 million new diagnoses arising each year (1). In order to effectively combat the onset and associated complications of the disease, new interventions must be developed toward improving skeletal muscle insulin resistance which is known to be the primary defect in T2DM (6). Recent evidence has shown that TXNIP exacerbates skeletal muscle insulin resistance and as such, may be a suitable target for the treatment of T2DM (13, 24). Preclinical and *in vitro* models have suggested that aerobic exercise may be capable of modulating TXNIP and its interacting partners, but *in vivo* investigations with human subjects with or without disease have yet to be completed. Following the completion of this study, we will have a better understanding of exercise's effect on TXNIP, TRX, and REDD1 in normal physiology, which can later be applied to a diseased population.

2. REVIEW OF THE LITERATURE

The development of T2DM is associated with chronic hyperglycemia increasing oxidative stress and inflammation in several tissues throughout the body. If left untreated, secondary complications can arise including heart disease, kidney failure, and augmented cognitive decline. Since the pathogenesis is instigated and propagated by insulin resistance, therapies should focus on increasing insulin sensitivity in skeletal muscle. Skeletal muscle is responsible for ~80% of insulin-mediated glucose uptake and recent evidence has suggested that upregulation of the pro-oxidant, TXNIP can attenuate glucose uptake and metabolism contributing the insulin-resistant phenotype. Therefore, the purpose of this review is to: 1. explain the link between oxidative stress and insulin resistance. 2. understand the role of exercise in improving antioxidant capacity and reducing oxidative stress 3. explore the TRX system and its endogenous inhibitor, TXNIP in the context of metabolism and diabetes 4. explain the mechanisms through which TXNIP protein levels can be regulated, and 5. explore the role of REDD1 and the REDD1/TXNIP axis in modulating skeletal muscle metabolism.

2.1   Role of oxidative stress in insulin resistance

Skeletal muscle insulin resistance marks the beginning of T2DM pathogenesis by placing increased demand on the β-cells to generate more insulin in response to attenuated glucose uptake rates. Chronically, this increased demand will accelerate the rate of β-cell death, decreasing their collective capacity to produce insulin thus leading to persistent hyperglycemia (25, 26). Currently, several mechanisms exist to explain the etiology of insulin resistance including mitochondrial dysfunction (27, 28), fatty acid overload (29, 30), inflammation (29, 31, 32) , and hyperinsulinemia (33, 34). However, a

common underpinning that exists behind insulin resistance is the increased production of reactive oxygen species (ROS) (35-38).

ROS is produced from several subcellular locations including the mitochondria, cytosol, peroxisomes, and lysosomes. Typically, ROS is scavenged rapidly by the endogenous antioxidant systems and can play a role in normal cell physiology (39-41), but these systems can be chronically overwhelmed in states of obesity and T2DM, which allows ROS to modify proteins, nucleic acids, and lipids leading to oxidative stress (42, 43). In addition, ROS activates several downstream enzymes including protein kinase C (PKC) and c-jun N terminal kinases (JNK) leading to serine phosphorylation of insulin receptor substrate (IRS) (36, 44). This post translational modification inhibits the recruitment of phosphatidylinositol-3-kinase (PI3K) and thus prevents activation of the distal segment of the insulin signaling pathway (45).

As mentioned above, overproduction of ROS is a shared consequence between all of the stimuli that cause insulin resistance. Importantly, in preclinical models, overexpression of certain endogenous antioxidants (46), deletion of genes that encode for pro-oxidant proteins (47), or treatment with exogenous antioxidants such apocynin (48) prevents insulin resistance induced by a high fat diet. Additionally, restriction of pro-oxidant compounds in food has been investigated in the context of glucose tolerance. Uribarri et al. recruited both healthy and T2DM subjects and randomly assigned them one of two isocaloric diets: a control diet or a diet that restricted advanced glycation end products (AGEs) for four months (49). AGEs are proteins, lipids, or DNA that have been non-enzymatically and irreversibly conjugated to a sugar molecule (50). These compounds have the ability to bind with the receptor for advanced glycation end products

(RAGE) triggering the production of ROS via NADPH oxidase (51). By restricting exogenous AGEs, circulating AGEs were reduced and insulin sensitivity was improved in the T2DM cohort (49, 52). Based on this data such as this, research has been devoted to understanding the effect of antioxidant supplementation for the treatment of metabolic disease. Short term studies have yield positive results in terms of glucose tolerance and insulin resistance (52-55), but the long-term efficacy has yet to be established. With this in mind, it is important to establish therapies that reduce oxidative stress for the treatment of insulin resistance and T2DM.

2.2   Effects of aerobic exercise on antioxidant capacity and oxidative stress

Aerobic exercise training has been used as a therapy to reestablish the redox balance in several clinical populations. For T2DM specifically, four studies have been conducted to investigate the effect of aerobic exercise on oxidative stress and antioxidant capacity, all of which have provided evidence that aerobic exercise elicits increases in antioxidant capacity as defined by enzyme activity and protein levels, and decreases in oxidative stress markers. Oliveira et al. recruited assigned 31 T2DM subjects to one of the following 12-week interventions: aerobic (AT), resistance (RT), combined (CT; resistance + aerobic), or no training. Following the intervention, the activity levels for the antioxidants, glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) were improved in the erythrocyte fraction. In addition, aerobic exercise training improved systemic redox markers by reducing thiobarbituric acid reactive substance (TBARS), a maker of oxidative stress and all exercise interventions increased total plasma sulfhydryl group, a marker of antioxidant capacity (56). These findings were substantiated further by Mitranun et al. who found that both continuous and interval

aerobic exercise was able to increase the levels of erythrocyte superoxide dismutase and glutathione peroxidase and decrease the levels of malondialdehyde, a marker for oxidative stress (57).

However, it is important to note that the effect of exercise on the antioxidant capacity in skeletal muscle has yet to be elucidated in T2DM. Further, most of the studies that have be conducted in skeletal muscle use preclinical and cell culture models. For example, Powers, et al. has investigated the effects of different aerobic exercise training intensities and time on antioxidant capacity in rat skeletal muscle. Following 120 days of aerobic training, SOD and GSH-Px activity were improved significantly in a fiber type, intensity and duration-dependent manner (58). This data suggests that skeletal muscle contains a higher proportion of oxidative, type I fibers has the greatest capacity to improve antioxidant capacity. Other studies have investigated the same antioxidant systems *in vivo* as well and have consistently shown that GSH-Px and SOD expression and activity increase following aerobic exercise training (59-63). Another antioxidant system that has been recently investigated in the context of metabolic disease, but has not been investigated in the context of skeletal muscle metabolism during exercise, is the thioredoxin (TRX) system.

2.3   TRX Antioxidant System

The thioredoxin (TRX) system is composed of NADPH, thioredoxin reductase (TRXr), TRX, and its endogenous inhibitor TXNIP. The general mechanism of this system is outlined in Figure 1. TRX contains a dithiol moiety that are reduced by electrons donated by NAPDH via the enzyme TXRr. Following the reduction, TRX is capable of reducing oxidized residues on proteins and scavenging ROS via the enzyme

peroxiredoxin (64-68). In mammals, three isoforms of TRX exist: TRX 1, 2, and 80. TRX1 and 2 are ubiquitously expressed (69-71), whereas TRX80 is the result of posttranslational cleavage of TRX1 (72).
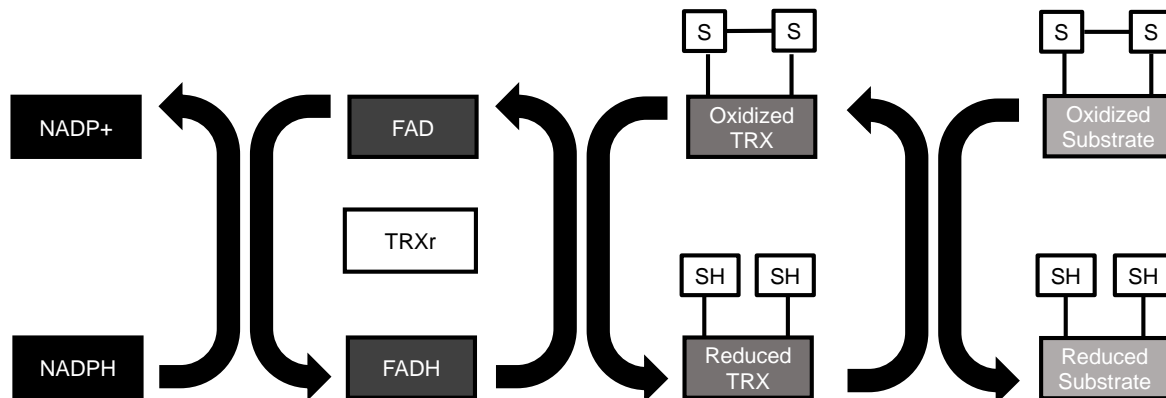
Figure 1. General Mechanism of the Thioredoxin Antioxidant System. TRX can be reduced by electrons donated by NADPH in a reaction catalyzed by TRXr. Following the reduction of the dithiol moiety, TRX is capable of reducing oxidize cysteine residues and disulfide bridges on various substrate proteins.

TRX1 is a 12kDa protein that contains a conserved Cys^{32}-Gly-Pro-Cys^{35} sequence, through which it is able to elicit its cellular effects (68). Within the cytosol, TRX1 can modulate the redox environment (73), calcium homeostasis (74), and can inhibit apoptotic signaling kinase (ASK1) and phosphatase tensin homolog 10 (PTEN) in order to inhibit apoptosis and promote growth signaling, respectively (75, 76). Although the structure of TRX1 does not contain an intrinsic nuclear localization signal, it is capable of translocating into the nucleus during oxidative stress and can interact with transcription factors including redox factor-1 (Ref-1), hypoxia-inducible factor-1 a (HIF-1a), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), p53, activator protein-1 (AP-1), nuclear factor E2-related factor 2 (Nrf-2), glucocorticoid receptor (GR), and estrogen receptor (ER) order to modulate gene expression (77-84). As mentioned previously, TRX1 can be cleaved post translational into a truncated isoform, which is secreted into the extracellular space and can serve as a growth factor and co-cytokine (85, 86). Additionally, full length TRX1 can be secreted as well via an unknown mechanism and can interact with lipid rafts in order to form redoxosomes that evoke certain intracellular responses following endocytosis (87).

TRX2 is a 12kDa protein that contains the same dithiol moiety as TRX1, however within the n-terminus it contains a signal peptide that directs it to the mitochondria (88). As such, TRX2 is responsible for protecting the mitochondria from the oxidative damage induced by ROS that is constitutively produced by the electron transport chain (ETC). Additionally, TRX2 is capable of interacting with ASK1 in order to stop the release of cytochrome c from the mitochondria, thereby preventing apoptosis (89, 90).

The importance of TRX for organismal survival was highlighted by the finding that

TRX1- and TRX2-null mice were highly susceptible to oxidative stress which led to early embryonic fatality (69, 91). Oppositely, overexpression of TRX1 in mice increased their ability to resist oxidative stress and extended their lifespan (92). In the context of diabetes, TRX1 transgenic overexpression models have been investigated to determine if it could attenuate the pathogenesis of the disease. Due to the enhanced antioxidant capacity, TRX1-transgenic mice were more resistant to streptozotocin-induced diabetes and ß-cell specific overexpression of TRX1 prevented apoptosis associated with streptozotocin treatment allowing them to maintain insulin output and prevent hyperglycemia (93, 94). Although overexpression models give important insight into the beneficial role of TRX, establishing how TRX1 expression can be modulated by pharmacological or physiological means is necessary for translation to human research.

Recent investigation by Li, et al. showed that TRX expression is increased in human endothelial following treatment with an AMPK agonist, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). As a result, ROS levels at basal and following treatment with palmitic acid (PA) were attenuated (95). These findings were substantiated in murine podocytes that were co-incubated with adriamycin and metformin. Metformin was capable of increasing AMPK activity which was necessary to promote increases in TRX expression, which prevented oxidative cell injury from adriamycin treatment (96).

Aerobic exercise has been shown to induce AMPK activity *in vivo* and thus may provide the necessary stimulus to increase TRX1 expression. There have been numerous studies that have investigated the effect of exercise on intracellular TRX expression. Sumida et al. was the first to show that an acute bout of swimming was able

to induce TRX expression at 12 and 24 hrs. post exercise in the peripheral blood mononuclear cells (PBMCs) of male mice (97). The effect of exercise intensity was explored further by Wadley et. al who enrolled young healthy human males and placed them through three aerobic exercise trials which consisted of two continuous sessions at either a moderate, high intensity, as well as low-volume high intensity interval training session done in randomized order. Similar to aforementioned study, TRX1 expression was transiently induced in PBMCs following the exercise trial, but returned to baseline 30 min. post (98). Lastly, the effect of aerobic exercise training on the TRX system was investigated in age matched sedentary, nondiabetic rats and streptozotocin-induced diabetic rats. Following 8 weeks of exercise training, the nondiabetic rats were able to increase their TRX expression, whereas the diabetic mice experienced no significant increase in their brain tissue homogenate, indicating that an experimental-diabetes model may have a reduced capacity to improve their antioxidant defense system (99). Although the effect of acute aerobic exercise on TRX protein expression has been investigated in human and murine models alike, it has yet to investigated in the context of human skeletal muscle biology. Additionally, TRX is under the control of its endogenous inhibitor TXNIP and the effect of exercise on the TRX: TXNIP interaction has yet to be determined in any model and warrants investigation in order to gain a more complete understanding of exercise's effect on the TRX system.

2.4. TXNIP Description and Structural Analysis

TXNIP, also referred to as Vitamin-D upregulated protein 1 was first discovered in HL-60 leukemia cells as it was shown to be upregulated in response to treatment with1,25 dihyroxyvitamin D_3 (100). Subsequent investigations utilizing two-hybrid yeast analysis

revealed that TXNIP is capable of binding to cysteine residues located within the active site of antioxidants Thioredoxin-1 (TRX-1 and 2 (TRX-2) inhibiting their ability to scavenge reactive oxygen species thus promoting oxidative stress (101) . Although TXNIP belongs to the ∝-arrestin protein family, the active cysteine residues that enable its interaction with TRX are not present in other members of the family, indicating that TXNIP has the distinct ability to bind with active site of TRX and affect the redox environment (7). In addition, Spindel et al. conducted three-dimensional (3D) analysis of TXNIP from which they revealed that TXNIP contains several domains that are conserved amongst other members of the ∝-arrestin family which are for necessary for its redox-independent functions. Within the n-terminus there are two SH3-binding domains which are known to interact with non-tyrosine kinase Src, and a mitogen activated protein kinase kinase kinase 5 (MAP3K-5) (102-104). Conversely, within the C-terminus lies three SH3-binding domains and two PPxY motifs. The PPxY motifs are necessary for TXNIP to interact with E3 Ubiquitin Ligase, Itch, enabling it to undergo polyubiquitination and subsequent proteasome degradation (105).   Recent evidence has suggested that following phosphorylation of a specific serine residue, ser308, TXNIP undergoes conformational change that increases TXNIP's susceptibility to ubiquitination via Itch, augmenting its rate of proteasome degradation (23). TXNIP also contains two additional domains including immune-receptor tyrosine-based inhibition domain (ITIM) and chromatin maintenance region 1 (CRM1) (102). ITIM mediates TXNIP's interaction with tyrosine phosphatases in order to regulate the intracellular activity of membrane-bound receptors (106). CRM1 mediates the interaction with hypoxia-inducible factor 1-∝ (HIF1-∝) and ubiquitin ligase von Hippel-Lindau protein (pVHL), leading to the exclusion of HIF1-∝ from the nucleus

and degradation in the cytosol (107). The domain analysis of TXNIP is displayed in Figure 2.
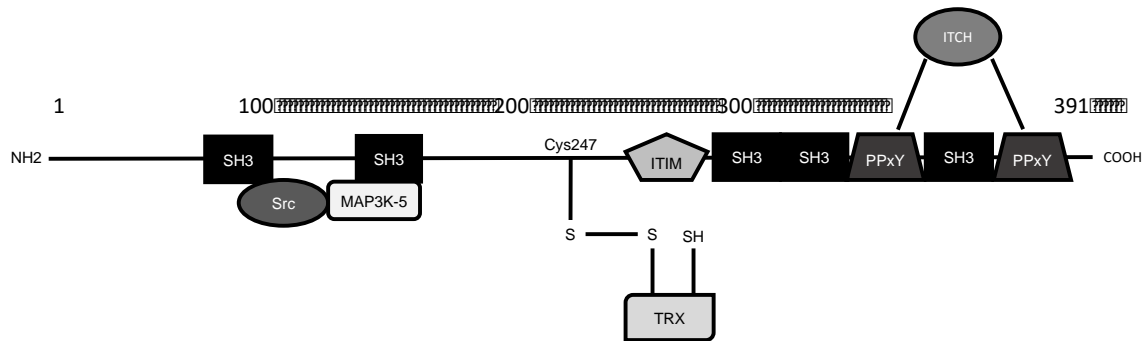
Figure 2. Structural Domain Analysis of TXNP. TXNIP contains a 391 amino acid sequence consisting of five SH3 domains, two in the n-terminus and three within the c-terminus. TXNIP has a cysteine residue (Cys247) which allows it to form mixed disulfide bridges with TRX.

2.5   Role of TXNIP in metabolic regulation

TXNIP's role in regulating metabolism was first investigated in HcB-19 mouse strain used a preclinical model to study familial hypercholesterolemia in humans. Interestingly, the hypocholesteremia was a result of a spontaneous mutation on the TXNIP gene leading to reductions at the mRNA level. HcB-19 mice had lower basal CO_2 production, increased triglyceride synthesis, and decreased flux of free fatty acids (FFA) through the tricarboxylic acid cycle (108). Considering that mitochondrial FFA flux is a contributor to insulin resistance, Chutkow et al. performed additional investigation to determine the effect of a TXNIP knockout on insulin sensitivity and substrate utilization. TXNIP null mice had a significant increase in adiposity compared to their wild-type littermates, however, they were protected from high-fat induced insulin resistance. Concomitantly, TXNIP-null mice saw improvements in clamp derived glucose uptake, glycolytic rate and glycogen synthesis, together indicating that TXNIP is a negative regulator of whole body glucose metabolism (109).

In order to delineate TXNIP's role further at the tissue and cellular level, a preclinical model with hepatocyte-specific deletion of TXNIP was developed. As a result of the deletion, hepatic glucose production was blunted which led to hypoglycemia during the fasting state. However, in response to a glucose challenge, liver-specific TXNIP knockout mice were incapable of achieving greater glucose clearance rates compared to their wildtype littermates. This finding is in contrast with systemic knockout models indicating that although fasting glucose homeostasis is modulated by hepatocyte TXNIP levels; postprandial glucose tolerance requires modulation of TXNIP biology in other tissues and cell types (110).

Investigation of TXNIP in modulating skeletal muscle metabolism has been investigated recently by DeBalsi, et al. through the development of skeletal muscle-specific knockout (SKM-/-) murine model. Cross sectional analysis between the whole-body knockout, skeletal muscle-specific knockout, and wild type revealed that glucose tolerance was increased significantly in the whole body knockout model following an intraperitoneal glucose tolerance test. Interestingly, these improvements were preserved in the SKM (-/-) specific knockout indicating that skeletal muscle expression of TXNIP contributes significantly to whole-body glucose tolerance. Although TXNIP knockout presented the mice with greater glucose tolerance, they also experienced reduced mitochondrial respiration, substrate oxidation, mitochondrial protein markers, and aerobic exercise capacity. These results indicate that TXNIP serves a metabolic switch between oxidative and glycolytic metabolism, and must be regulated appropriately in order to achieve proper balance between energy systems (111).

2.6   Regulation of TXNIP Expression

As mentioned previously, the first known regulation of TXNIP gene expression was discovered in 1,25 dihyroxyvitamin D_3-treated HL-60 leukemia cells (100). Subsequent investigations revealed that TXNIP is sensitive to stimuli indicative of the metabolic state. TXNIP is positively regulated by intracellular glucose concentration and glycolytic metabolites. *In vitro* glucose titrations have been conducted in endothelial cells which show that TXNIP gene and protein expression is increased in a stepwise fashion. This effect was mediated via activation of p38 mitogen-activated protein kinase (p38 MAPK) and forkhead box 01 transcriptional factor (FOXO1) (8). ß-cells treated with high glucose increased TXNIP expression, however the investigators determined via gene silencing,

that the effect was dependent on the recruitment of transcription factor, carbohydrate response element binding protein (ChREPB) and histone acetyltransferase p300 (p300), to the promoter site of TXNIP (112). Although *in vitro* or *ex vivo* glucose treatments have yet to be completed in skeletal muscle, protein and mRNA analysis of skeletal muscle tissue from NGT, IGT, and T2DM have shown that TXNIP expression increases as glucose tolerance decreases, indicating that increasing plasma glucose levels may be a potent stimulus to promote TXNIP gene expression in skeletal muscle as well (13).

In addition to glucose, free fatty acids augment the gene expression of TXNIP. C2C12 skeletal muscle cells treated with the long-chain saturated acid, palmitic acid (PA) underwent increases in ROS production and TXNIP expression in a dose-dependent manner. However, this effect was attenuated following co-incubation with fenofibrate or N-Acetyl-L-cysteine (NAC), a ROS-scavenging compound (24). In agreement, others have shown that an increase in ROS production following treatment with glucose or known oxidative stressors such as paraquat, ultraviolet (UV) radiation, or H_2O_2 is essential for the upregulation of TXNIP expression (8, 113, 114). Although this explains the blunted gene expression response following treatment with the antioxidant NAC, fenofibrate was able to mediate its effect via other signaling pathways. In order to tease out the specific mechanism, the investigators co-incubated cells with PA, fenofibrate and inhibitors of its known downstream effectors, AMP-activated protein kinase (AMPK), and sirtuin 1 (SIRT1). Following the addition of compound C (AMPK inhibitor), fenofibrate was no longer capable of downregulating TXNIP expression in response to PA treatment (24).

The role of AMPK activation in reducing TXNIP expression has been investigated

further. Shaked et al. performed a dose response curve with metformin in glucose-treated rat hepatocytes. As metformin concentration increased, the ability for glucose to stimulate TXNIP gene expression decreased concurrently. Chromatin immunoprecipitation analysis revealed that AMPK activation following metformin treatment was capable of excluding ChREBP from the nucleus preventing its binding to the promoter site of TXNIP (22). Additionally, AMPK can alternatively affect TXNIP protein levels via post translational mechanisms. Primary rat hepatocytes treated with AMPK agonists 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) and phenformin resulted in the phosphorylation of TXNIP at ser308 inducing a conformational change that increased TXNIP's susceptibility to ubiquitination and proteasome degradation (23).

Although AMPK can be activated via pharmaceutical means, its physiological role is to sense and respond to the cellular energy state. Elevated [AMP], [ADP], and [NAD$^+$], hypoxia, and glycogen depletion have been shown to activate AMPK which initiates glucose and fat uptake in order to increase substrate availability for ATP production. TXNIP has been shown to inhibit glucose uptake (13), so AMPK's ability to signal for its degradation may be an indirect mechanism to improve glucose availability. Physiological stressors that have been shown to activate AMPK *in vivo* include caloric restriction and aerobic exercise (115-118). Johnson et al. completed a 16-week caloric restriction intervention in order to understand the mechanisms that lead to improvements in insulin sensitivity in obese subjects. Interestingly, TXNIP protein levels decreased as a result of the intervention, which was significantly correlated with clamp-derived glucose disposal rate (119). Additionally, a recent global gene analysis was conducted in young, lean, healthy males following nine-days bedrest and subsequent retraining period showed that

TXNIP gene expression was significantly decreased following four weeks of extensive aerobic exercise retraining, which positively correlated with improvements in insulin sensitivity (120) . From this analysis it is still not clear the effect of exercise on TXNIP protein expression, intermolecular interactions through which it modulates its cellular effects including REDD1 and TRX, and the mechanisms by which its protein expression is modulated following exercise training.

2.7   REDD1 and skeletal muscle metabolism

REDD1 was originally classified as a gene whose expression was inducible by hypoxia and DNA damage (121, 122).   Following, it has been investigated as a regulator of nutrient sensitive pathways including insulin/insulin-like growth factor (IGF) and mammalian target of rapamycin (mTOR) signaling (123-125). Coradetti et al. determined that REDD1 overexpression was able to reduce the phosphorylation status of p70 ribosomal S6 kinase (S6K), a known mTOR substrate, in a dose-dependent fashion (126). It was postulated that REDD1 can inhibit mTOR by scavenging 14-3-3 proteins from tuberous sclerosis complex (TSC1/2). In doing so, TSC1/2 remains unbound and is capable of functioning as a GTPase on Rheb. Following the conversion of Rheb from GTP to GDP bound, Rheb is incapable of activating mTOR (124). However, when structural analysis was conducted to verify this interaction, there was no evidence to support that REDD1 physically interacts with REDD1, but rather through another upstream mechanism (127). Dennis et al. determined that REDD1 is capable of interacting with TSC2 and 14-3-3 complex via co-immunoprecipitation experiments indicating that a multimeric complex can form between all three proteins. However, they presented novel evidence that REDD1 actively recruits both protein phosphatase 2

(PP2A) and protein kinase B (AKT). This enabled PP2A to inactivate AKT, preventing it from inhibiting TSC2 (128).

The role of REDD1 *in vivo* has been investigated further in both physiological and pathophysiological models. Murine models of both obesity and diabetes have indicated that REDD1 protein and gene expression is elevated compared to the lean, healthy littermates (129, 130). However, in human trials, skeletal muscle expression does not seem to vary between T2DM and their lean, healthy counterparts (12). However, T2DM undergo a significant elevation in REDD1 protein expression in response to the hyperinsulinemic-euglycemic clamp, which led to a blunting of insulin-mediated mTOR activation (12). Physiological stressors such as resistance and aerobic exercise have been investigated to elucidate the relationship between REDD1 protein and gene expression changes and mTOR activation following exercise bouts. Resistance training seems to promote a decrease in REDD1 gene expression, whereas aerobic exercise promotes a rapid increase in REDD1 gene and protein expression (131-133). However, the metabolic controls for these studies remains a confounder as the amount of time that subjects were fasted varied across investigations. Nutrient availability has a large impact on REDD1 expression as periods of fasting and refeeding can acutely modulate REDD1 protein levels (134, 135). Additionally, most of the acute aerobic exercise studies were conducted in murine models and all mice were prescribed the same exercise parameters, thus the role of relative intensity cannot be isolated from these investigations (131). The only investigation of REDD1 expression in response to acute aerobic exercise in humans investigated the effect aerobic exercise in a high altitude/hypoxic vs. normoxic environment. During the exercise bouts, subjects trained at ~50% VO_{2max} during the

normoxic bout and ~80% during the hypoxic bout. In response to either training bout, there was not a significant change in REDD1 expression, however hypoxia independently promoted a significant increase in REDD1 protein expression (136). From this investigation, it is impossible to clarify the independent effect that high intensity aerobic exercise has on REDD1 expression.

Another mechanism through which REDD1 protein expression can be modulated is through its interaction with TXNIP. REDD1 can dimerize with TXNIP forming a pro-oxidant complex that can suppress mTOR signaling (137). Jin et al. recently investigated the effect of TXNIP on REDD1 expression and cellular outcomes. Deletion of TXNIP resulted in a decrease in REDD1 stability and thus a decrease in its protein expression, whereas overexpression of TXNIP promoted REDD1 protein expression and increased its ability to inhibit mTOR in response to glucose treatments (10). It is important to note that these investigations were conducted in HeLa cells, and the dynamics of the REDD1/TXNIP axis have yet to be elucidated in human skeletal muscle. Further, acute aerobic exercise has been shown to affect REDD1 protein levels, but it remains to be seen whether it may individually affect TXNIP protein levels. The established link between TXNIP expression and the cellular stability of REDD1 warrants investigation to determine the effect of acute aerobic exercise on REDD1 and TXNIP in order to provide a potential mechanism through which exercise can modulate REDD1 expression via the REDD1/TXNIP axis.

3. METHODS

3.1   Participants

Fifteen healthy adults, (8 M, 7 F, Age: 25 ± 4) volunteered to participate in the study. An eligibility check list was used to screen potential participants over the phone to determine their eligibility and gather health history information (see Appendix A). Criteria for exclusion included being a current smoker, quit smoking with in the past year, and/or if they were previously diagnosed with any major disease such as diabetes, cardiovascular disease, kidney disease, major depression, high blood pressure, or high blood cholesterol (see appendix A for full list). Females who were currently pregnant were excluded, which was confirmed by an over-the-counter pregnancy test. Menstrual status was not recorded for all female subjects. Criteria for inclusion were the participants had to be 18-35 years of age, BMI between 18-26 kg/m^2, and absence of any chronic disease. Baseline characteristics for all participants are presented in Table 1.

TABLE 1.

N	15
Age (y)	25.7 ± 4
Gender (%F)	47%
Weight (kg)	68.3 ± 8.3
BMI (kg/m^2)	22.4 ± 2.6
BF%	23.1 ± 5.7
VO$_{2Max}$ (mL/kg/min)	47.7 ± 7.4
80% VO$_{2max}$ Treadmill Speed (mph)	6.5 ± 1.1
80% VO$_{2max}$ Treadmill Grade (%)	1.0 ± 1.0

Table 1. Subject Baseline Characteristics and Treadmill Settings for 80% VO$_{2max}$ trial. Data are depicted as mean ± SD

3.2   Study Design

The current investigation is a retrospective analysis of a larger study that consisted of four sequential visits. During the first visit all procedures and potential risks involved with the study were explained to the participants. Upon giving their verbal and written informed consent (see Appendix B) the subjects were enrolled in the study and baseline measures including height, weight, body fat percentage via dual x-ray absorptiometry (DXA), and maximal aerobic capacity (VO_{2max}) were collected. Visits 2, 3, and 4 consisted of aerobic treadmill exercise at 40, 65, and 80% of VO_{2max}, respectively. Each study visit was separated by at least four days. Three days before each visit subjects completed diet and physical activity logs (see Appendices C and D), which they were asked to replicate in the days leading up to their subsequent visits. Subjects were also instructed to abstain from vigorous exercise, alcohol consumption 48 hours prior to each visit, and caffeine consumption 24 hours prior to each visit. In addition, subjects were asked to arrive at each visit fasted for at least 12 hours.

3.3 VO_{2max} and Treadmill Testing

VO_{2max} was determined using a treadmill ramp protocol during which the subjects ran at a uniform speed while the treadmill grade increased two percent after every two-minute stage, until volitional fatigue was reached. Expired air was collected for the duration of the test and was analyzed via the PARVO Medics metabolic cart (Salt Lake City, UT). As a further validation of exercise intensity, subject's heart rate was monitored via Polar heart rate monitors that was fitted to the subject's chest prior to testing. Subjects were also asked their rating of perceived exertion (RPE) on the Borg scale (6-20) at the end of each two-minute interval. VO_{2max} was achieved if the subjects met 3 of the 4

criteria: a plateau in VO_2, despite an increasing workload, an RPE >17, RER >1.1, and a HR >85% age-predicted maximal heart rate.

Following the determination of the subject's VO_{2max}, oxygen consumption at 40, 65, and 80% of their VO_{2max} was calculated and inserted into the appropriate ACSM metabolic equations in order to derive the proper speed and grade that the treadmill was to be set in order for them to reach the appropriate VO_2. The average speed and grade performed during the final visit is displayed in Table 1.

3.4   Skeletal Muscle Biopsy

Skeletal muscle biopsies were taken from the vastus lateralis before, immediately after, and 3hrs after exercise on the final visit.   Prior to the biopsy, local anesthetic (lidocaine HCl 1%) was administered followed by a small incision (~0.5 cm) at the biopsy site. Through this incision, a Bergstrom needle was inserted with suction extracting ~100mg of muscle tissue. Muscle tissue was cleared of all visible connective tissue and fat, blotted with gauze to remove blood, and immediately flash frozen in liquid nitrogen and stored at -80°C until further analysis was performed.

3.5   Tissue Homogenization and Protein Quantification

Approximately 10mg of muscle tissue was weighed and homogenized with ceramic beads (Lysing Matrix D; FastPrep®-24; MP Bio, Santa Ana, CA) in 20 volumes of 1x cell lysis buffer (20mM Tris-HCl (pH 7.5), 150 mM NaCl, 1mM Na2EDTA, 1mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM-glycerophosphate, 1mM Na3PO4, 1 g/mL leupeptin; Cell Signaling Technology, Danvers, MA) supplemented with protease/phosphatase inhibitor cocktail (1:10,000: MSSAFE; Sigma, St. Louis, MO). Protein concentration for each sample homogenate was determined by a commercially

available bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL).

3.6   Western Blotting

Aliquots containing 30 µg of total protein were diluted in equal volumes of 2x Laemmli Buffer (BioRad, Hercules, CA) with 5% ß-mercaptoethanol (ßME) prior to heating at 70°C for 10 min. Denatured samples were brought to room temperature, loaded onto a 12.5% polyacrylamide gel, separated by SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were blocked with Odyssey Blocking Buffer (OBB; Li-Cor, Lincoln, NE) for 1 h and were incubated with primary antibodies TXNIP (1: 1,000; Abcam, ab188865, Cambridge, UK), TRX (1: 1,000; Abcam, ab16965), REDD1 (1: 1,000; Proteintech, 10638-1-AP), and GAPDH (1: 5,000; Cell Signaling, 2118) overnight at 4°C in OBB with 0.1% Tween-20. Membranes were washed with TBST and incubated with an anti-rabbit or anti- mouse fluorophore-conjugated secondary antibody (1:20,000, Li-Cor) in OBB supplemented with 0.1% Tween-20 for 1 h. The membranes were washed with TBST followed by TBS prior to being scanned on the Odyssey CLx Imaging System (Li-Cor) and quantified on Image Studio software (V4.0.21; Li-Cor). GAPDH was used as a loading control.

3.7   Immunoprecipitation

Aliquots containing 100-200 µL of total protein was incubated overnight, while rotating at 4°C with an anti-TRX antibody (2 µg antibody: 100µg total protein; Abcam) or anti-TXNIP antibody (4 µL antibody: 200 µg total protein; Cell Signaling), which were bound to magnetic beads (Dynabeads Protein G; Thermo Fisher, Waltham, MA). The beads containing the antigen-antibody complex were washed three times with ice cold 1x PBS. Following the last wash, the beads were resuspended in Laemmli Buffer (BioRad)

with 5% ßME and heated at 70°C for 10 min in order to denature and elute the proteins from the beads. The eluate was then loaded onto a 12.5% polyacrylamide gel, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. Membranes were blocked in OBB (Li-Cor) for 1 h followed by incubation with primary antibodies overnight at 4°C. Membranes containing TXNIP immunoprecipitate were probed with anti-phosphoserine (1:500; EMD Millipore, AB1603), anti-ubiquitin (1: 500; Santa Cruz, sc-8017), REDD1 (1:1000) and TXNIP (1: 1,000; Cell Signaling, 14715). Membranes containing TRX immunoprecipitate were probed for TXNIP (1: 1,000; Cell Signaling, 14715) and TRX (1: 1,000; Abcam, ab16965) and TXNIP (1: 1,000; Cell Signaling, 14715). Membranes were washed with TBST and were incubated with appropriate fluorophore-conjugated secondary antibody in OBB supplemented with 0.1% Tween-20 for 1 h. In order to avoid detection of the IgG heavy chain (50 kDa) from the antibody co-eluted with the protein of interest, a light chain specific anti-rabbit secondary (1: 100,000, Jackson ImmunoResearch, 211-622-171) was utilized. The membranes were washed with TBST followed by TBS prior to being scanned and quantified on the Odyssey CLx Imaging System (Li-Cor). All protein signals were normalized to the protein target that was immunoprecipitated from the sample.

3.8 Statistical Analysis

Baseline data are presented as mean ± standard deviation and western blot data are presented as the mean ± standard error of the mean. Immunoprecipitation analysis is presented as mean fold change over pre ± standard error of the mean. Statistical analysis was performed using Prism 4.0 software (GraphPad Software, Inc., La Jolla, CA). Differences in protein signals across the three time points were analyzed via one-

way repeated measures ANOVA.   Pearson's r was performed to analyze the relationship between dependent variables.   Outliers identified as being two standard deviations outside of the means were excluded from analysis.   Significance was set at $p < 0.05$.

4. RESULTS

4.1 Effect of Acute Aerobic Exercise on TXNIP, TRX, and REDD1 Protein Expression in Skeletal Muscle

There was a significant decrease in skeletal muscle protein expression of TXNIP immediately following acute aerobic exercise ($p<0.05$), but this effect was abolished three hours post exercise. Furthermore, there was a significant decrease in REDD1 protein expression three hours following the aerobic exercise bout ($p<0.05$). Additionally, pre-exercise REDD1 protein expression significantly correlated with pre-exercise TXNIP expression (Figure 4.). TRX protein expression was not significantly altered following acute aerobic exercise (Figure 3). However, the change in TRX was significantly correlated with the change in REDD1 protein expression from pre to post exercise (Figure 5).
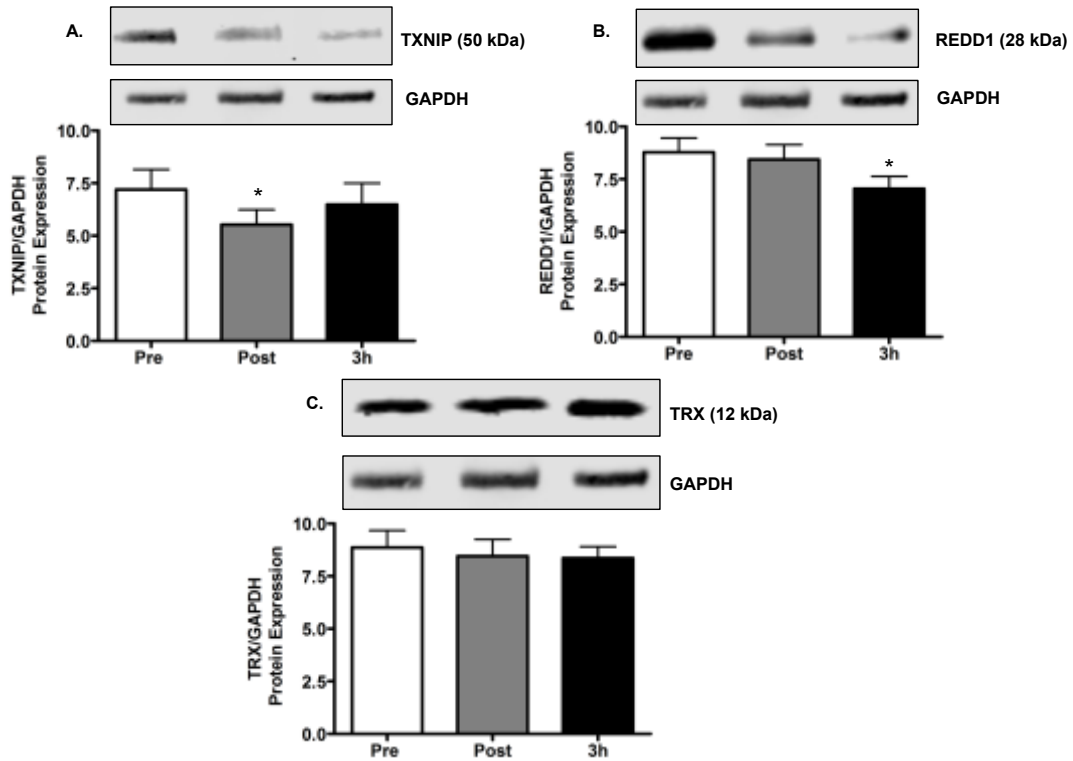
Figure 3. Effect of Acute Aerobic Exercise on Skeletal Muscle TXNIP, REDD1, and TRX Expression. A) TXNIP skeletal muscle protein expression. B) REDD1 skeletal muscle protein expression. C) TRX skeletal muscle protein expression. Expression of all three protein targets are presented as a percentage of GAPDH. All values are presented as mean ± SEM. *p<0.05 vs. Pre. n=15.
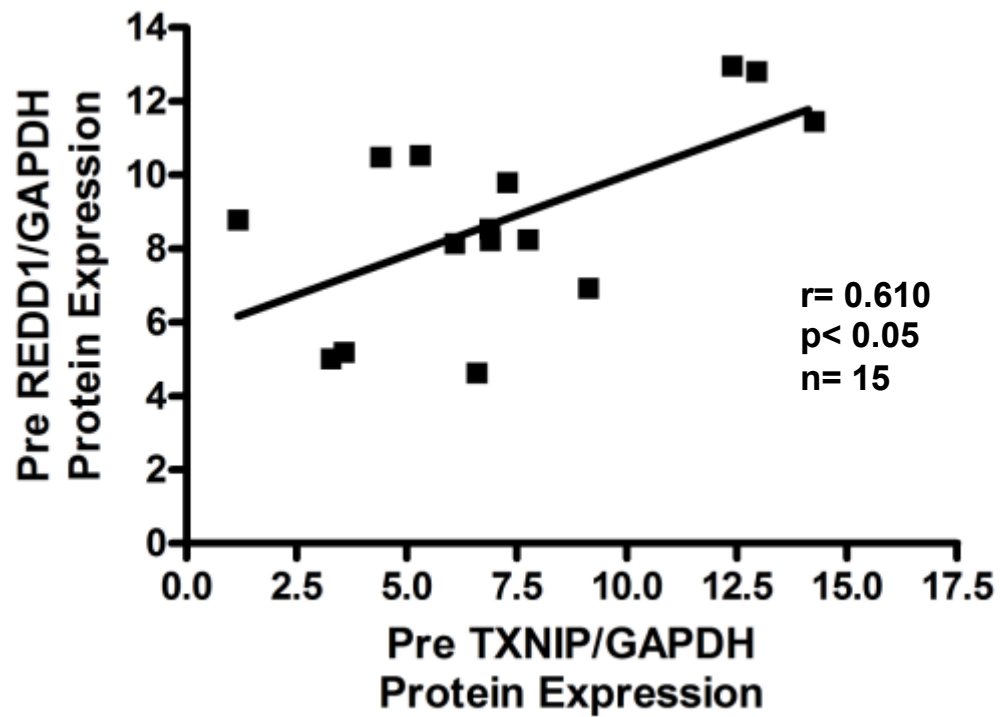
Figure 4. Relationship Between Pre-Exercise Protein Expression. REDD1 protein significantly correlated with TXNIP protein expression pre-exercise. All protein expression values were quantified via western blotting and normalized to GAPDH. n=15.
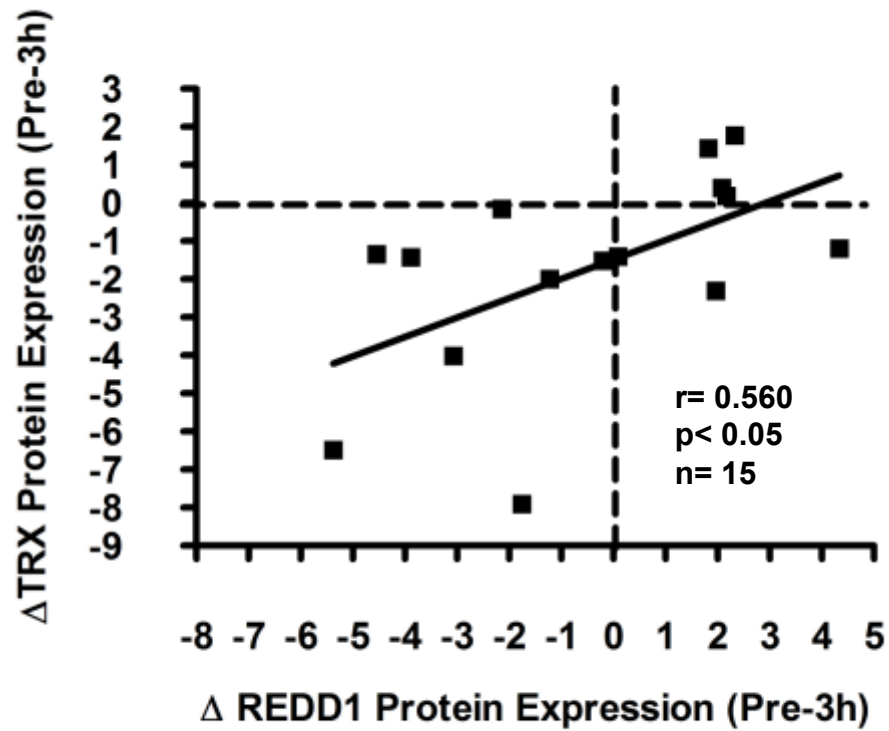
Figure 5. Relationship Between Change in Protein Expression Three Hours Following Exercise. Change in REDD1 protein expression significantly correlated with change in TRX protein expression three hours following acute aerobic exercise. n=15.

4.2   Effect of Acute Aerobic Exercise on Posttranslational Modifications and Protein: Protein Interactions of TXNIP in Skeletal Muscle

Immunoprecipitation analysis was conducted in order to isolate TXNIP and quantify posttranslational modifications (PTM) including serine phosphorylation and ubiquitination status via western blotting. Acute aerobic exercise did not have a significant effect on serine-phosphorylation status nor ubiquitination status (Figure 6). However, there was a significant correlation between the change in serine phosphorylation and ubiquitination status from the pre to post exercise state (Figure 7). Unfortunately, with our current immunoprecipitation protocol, we were unable to detect an interaction between REDD1 and TXNIP.
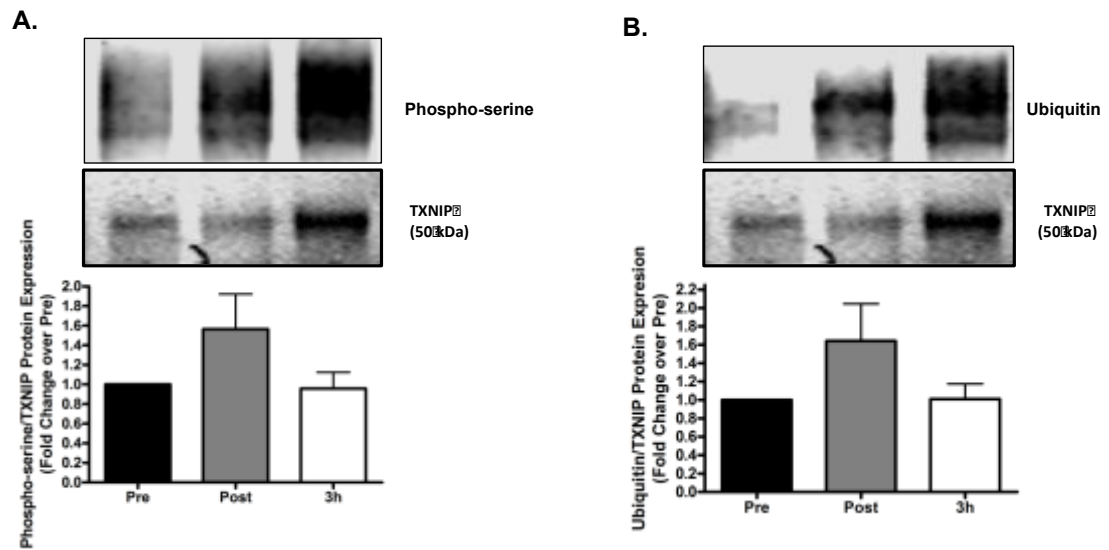
Figure 6. Effect of Acute Aerobic Exercise on Posttranslational Modifications of TXNIP in Skeletal Muscle. Immunoprecipitation of TXNIP was analyzed via western blot analysis. Exercise did not have a significant effect on either serine phosphorylation or ubiquitination status of TXNIP at either the 30-minute post or 3-hour post exercise state. Data is presented as mean fold change over pre-exercise values ± SEM. n=9
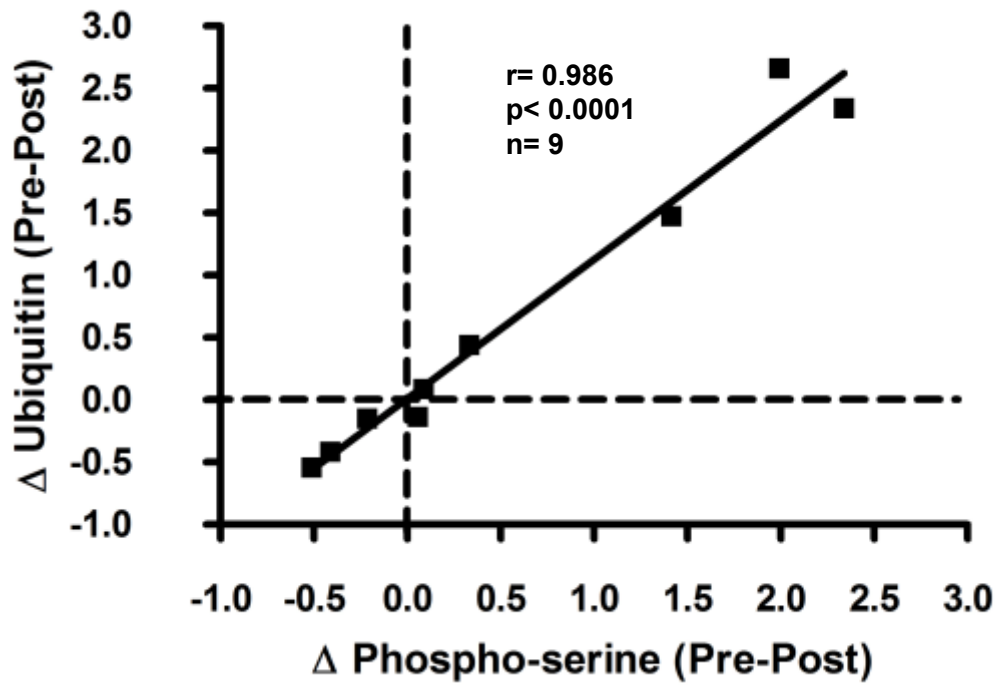
Figure 7. Relationship Between Change in Serine Phosphorylation and Ubiquitination Status Following Acute Aerobic Exercise. Change in serine phosphorylation significantly correlated with change in ubiquitination of TXNIP following acute aerobic exercise. n=9.

4.3   Effect of Acute Aerobic Exercise on TXNIP:TRX Protein Interaction in Skeletal Muscle.

Immunoprecipitation analysis was performed in order to isolate TRX and other protein targets that it could be interacting with during each time point. Specifically, we used an antibody that detected TXNIP and compared the TXNIP: TRX ratio which was detected via western blot of the immunoprecipitated protein complexes. Following acute aerobic exercise there was no significant change in TRX: TXNIP interaction (Figure 8).
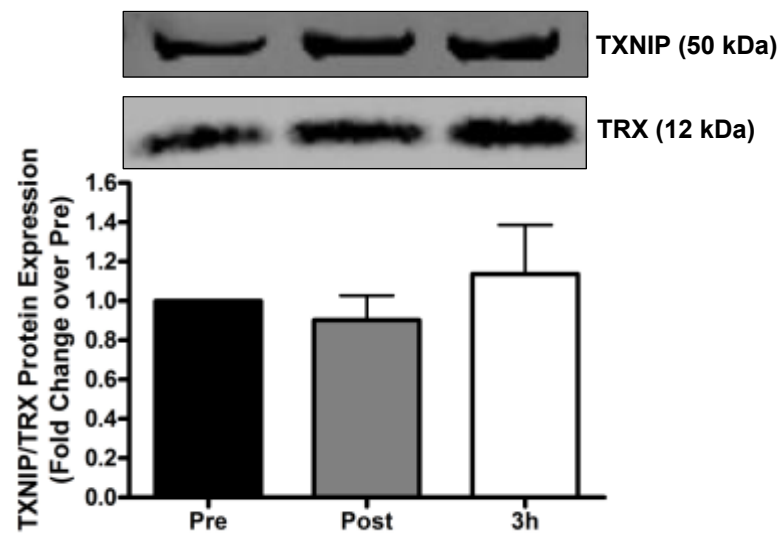
Figure 8. Effect of Acute Aerobic Exercise on TXNIP: TRX Protein Interaction in Skeletal Muscle. Immunoprecipitation of TRX was analyzed via western blot analysis. Exercise did not have a significant effect on the interaction between TRX and TXNIP at either the 30-minute post or 3-hour post exercise state. Data is presented mean fold change over the pre-exercise values ± SEM. n=13.

5. DISCUSSION

The present study aimed to determine the effect of acute aerobic exercise on the expression, posttranslational modifications, and interactions of TXNIP. Additionally, we also were interested in understanding the effect of exercise on the expression of the antioxidant TRX and its interaction with TXNIP. Our hypotheses were that acute aerobic exercise would decrease the expression of TXNIP which would reduce its ability to interact with REDD1. As a result, REDD1's cellular stability would be reduced and there would be a similar decrease in its protein expression. In order to provide a mechanism through which exercise could regulate TXNIP protein levels, we postulated that there would be an increase in serine-phosphorylation and subsequent ubiquitination status following exercise which would increase its susceptibility to proteasome degradation. Furthermore, exercise would provide an adequate stimulus to increase TRX expression which would independently generate an imbalance in the TRX: TXNIP ratio, decreasing their relative interaction thus leading to a greater proportion of unbound TRX.

5.1   Exercise and TXNIP expression, Posttranslational Modifications, and Protein: Protein interactions

In order to test the first hypothesis, we obtained skeletal muscle biopsy samples prior to exercise, 30-minutes post, and 3h post exercise at $80\%VO_{2max}$. Immunoprecipitation and western blotting were used to quantify TXNIP and REDD1 protein expression, posttranslational modifications, and TXNIP's interaction with REDD1. In agreement with our hypothesis TXNIP and REDD1 expression decreased, but at different time points. TXNIP expression decreased 30-minutes post exercise whereas REDD1 did not decrease significantly until 3-hours post exercise. However, we did not

notice significant increases in serine phosphorylation status nor ubiquitination status, but the change in these two posttranslational modifications were significantly correlated with each other. Lastly, we were not able to detect REDD1 following western blot analysis of TXNIP immunoprecipitate, and therefore we were not able to quantify REDD1: TXNIP interaction.

To our knowledge, we are the first to show that TXNIP protein expression decreases following acute aerobic exercise. However, the mechanism through which this occurred could not be explained by changes in posttranslational modifications. The discrepancy between the change in TXNIP protein expression and posttranslational modifications could have been a result of the time gap between the end of exercise and skeletal muscle biopsy. Posttranslational modifications are transient in nature and from our current analysis we are not certain which enzymes are eliciting such modifications. Consequently, we could have missed the window during which serine phosphorylation and ubiquitination status were at their peak. Interestingly, the significant correlation that exists between them demonstrates that the regulation of these two modifications occurs via similar mechanisms, which is in line with our previous hypothesis. An alternative explanation is that we used a non-specific phospho-serine and ubiquitin antibody. There are numerous serine residues located on TXNIP capable of being phosphorylated and are regulated by distinct mechanisms (23, 138) . From our current analysis we are unable to determine the degree to which each individual residue is phosphorylated which could be diluting our results, considering only one (ser308) has been distinctly shown to increase its susceptibility to degradation (23). Lastly, it is important to note that the n size for this analysis was reduced compared to other analyses in this investigation as a result

of limitations in sample. This may have caused our analysis to be underpowered, thus making it harder to detect significance.

To our knowledge, we are also the first to show that acute high intensity aerobic exercise is able to reduce REDD1 protein expression. Most studies that have used aerobic exercise to study REDD1 have used murine models and have shown aerobic exercise is able to increase its expression at both the mRNA and protein level (131, 132, 139). However, a limitation to these investigations is that their exercise prescription is standard for each mouse and as such, the effect of relative exercise intensity cannot be isolated. The only aerobic exercise intervention that has been conducted in human skeletal muscle used both a low and high intensity exercise bout. However, the high intensity bout was conducted in a hypoxic environment, which independently has been shown to upregulate REDD1 protein levels, and we can therefore not conclude anything about the effect of intensity alone on REDD1 protein expression (136). Our study design utilized a high intensity (80% VO_{2max}) aerobic exercise bout and we found a significant decrease in REDD1 protein expression. One of the ways through which REDD1 protein expression can be regulated posttranslational is via an interaction with TXNIP. Recent evidence has suggested that TXNIP is able to dimerize with REDD1, increasing its cellular lifespan (10). However, when TXNIP gene expression was silenced, this led to concomitant reduction in REDD1 protein expression. Based on this evidence we developed the hypothesis that exercise would reduce TXNIP protein expression and in doing so, reduce its interaction with REDD1 thus diminishing its cellular stability leading to a decrease in its protein expression as well. Unfortunately, we were not able to detect REDD1 from the western blot analysis of the TXNIP immunoprecipitate. REDD1 is a 25

kDa protein that we were able to detect at ~28 kDa via western blot analysis of skeletal muscle homogenate. However, when conducting immunoprecipitation, the antibody that was used to isolate the protein of interest was co-eluted with antigen and was going to be detected during the western blot analysis. Specifically, the light chain monomer of the antibody is detectable with a robust protein band at 25 kDa which may have overlapped with the REDD1 protein band making it impossible to determine to determine the degree to which REDD1 was interacting with TXNIP.

5.2   Exercise and TRX Expression and TXNIP: TRX Protein Interaction

In order to test the second hypothesis, we obtained skeletal muscle biopsy samples prior to exercise, 30-minutes post, and 3h post exercise at $80\%VO_{2max}$. Immunoprecipitation and western blotting were used to quantify TRX protein expression as well as its protein interaction with TXNIP. Contrary to our hypothesis, we did not find any significant changes in TRX protein expression following acute aerobic exercise, nor did we find a significant change in its relative interaction with TXNIP. However, there was a significant correlation that developed between the change in REDD1 and change in TRX protein expression from the pre to 30-minute post exercise.

Other studies have shown that TRX expression increases following acute and chronic aerobic exercise, however this was the first investigation in human skeletal muscle, which points to a tissue-specific regulation of this protein target. It is possible that the window of time during which we would see TRX protein accretion could be delayed in skeletal muscle tissue. Additionally, the subjects in this cohort were young, healthy, and aerobically fit (VO_{2Max} 47.7 ± 7.4 mL/kg/min, mean ± SD). Due to this, it is possible that their antioxidant capacity could be optimal and the level of oxidative stress

that they underwent during the exercise bout was not a high enough stimulus to elicit a detectable increase in antioxidant capacity. Another potential explanation is that the window of time during which we would see increases in TRX could be delayed in skeletal muscle and therefore reverse transcriptase-polymerase chain reaction (RT-PCR) may have been a better analysis to detect changes in TRX at the level of gene expression.

In agreement with the lack of significant change in TRX expression, we were unable able to detect changes in its interaction with TXNIP following acute aerobic exercise. It has been shown that TXNIP dissociates rapidly from TRX following treatment with oxidative stressors such as uric acid (140). Aerobic exercise has been shown to induce transient oxidative stress which we postulated would elicit a similar dissociation. However, it is possible that individuals with this level of fitness had the ability to resist such changes in the redox state allowing the disulfide bridges between TRX and TXNIP to be remain preserved following the exercise bout. Similar to the argument pertaining to posttranslational modifications, it is also conceivable that the 30 minutes following aerobic exercise was enough time for interactions between TXNIP and TRX to re-equilibrate to pre-exercise values. Although this is the first investigation that focused on the redox-dependent TRX: TXNIP interaction, others have investigated the redox state of other cysteine-containing molecules in response to aerobic exercise. Sastre et al. investigated the effect of exhaustive aerobic exercise in younger trained males and found that oxidized glutathione levels in the plasma were significantly elevated following exhaustive exercise, but these values returned to baseline 1-hour following (141). This supports our findings and in fact it may have been more appropriate to investigate the effect of exercise on the TRX: TXNIP interaction immediately following exercise.

5.3   Limitations and Future Directions

This is first study to examine the effect of acute aerobic exercise on TXNIP protein expression and posttranslational modifications in the skeletal muscle of lean, healthy individuals. Although other investigations have looked at the effect of acute aerobic exercise on REDD1 and TRX independently, this is first study to examine its effect on their interaction with TXNIP, which is important for determining their cellular fate. Lastly, this is first investigation to examine the effect of high intensity acute aerobic exercise alone on REDD1 protein expression.

Although this study examined several molecular targets that have clinical significance for the outcome of T2DM, it was not void of limitations. The first of which is the small sample size as there was only an n of 15 for the western blot analysis. Due to sample limitations there was only an n of 12 for the TRX immunoprecipitation analysis and an n of 9 for the TXNIP immunoprecipitation analysis.   Furthermore, the high level of fitness for the subjects that we tested may have mitigated the effect of acute aerobic exercise on these protein markers as they may have already achieved optimal level of antioxidant status in their skeletal muscle. Future investigations should utilize a diseased cohort in order to determine if exercise can elicit more robust changes in individuals with aberrant TXNIP/REDD1 signaling and reduced TRX antioxidant capacity. In terms of our exercise modality, another potential limitation and confounding variable is the % incline that was used during the exercise bout. Recent data has shown that treadmill grade may have a significant effect on altering muscle group activation even when conducting exercise at the same relative intensity. For our purpose, we took skeletal muscle biopsies from the vastus lateralis and it has been shown that increasing the grade of the treadmill

significantly increases the EMG graded muscle activity of the vastus lateralis (142). Although we are not aware of how this increased electrical activity may impact AMPK activity, it is worth noting as a potential source of error and should be controlled for in future studies.

Even though we determined the effect of acute aerobic exercise TRX protein expression and interaction with TXNIP, we did not investigate changes in its activity nor the activity of TRXr, which is responsible for activating TRX. Another limitation pertained to the capabilities of our current analysis. Immunoprecipation was conducted to detect changes in posttranslational modifications, but we could not determine the specific residues that these changes were occurring on. This is vital to our outcome measures as there are multiple serine residues that TXNIP can be phosphorylated on, but on one (ser308) has been shown to make it susceptible to ubiquitination and subsequent degradation. Another limitation of our immunoprecipation analysis is that the light chain of the antibody used to pull down TXNIP inhibited us from being able to quantify or detect REDD1's interaction. REDD1's stability is dependent on its interaction with TXNIP and if this interaction were to change as a result of acute exercise, this would provide a potential mechanism to explain the decrease in REDD1 protein expression three hours following exercise. Another limitation of our current study is that we did not conduct additional analysis to determine relationships between changes in our protein targets with relatable outcomes such as insulin sensitivity or oxidative stress. Future analysis, whether *in vivo* or *in vitro*, should aim to address this limitation in order to elucidate the clinical importance of changes in TXNIP, REDD1, and TRX biology following aerobic exercise.

5.4   Conclusion

Acute aerobic exercise was able to significantly reduce TXNIP and REDD1 protein expression. However, the change in TXNIP protein expression could not be explained by changes in posttranslational modifications as there was no significant change in ubiquitination or serine phosphorylation status following the exercise bout. It is worth noting that although they were independently insignificant, the change in these two posttranslational modifications significantly correlated with one another, indicating that they may be regulated through a convergent mechanism. Additionally, we observed a significant correlation between REDD1 and TXNIP in the pre-exercise state, but due to limitations in our methodology, were unable to determine whether the interaction between REDD1 and TXNIP is altered as a result of exercise. Lastly, we did not observe changes in TRX expression following acute aerobic exercise and likewise we did not observe changes in its interaction with TXNIP. Futures studies should be conducted in a diseased population in order to determine if acute exercise can elicit more robust changes in TXNIP expression and its interactions with REDD1 and TRX. Further, future analysis should incorporate other outcome measures in order to understand if changes in TXNIP, REDD1, and TRX biology has any implications for oxidative stress and insulin sensitivity following acute aerobic exercise in order to expand on its efficacy in the prevention and treatment of skeletal muscle insulin resistance that is associated with T2DM.

APPENDICES

APPENDIX A
TELEPHONE ELIGIBILITY CHECKLIST

Screen	OUT	IN	FOLLOW-UP	
	☐	☐	☐	Reason:_____

STARTS **APPROVAL** EXPIRES

MAR 1 9 2015 TO MAR 1 8 2016

UNIVERSITY OF ILLINOIS AT CHICAGO
INSTITUTIONAL REVIEW BOARD

Telephone Eligibility Checklist

Study Title: Effects of acute aerobic exercise intensity on plasma sRAGE in lean healthy individuals
Principal Investigator: Jacob M. Haus, PhD

Team Member performing screening:_____ Date:_____

Subject Name:_____ Date_____

Hello, my name is _____ and I am calling from the University of Illinois at Chicago in regards to your interest in an exercise study. Is this correct?
      YES → Continue
      NO → Thank you for your time

You are being contacted because you have responded to a study advertisement related to exercise intensity. If you are still interested then I will give you some additional information about the research study and I will also need to ask you a few questions. The questions are arranged so that if you are not eligible to participate you will be screened out as we go down the list of questions. This will save your time and avoid any inconvenience. All information collected and discussed during this conversation will be kept strictly confidential. It will take about 15 minutes to complete this initial screening. Is this ok to do now?
      YES → Continue
      NO → Is there a more convenient time I can call you back? When?_____

First, I need to verify your contact information. (*Verify contact info against what we have.*)

1. Mailing address (necessary for mailing study information and the informed consent for their review

    prior to study)

Street:_____

City:_____ State:_____ Zip Code:_____

Phone:_____ email:_____

Do you have an alternate telephone number you'd like to be contacted at (such as a cell or (*work/home*) phone)?

Alternate contact number(s):_____

2. Where did you see this ad or where did you hear about the study (used for tracking recruitment dynamics)

_____

Now we need go over your background/health information to see if you qualify for the study and then I will briefly explain the study to you. Again, all health and personal information will remain confidential.

3. Date of Birth _____ Age _____ (*must be 18-35 yrs*)   Male   Female

APPENDIX A (CONTINUED)

4. Height _____ inches_____cm   (multiply inches by 2.54)

5. Weight _____lbs_____kg (divide lbs by 2.2)

    5a. BMI _____(*Calculate BMI (kg/m²)* → *must be greater than 18 kg/m² and less than 26 kg/m².*
    Does subject meet BMI criteria?
    YES→ Continue to 6.
    NO→ I'm sorry you are not eligible to participate, would you mind if we held onto your name and contact
    information in the event you qualify for a future study?   YES   NO

6. Are you fluent in both verbal and written English?
    YES→ Continue to 7.
    NO→ I'm sorry you are not eligible to participate, would you mind if we held onto your name and contact
    information in the event you qualify for a future study?   YES   NO

7. Do you currently smoke, use smokeless tobacco or use any other tobacco products?   YES   NO
    NO → Continue to 7a.
    YES → I'm sorry you are not eligible to participate, tobacco use is part of the exclusion criteria for this
    particular study. Would you mind if we held onto your name in the event you qualify for a future study?
    YES   NO

    7a.   Have you abstained from tobacco use for at least 1 year?   YES   NO
    YES→ Continue to 8.
    NO→ I'm sorry you are not eligible to participate, smoking in the past 1 year is part of the exclusion criteria
    for this particular study. Would you mind if we held onto your name in the event you qualify for a future
    study?

8. Do you have, or have you <u>ever</u> had any of the following conditions?

Type 1 Diabetes (insulin dependent)	Yes _____	No _____
Heart Disease/Failure	Yes _____	No _____
Stroke or Aneurism	Yes _____	No _____
Peripheral arterial disease (PAD)	Yes _____	No _____
Pacemaker	Yes _____	No _____
Liver Disease/Hepatitis/Cirrhosis	Yes _____	No _____
Lung or Respiratory Disease (except asthma)	Yes _____	No _____
Kidney Disease	Yes _____	No _____
Epilepsy	Yes _____	No _____
Bleeding or Clotting Disorders	Yes _____	No _____
Immune Disorder	Yes _____	No _____
Cancer or Malignancy (in the past 5 yrs)	Yes _____	No _____
If yes, what kind: _____		
Mental illness	Yes _____	No _____
Severe Depression	Yes _____	No _____
High blood pressure (SBP≥180mmHg, DBP≥110mmHg)	Yes _____	No _____ Don't Know_____
High blood cholesterol > 260 mg/dl	Yes _____	No _____ Don't Know_____
High blood triglycerides > 400 mg/dl	Yes _____	No _____ Don't Know_____

    NO → Continue to 9.
    YES→ I'm sorry you are not eligible to participate, _____condition is part of the exclusion criteria for this
    particular study. Would you mind if we held onto your name in the event you qualify for a future study?
    YES   NO

9. <u>Females only</u>: Are you pregnant?
                YES   NO
    NO → Continue 10
    YES → I'm sorry you are not eligible to participate. Pregnancy is an exclusion criterion for this study. Would
    you mind if we held onto your name in the event you qualify for a future study?   YES   NO

APPENDIX A (CONTINUED)

10. Are you comfortable giving blood and muscle tissue samples?   YES   NO

11. Are you able to come to UIC for 4 study visits? This study visit may last up to 4 hrs?

    YES   NO   MAYBE

    Comments (e.g.) work, children, transportation, etc:

    _____

12. Additional comments on potential subject:

    _____

    _____

    _____

    _____

    _____

From the initial answers you have provided, you are a likely candidate to qualify for the current study but final eligibility will be determined by the Principal Investigator and the study physician. Would you like to continue and hear some additional information about participating in the study at this time?
　　　YES→ Continue to Study Overview
　　　NO → Thank you for your time

Do you have any other questions?

Do not hesitate to contact me at (your phone and email) or 312-413-1913 with any additional questions or concerns. Thank you for your time.

NOTE: Give this screening form to Dr. Haus for review.

APPENDIX B
INFORMED CONSENT

University of Illinois at Chicago
Research Information and Consent for Participation in Biomedical Research

Effects of acute aerobic exercise intensity on plasma sRAGE in lean healthy individuals

You are being asked to participate in a research study. Researchers are required to provide a consent form such as this one to tell you about the research, to explain that taking part is voluntary, to describe the risks and benefits of participation, and to help you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Principal Investigator Name and Title: **Jacob M. Haus, Ph.D., Assistant Professor**

Department and Institution: **Department of Kinesiology and Nutrition, at the University of Illinois at Chicago**

Address and Contact Information: **1919 West Taylor St, Rm 530, (MC 517), Chicago, IL 60612; Phone: 312-413-1913, email: hausj@uic.edu**

Emergency Contact Name and Information: **Jacob Haus, phone: (330) 518 – 8225**

Sponsor: **none**

Why am I being asked?

You are being asked to be a subject in a research study about how different aerobic exercise intensities change the amount of a beneficial protein that is found in your body.

You have been asked to participate in the research because you have responded to an advertisement and are interested in being a participant

Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future dealings with the University of Illinois at Chicago. **If you decide to participate, you are free to withdraw at any time without affecting that relationship.**

Approximately 20 subjects may be involved in this research at UIC.

APPENDIX B (CONTINUED)

What is the purpose of this research?

A protein found in the body called sRAGE (soluble receptor for advanced glycation endproducts) may play a protective role in the development of future disease. Exercise has been shown to increase the amount of this protein in your body. This study is being done to find out if exercise intensity plays a role in the extent at which these protein level changes occur.

What procedures are involved?

This research will be performed at the Integrative Physiology Laboratory in the Disabilities, Health and Social Policy Building (DHSP) at 1640 W. Roosevelt Rd.

You will need to come to the study site at 4 separate times (4 study visits).

The total time of these combined visits is estimated to be approximately 10-11 hrs.

The study procedures are as follows:

VISIT 1:

For this visit you will be asked to report to the Integrative Physiology Laboratory in the Disabilities, Health and Social Policy Building (DHSP) at 1640 W. Roosevelt Rd.

This visit must be performed in the morning. This visit will be comprised of the procedures listed below. The total time for completing this visit is expected to be about *2 hrs*.

We ask that you fast for a period of 10-12 hrs prior to this visit and that you refrain from consuming alcohol for 48 hrs prior to participating in this study.

You will read and sign two copies of this informed consent document in order to enroll in the study. A study team member go through the informed consent document in detail with you, answering any questions you may have relating to the study. Once this document has been read and signed, confirming that you understand the study, you will be enrolled. Then, these procedures will occur in the following order.

- A study team member will take your height and weight

- *Dual Energy Absorptiometry (DEXA)* (approximately 30 minutes): This test is used to measure the amount of fat distributed throughout your whole body and will allow us to measure your percent body fat. During this test, you will lay on your back on a table for 10-15 minutes while a scanner passes over your body. This test also involves a very small amount of radiation exposure.

APPENDIX B (CONTINUED)

- *Blood Draw*: We will collect blood samples *two times* during this visit. The total amount of blood we will take from you is about 20 cc or 1 tablespoon. The first blood draw will be taken before the exercise capacity test (below) and the second blood draw will be taken after the exercise capacity test.

- *Exercise Capacity test* (approximately 30 minutes): This test will be used to evaluate your current level of physical fitness. You will be encouraged to walk on a treadmill as much as you can, safely. During this test you will breathe into a mouthpiece and a clip will be placed over your nose so that all of the air you breathe in and out, will pass through the mouthpiece and into a machine alongside the treadmill. The machine allows us to measure your oxygen consumption and provides a measure of your maximum oxygen capacity (VO2max), or maximum exercise capacity. During this test your heart rate and feelings of how hard you are working will also be monitored. You will walk or jog on a treadmill, with the incline progressively increased every few minutes, until fatigue, breathlessness, and/or symptoms indicate to the research staff, or yourself, that you should stop the exercise.

- *Questionnaires*: Following the procedures described above you will be given 2 separate questionnaires and instructions for their use on how to record your eating and physical activity habits. You will be asked to complete each of these questionairres for every day until the next study visit.

VISIT 2:

Approximately 4 days after *visit 1* or when your schedule permits you will be asked to return to the Integrative Physiology Laboratory in the Disabilities, Health and Social Policy Building (DHSP) at 1640 W. Roosevelt Rd.

This visit must be performed in the morning. This visit will be comprised of the procedures listed below. The total time for completing this visit is expected to be about *2 hrs*.

We ask that you fast for a period of 10-12 hrs prior to this visit and that you refrain from consuming alcohol for 48 hrs prior to participating in this study.

- *Blood Draw*: We will collect blood samples *three times* during this visit. The total amount of blood we will take from you is about 30 cc or 1.5 tablespoons. The first blood draw will be taken before the low intensity exercise test (below), the second blood draw will be taken during the exercise test and the final blood draw will be taken after completing the exercise test

- *Low Intensity Exercise Test* (approximately 60 minutes): During this visit you will perform treadmill exercise at low intensity (40% of your maximum capacity) for one hour. During this test you will breathe into a mouthpiece and a clip will be placed over your nose so that all of the air you breathe in and out, will pass through the mouthpiece and into a machine alongside the treadmill. The machine allows us to measure your

oxygen consumption and provides a measure of your metabolism. During this test your heart rate and feelings of how hard you are working will also be monitored.

- *Questionnaires*: Following the procedures described above you will be given 2 separate questionnaires and instructions for their use on how to record your eating and physical activity habits. You will be asked to complete each of these questionnaires for every day until the next study visit.

VISIT 3:

Approximately 4 days after *visit 2* or when your schedule permits you will be asked to return to the Integrative Physiology Laboratory in the Disabilities, Health and Social Policy Building (DHSP) at 1640 W. Roosevelt Rd.

This visit must be performed in the morning. This visit will be comprised of the procedures listed below. The total time for completing this visit is expected to be about *2 hrs*.

We ask that you fast for a period of 10-12 hrs prior to this visit and that you refrain from consuming alcohol for 48 hrs prior to participating in this study.

- *Blood Draw*: We will collect blood samples *three times* during this visit. The total amount of blood we will take from you is about 30 cc or 1.5 tablespoons. The first blood draw will be taken before the moderate intensity exercise test (below), the second blood draw will be taken during the exercise test and the final blood draw will be taken after completing the exercise test

- *Moderate Intensity Exercise Test* (approximately 60 minutes): During this visit you will perform treadmill exercise at moderate intensity (65% of your maximum capacity) for a period of time that equals the energy you burned during the *visit 2* exercises test. During this test you will breathe into a mouthpiece and a clip will be placed over your nose so that all of the air you breathe in and out, will pass through the mouthpiece and into a machine alongside the treadmill. The machine allows us to measure your oxygen consumption and provides a measure of your metabolism. During this test your heart rate and feelings of how hard you are working will also be monitored.

- *Questionnaires*: Following the procedures described above you will be given 2 separate questionnaires and instructions for their use on how to record your eating and physical activity habits. You will be asked to complete each of these questionnaires for every day until the next study visit.

VISIT 4:

Approximately 4 days after *visit 3* or when your schedule permits you will be asked to return to the Integrative Physiology Laboratory in the Disabilities, Health and Social Policy Building (DHSP) at 1640 W. Roosevelt Rd.

APPENDIX B (CONTINUED)

This visit must be performed in the morning. This visit will be comprised of the procedures listed below. The total time for completing this visit is expected to be about *4 hrs*.

We ask that you fast for a period of 10-12 hrs prior to this visit and that you refrain from consuming alcohol for 48 hrs prior to participating in this study.

- *Blood Draw*: We will collect blood samples *four times* during this visit. The total amount of blood we will take from you is about 40 cc or 2 tablespoons. The first blood draw will be taken before the high intensity exercise test (below), the second blood draw will be taken during the exercise test, the third blood draw will be taken immediately after completing the exercise test and the final blood draw will be taken 3 hrs after completing the exercise test.

- *High Intensity Exercise Test* (approximately 30-40 minutes): During this visit you will perform treadmill exercise at high intensity (80% of your maximum capacity) for a period of time that equals the energy you burned during the *visit 2* exercises test. During this test you will breathe into a mouthpiece and a clip will be placed over your nose so that all of the air you breathe in and out, will pass through the mouthpiece and into a machine alongside the treadmill. The machine allows us to measure your oxygen consumption and provides a measure of your metabolism. During this test your heart rate and feelings of how hard you are working will also be monitored.

- *Muscle Biopsy* (approximately 1.5 hrs): We will collect muscle biopsy samples three times during this visit. The first muscle will be taken before the high intensity exercise test, the second muscle biopsy will be taken immediately after completing the exercise test and the final muscle biopsy will be taken 3 hrs after completing the exercise test.

   The biopsy involves removal of a very small piece of muscle (1/3 the size of an eraser on a pencil) by inserting a needle into your outer thigh muscle through a quarter inch skin incision. Local anesthesia will be used to numb the area where the incision will be made. Muscle biopsies will be performed on both legs, alternating right and left. The biopsy involves the following:

   First, the skin on the outside portion of your lower thigh will be cleansed with an cleaning solution to sterile the area. Once thoroughly cleansed and dry, a small amount of numbing agent (about 3-4 cc, or less than ½ teaspoon of Lidocaine), will be injected into the area to be biopsied. If you are allergic to Lidocaine or drugs in the 'caine' family (e.g., Novocaine), tell the research team.

   Once the area is sufficiently numbed, a small incision will be made (approximately 5 mm, or less than ¼ of an inch) and a biopsy needle will be inserted in order to obtain approximately 200 mg per biopsy (400mg total) or about 0.007 ounces of muscle per biopsy. Once the biopsy has been completed, slight pressure will be applied to the biopsy area to minimize any bleeding. The area will then be cleansed and a special bandage will be applied to the biopsy site. An ACE bandage will be wrapped over the

APPENDIX B (CONTINUED)

site and you will be asked to wear this bandage for the next 24 hours to reduce the risk of bleeding.

One of the study team members will call you within 24 hours to follow-up on how your biopsy is healing and to ask if you are having any pain or discomfort that might limit your activity.

- After completion of the final muscle biopsy, you will have completed the study.

Tissue Banking and Participating in Future Studies

We would like to save tissue left-over after your muscle biopsy is tested to be used in future research. This excess tissue may be used to further explore mechanisms, which may contribute to the development of diabetes. Samples will be de-identified, and stored in the PI's laboratory to which only he and his personnel have access. Samples will be stored until utilized.

☐ I agree to allow my tissue sample to be kept by **Jacob Haus, PhD** for use in future research to learn more about how to prevent, detect, or treat disease.

☐ I do not agree to allow my tissue sample to be kept by **Jacob Haus, PhD** for use in future research to learn more about how to prevent, detect, or treat disease.

Initials _____

We plan future studies about how the body uses energy and stores fat. We would like to be able to contact you about future studies. Please indicate your interest about being contacted for future studies.

☐ I agree to allow the researchers to contact me about future research studies.

Initials _____ ;

How Long Will I Be In The Study?

Your participation in this study will last approximately 12 days and you will be asked to visit the west campus of University of Illinois at Chicago 4 times depending upon your schedule. The time required for these visits will vary depending upon the specific tests performed but we estimate that the total time required will be about 10-11 total hours.

What are the potential risks and discomforts?

Your participation in this study may involve the following risks:

Blood Draw: The risks of drawing blood from a vein includes discomfort at the site of the needle stick, possible bruising and swelling around the site of the needle stick, rarely an infection, and uncommonly feeling faint from the procedure.

APPENDIX B (CONTINUED)

Muscle Biopsy: You will feel pain, cramping, or bleeding where the sample is taken. Infection is very rare as your skin is cleansed with alcohol and the needle used is sterile. It is very rare, but you could have an allergic reaction to the lidocaine that is used to numb your skin. Tell the research team if you are allergic to any drugs in the "-caine" family (for example, lidocaine, novocaine).

Activity is good for your muscle after the biopsy. Walking is required after the biopsy procedure to help prevent additional stiffness and blood clot formation. (The development of a blood clot is related to inactivity, and may occur in less than 1% of biopsy procedures).

There may be additional pain at the biopsy site during or after exercise and there is also a possible risk of scarring.

Exercise capacity test/High intensity exercise: With a maximal effort exercise tests such as a VO2MAX there is an inherent risk of complications such as chest pain, shortness of breath, dizziness and rarely heart attack. However the occurrence of such events is highly unlikely in younger lean healthy individuals such as those who will be recruited for this study. You will be constantly monitored by exercise professionals who are CPR and advanced cardiac life support (ACLS) certified and will terminate any of the exercise trials at any time if they should feel it necessary. The apparatus/mask and nose clip used to measure VO2max may make you uncomfortable.

Exercise testing on the treadmill: You might experience tiredness or shortness of breath. You might feel like your heart is pounding very fast or very hard. You might feel dizzy or experience chest pain. You could stumble and fall off the treadmill. If you have any of these experiences, tell the research team. Also, when the breathing tube is in your mouth and you are wearing the nose clip, this might feel uncomfortable.

Radiation Exposure from DEXA scan: One of the risks associated with radiation exposure is cancer. The natural incidence of fatal cancer in the U.S. is about 1 chance in 5. Everyday radiation exposure from natural occurring background radiation (sun, radon exposure in the home) is approximately 3.0 mSv per year. In this research study, you will be receiving a DEXA scan. One DEXA scans amount to 0.6 mSv. The total radiation exposure in this study is about 0.6 mSv which is equal to approximately 0.2 years' worth of natural radiation exposure. This amount of radiation is very low as to make an accurate risk estimate meaningless. There is also no chance for skin injury. If you have already had many x-rays you should discuss the potential added risk from more radiation with the researchers before agreeing to be in the study.

Pregnant women, fertile females/males: There may be unforeseen risks to an unborn child associated with some of the study testing. Therefore, if you are capable of giving birth to or fathering a child, you and your sexual partner should use adequate birth control measures while you are in the study. These measures may include abstinence, oral contraceptives (birth control pills), IUD, diaphragm, approved hormone injections, condoms, or documentation of medical sterilization. If you are unwilling to do this, we ask that you not participate in this study.

Pregnancy tests will be performed on all women of child-bearing potential before beginning the study and before each DEXA scan. If you or your spouse becomes pregnant while taking part in this study you must notify the research staff immediately.

Questionnaires: You might find it boring or time-consuming to complete the questionnaires. There is the potential risk of loss of confidentiality. Every effort will be made to keep your information confidential, however, this cannot be guaranteed. Some of the questions we will ask you as part of this study may make you feel uncomfortable.

Dietary restrictions: You may find it burdensome to adhere to abstain from eating or drinking food or drink items containing caffeine or alcohol during the study periods. You might have to fight cravings to eat or drink items that are not permitted.

Unforeseeable risks: There may be risks or side effects related to the study that are unknown at this time. You will be notified of any significant new findings that become known that may affect your willingness to continue in the study.

Loss of Confidentiality: There is the potential risk of loss of confidentiality. Every effort will be made to keep your information confidential, however, this cannot be guaranteed.

Will I be told about new information that may affect my decision to participate?

Taking part in this study is voluntary. You will be told of any new, relevant information from the research that may affect your health, welfare, or willingness to continue in this study. You may choose not to take part or may leave the study at any time. Withdrawing from the study will not result in any penalty or loss of benefits to which you are entitled.

Are there benefits to taking part in the research?

You will not benefit from participating in this research

What other options are there?

You have the option to not participate in this study.

What about privacy and confidentiality?

The people who will know that you are a research subject are members of the research team, and if appropriate, your physicians and nurses. No information about you, or provided by you, during the research, will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care or when the UIC Office for the Protection of Research Subjects monitors the research or consent process) or if required by law.

Study information which identifies you and the consent/authorization form signed by you will be looked at and/or copied for examining the research by:

- UIC Office for the Protection of Research Subjects, and State of Illinois Auditors

A possible risk of the research is that your participation in the research or information about you and your health might become known to individuals outside the research.

Personal information collected will be kept secure by the investigative team in locked file cabinets in a controlled access room. Once you enroll into the study your name will be given a special code and then we will no longer use your name or personal information to identify the data we collect from you. Only the principal investigator will have access to the coded information

The final information collected from you will be stored in a secured manner as mentioned above to protect your information and ensure your privacy

At the completion of the study, any documents with personal information will be stripped of any information that can identify you only the coded data will remain. We will keep the coded information until we no longer need this information or until this information is of no further benefit in the discovery for the mechanisms and treatment of disease.
When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity.

What if I am injured as a result of my participation?

You may have medical problems or side effects from taking part in this research study. If you believe that you have become ill or been injured from taking part in this study, treatment may be obtained through:

- The UIC Medical Center OR
- Your regular doctor OR
- The treatment center or clinic of your choice.

If you do seek medical treatment, please take a copy of this document with you because it may help the doctors where you seek treatment. It will also provide the doctors where you seek treatment with information they may need if they want to contact the research doctors.

You may contact the researcher, **Jacob Haus, PhD at (330)-518-8225**, to talk to them about your illness or injury or in the case of an emergency.

You or your insurance company will be billed for this medical care. Your insurance company may not pay for some or all of this medical care because you are participating in a research study. There are no plans for the University to provide free medical care or to pay for research-

related illnesses or injuries, or for the University to provide other forms of compensation (such as lost wages or pain and suffering) to you for research related illnesses or injuries.

By signing this form you will not give up any legal rights.

What are the costs for participating in this research?

There are no costs to you for participating in this research.

Will I be reimbursed for any of my expenses or paid for my participation in this research?

You will be compensated $100 for completion of the study. Payments will be disbursed in 4 installments. You will receive $25 following completion of each visit. Compensation will not be provided for visits that you do not complete should you withdraw from the study.

Can I withdraw or be removed from the study?

If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC.

The researchers also have the right to stop your participation in this study without your consent if:
- You fail to follow the instructions of the study doctor or study staff.
- The study doctor decides that continuing participation could be harmful to you.
- The study is cancelled.
- Other administrative reasons.

In the event you withdraw or are asked to leave the study, you will be compensated as described above.

Who should I contact if I have questions?

Contact the researchers **Jacob M. Haus, PhD, Assistant Professor of Kinesiology and Nutrition at 312-413-1913 or email address: hausj@uic.edu**
- if you have any questions about this study or your part in it,
- if you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
- if you have questions, concerns or complaints about the research.

What are my rights as a research subject?

If you have questions about your rights as a research subject or concerns, complaints, or to offer input you may call the Office for the Protection of Research Subjects (OPRS) at 312-996-1711 or 1-866-789-6215 (toll-free) or e-mail OPRS at uicirb@uic.edu.

What if I am a UIC student?

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APPENDIX B (CONTINUED)

You may choose not to participate or to stop your participation in this research at any time. This will not affect your class standing or grades at UIC. The investigator may also end your participation in the research. If this happens, your class standing or grades will not be affected. You will not be offered or receive any special consideration if you participate in this research.

What if I am a UIC employee?
Your participation in this research is in no way a part of your university duties, and your refusal to participate will not in any way affect your employment with the university, or the benefits, privileges, or opportunities associated with your employment at UIC. You will not be offered or receive any special consideration if you participate in this research.

Remember:

Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future relations with the University. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

APPENDIX C
DIETARY LOG

24-HOUR DIET RECORD

NAME:_____ ID#_____

Write down everything that you eat and drink for_____day(s) during the following time period:

_____

Please include:_____weekdays _____weekend days

Choose days that are typical of your current eating habits.

DIRECTIONS

- Start with your first meal of the day. Record all foods, beverages, and supplements that you consume (except water) during the next 24 hours. Record the time of day that you consume each item.

- Record each item right after you consume it, rather than later in the day.

- If possible, list separately the different foods that compose one food item. *Example:* ham sandwich with ham, mayonnaise, and lettuce.

- If possible, specify the brand name, and how the item was prepared. *Example:* broiled, steamed, fried, poached, toasted, grilled, baked, or raw.

- If you eat at a restaurant, write the name of the restaurant.

- Include side items like gravy, jams, sauces, salads dressing, butter, margarine, sugar, and milk on cereal. Include alcohol-containing beverages.

- YOU MAY HAVE WATER on the evening prior to and the morning of your visits! Water is actually encouraged. Do not consume flavored, or carbonated water on the evening prior to or on the morning of your Glucose Tolerance Test.

- Describe amounts as accurately as possible. Record amounts in terms of dimensions, weight, or portion size. See below for some tips:

Meats, poultry and fish
Record in ounces, or measure the dimensions.

Example: Beef, 3 oz.
Example: Beef, 1 piece, 2" x 3" x 1"

Cereals, fats, & many snack items
Record in ounces, or in measuring cup or measuring spoon sizes.

Example: Cole slaw, 1/2 cup
Example: Margarine, 1 tsp.

Beverages
Record in fluid ounces or measuring cup sizes.

Example: Cola, 12 oz. can
Example: Coffee, 1 cup

Fruits and vegetables
Record in number of items or in measuring cup sizes.

Example: Cole slaw, 1/2 cup
Example: Apple, 1 item.

Version #2   24 Hour Diet Log

APPENDIX C (CONTINUED)

NAME:_____DATE:_____   DAY OF THE WEEK (please circle one)

STUDY:_____   Sun Mon Tues Wed Thurs Fri Sat

Time	Food Item	Amount Eaten	How Prepared	Brand Name
EX: 8:00 a.m.	Cereal, oatmeal	1 cup (cooked)	in microwave	Quaker Oats
EX: 8:00 a.m.	Milk, 1% fat (with cereal)	¼ cup	added to cereal	

Version #2   24 Hour Diet Log

APPENDIX D
PHYSICAL ACTIVITY LOG

Physical Activity Log

You can complete the highlighted fields on this form online and then print the form for easy reference. Only text that is visible on the form is printed; scrolled text will not print. Any text you enter into these fields will be cleared when you close the form; you cannot save it.

Marking progress

Use the chart below to keep track of how much activity you are getting. Include not only long walks or workouts, but also anytime you do something extra, like taking the stairs instead of the elevator, or parking at the far end of the lot at the store.

Physical activity log

Day of week	Activity (walk, bike, play, other)	Time (minutes)	Intensity (easy, moderate, hard)
Monday 1. 2. 3.			
Tuesday 1. 2. 3.			
Wednesday 1. 2. 3.			
Thursday 1. 2. 3.			
Friday 1. 2. 3.			
Saturday 1. 2. 3.			
Sunday 1. 2. 3.			

Print a new chart each week and compare your results. The time spent each day and the intensity should increase gradually over time. You may want to bring these charts to your doctor visits.

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VITA

Education

Aug. 2017   Master of Science
Major: Kinesiology
Track: Applied Exercise Physiology
GPA: 4.00
University of Illinois at Chicago, Chicago,
IL Advisor: Dr. Jacob Haus

May 2015   Bachelor of Science
Major: Exercise Science Allied Health
Track GPA: 3.97
Salisbury University, Salisbury, MD
Advisor: Dr. Scott Mazzetti

Professional Appointments

June 2016-present   Graduate Research Assistant
Department of Kinesiology and
Nutrition University of Illinois at
Chicago Chicago, IL
Advisor: Dr. Jacob Haus

Jan. 2016- present   Anatomy Workshop Instructor
Department of Kinesiology and
Nutrition
University of Illinois at Chicago
Chicago, IL

Aug. 2015- present   Graduate Teaching Assistant
Department of Kinesiology and
Nutrition
University of Illinois at Chicago
Chicago, IL

Jan. 2014 - May 2015   Research Assistant
The Laboratory for Human Performance
Salisbury
University
Salisbury, MD

Aug. 2014 - May 2015   Academic Tutor
Center for Student Achievement
Salisbury
University
Salisbury, MD

May 2014- Sept. 2014 Undergraduate Intern
         NRH Regional Rehabilitation
         MedStar National Rehabilitation
         Network Salisbury, MD

Courses Instructed

KN 251/252- Human Physiological Anatomy Laboratory
KN 496- Occupational Therapy Anatomy

Professional Service

May 2016   Faculty Selection Committee
         Department of Kinesiology and Nutrition
         University of Illinois at Chicago
         Chicago, IL

Aug. 2014 – May 2015 Exercise Science Club Executive Officer
         Student Government Association
         Salisbury University
         Salisbury, MD

Professional Certifications

Certified Strength and Conditioning Specialist-NSCA, *current*
Protecting Human Research Participants-NIH, *current*
Certified Tutor Level I-CRLA, *current*
Adult and Pediatric First Aide/CPR/AED-American Red Cross, *current*

Professional Memberships

American College of Sports Medicine-Midwest Chapter
National Strength and Conditioning Association
Phi Kappa Phi Professional Fraternity

Honors & Awards

National Student College Bowl-1st Place
American College of Sports Medicine, Awarded Spring 2015

Exercise Science Outstanding Major Award
Salisbury University, Awarded Spring 2015

MARC College Bowl- 1st Place
American College of Sports Medicine, Awarded, Fall 2014

Good Standing, Salisbury University

Awarded, Fall 2011-Spring 2015

Dean's List
Salisbury University, Awarded, Fall 2011- Spring 2015

Division II National Champion
USA Rugby, Awarded, Fall 2013, Bowling Green, OH

Presidential Scholarship
Salisbury University, Fall 2011-Spring 2015

Scientific Abstracts and Oral Presentations

Validity of a Weightlifting Accelerometer for Measuring Average Power:
William M. Castor, Joseph C. Watso, Samantha R. Guarnera, Josh M. Bock, Edwin R. Miranda, Victoria R. Meyers, Alec B. Chaves, Scott A. Mazzetti. Salisbury University, Salisbury, MD *Mid-Atlantic Regional Conference-American College Sports Medicine, Harrisburg, PA,* November, 2014.

Comparability of Tendo Weightlifting Analyzer with Recreational and Explosive Bench Press Exercise: Joseph Watso, William M. Castor, Alec B. Chaves, Victoria R. Meyers, Patrick J. Ferrara, Asif Shakur, Thomas C. Heinbockel, Scott A. Mazzetti. Salisbury University, Salisbury, MD, *Mid-Atlantic Regional Conference-American College Sports Medicine, Harrisburg, PA,* November, 2014.

Reliability of a Weightlifting Accelerometer During Repeated 1 KG Drop Trials: Alec B. Chaves, Pat J. Ferrara, Edwin R. Miranda, William M. Castor, Joseph C. Watso, Samantha R. Guarnera, Joshua M. Bock, Thomas C. Heinbockel, Scott A. Mazzetti. Salisbury University, Salisbury, MD *Mid-Atlantic Regional Conference- American College Sports Medicine, Harrisburg, PA,* November, 2014.

Poster Presentations

Validity of a Weightlifting Accelerometer for Measuring Average Power:
William M. Castor, Joseph C. Watso, Samantha R. Guarnera, Josh M. Bock, Edwin R. Miranda, Victoria R. Meyers, Alec B. Chaves, Scott A. Mazzetti. Salisbury University, Salisbury, MD, *National Annual Conference-American College of Sports Medicine,* San Diego, CA, May, 2015.

Comparability of Tendo Weightlifting Analyzer with Recreational and Explosive Bench Press Exercise: Joseph Watso, William M. Castor, Alec B. Chaves,

Victoria R. Meyers, Patrick J. Ferrara, Asif Shakur, Thomas C. Heinbockel, Scott A. Mazzetti. Salisbury University, Salisbury, MD, *National Annual Conference- American College of Sports Medicine,* San Diego, CA, May, 2015.

Educational Presentations

Understanding Spondylolysis and Spondylolisthesis. Alec Chaves. *MedStar National Rehabilitation Hospital,* Salisbury, MD*.* August 2014*.*

Collegiate Sports Appointments

Sept. 2012 – Nov. 2013   Forward's Captain
                                Salisbury University Men's Rugby Club
                                Salisbury
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Professional References

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