Effects of Acute Aerobic Exercise Intensity on Plasma sRAGE in Lean Healthy Individuals

BY

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THESIS

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LIST OF ABBREVIATIONS

ADAM10	A Disintigrin And Metalloproteinase 10
AGE	Advanced Glycation End Product
AGER	Advanced Glycation End Product Receptor Gene
AMPK	AMP-Activated Protein Kinase
BF%	Body Fat Percentage
BMI	Body Mass Index
Ca^{2+}	Calcium
CAM	Cell Adhesion Molecule
CAMKII	Calcium Calmodulin Dependent Protein Kinase II
CML	Nε–Carboxymethyllysine
COX	Cyclooxygenase
CVD	Cardiovascular Disease
Dial.	Hemodialysis
DN	Diabetic Nephropathy
DXA	Dual X-ray Absorptiometry
esRAGE	Endogenous Secretory RAGE
ESRD	End-Stage Renal Disease
GFR	Glomerular Filtration Rate
HEK	Human Embryonic Kidney Cells
hnRNPA1	Heteronuclear Ribonuclear Protein A1
hs-CRP	High-Sensitivity C-Reactive Protein
IMT	Intermedia Thickness
IRS	Insulin Receptor Substrate

LIST OF ABBREVIATIONS (continued)

kcal	Kilocalorie
kDa	Kilodaltons
MicroA.	Microalbuminuria
MMP-9	Matrix Metalloproteinase-9
mRAGE	Membrane-Bound RAGE
NAD^+	Nicotinamide Adenine Dinucleotide
Neg. Corr.	Negative Correlation
Neph.	Nephropathy
NF-kB	Nuclear Factor Kappa B
NormoA.	Normoalbuminuria
NPDR	Nonproliferative Diabetic Retinopathy
PBMC	Peripheral Blood Mononuclear Cells
PDR	Proliferative Diabetic Retinopathy
PKC	Protein Kinase C
RAGE	Receptor for Advanced Glycation End Products
RARβ	Retinoic Acid Receptor Beta
ROS	Reactive Oxygen Species
RRM	RNA Recognition Motif
RYR	Ryanodine Receptors
SIRT1	Sirtuin 1
SOD	Super Oxide Dismutase
SR	Sarcoplasmic Reticulum

LIST OF ABBREVIATIONS (continued)

sRAGE	Soluble RAGE
sRAGEc	Soluble Cleaved RAGE
SRSF	Serine-Arginine Splicing Factor
STZ	Streptozotocin
T2DM	Type 2 Diabetes Mellitus
TNF-α	Tumor Necrosis Factor-alpha
Tra2β	Transformer 2 Homolog Protein Beta
TRX	Thioredoxin
TXNIP	Thioredoxin Interacting Protein
VO_{2Max}	Maximal Oxygen Consumption

1. INTRODUCTION

In 2014 the Center for Disease Control stated that 29.1 million Americans were diagnosed with type 2 diabetes mellitus (T2DM) [1]. T2DM is characterized by insulin resistance, hyperglycemia, dyslipidemia, enhanced oxidative stress and chronic low-grade inflammation. If left untreated this diabetic milieu induces secondary complications such as neuropathy, nephropathy, retinopathy, and cardiovascular disease (CVD). The later of these complications is of critical importance as individuals with T2DM have a 50% greater chance of CVD related death than non-T2DM individuals [1]. These complications associated with T2DM are largely the result of unabated oxidative stress and low-grade chronic inflammation associated with T2DM, eventually resulting in cellular dysfunction and tissue damage. While the pathogenesis of T2DM is complex and multifactorial, one area that has gained much recent attention for its contribution to T2DM and the development of diabetes related complications is the formation advanced glycation end products (AGEs) and their interaction with their receptor (RAGE).

AGEs are glycotoxins that form as a result of non-enzymatic oxidation of a reducing sugar and a protein, lipid or nucleic acid through a series of reactions collectively known as the Maillard reaction [2]. AGEs are an irreversible product that once formed are difficult to remove due to their resistance to proteolytic degradation [3]. AGE formation is a result of normal metabolism and accumulation often occurs slowly over the course of months or years on proteins with relatively low turnover rates such as those in the lens of the eye [4, 5]. As such AGEs have been shown to accumulate in the skeletal muscle of older adults and may be related to age-induced decline in skeletal

muscle function by forming crosslinks between proteins that enhance tissue stiffness [6]. However, hyperglycemia and increased production of reactive oxygen species accelerate the formation of AGEs and therefore have been shown to accumulate in individuals with T2DM [7, 8]. In addition to altering protein structure and function, circulating AGEs also serve as ligands for RAGE, the activation of which initiates an inflammatory pathway resulting in cellular damage as well as further upregulation of RAGE expression [9].

RAGE is a transmembrane protein belonging to the immunoglobulin super family of proteins. RAGE is ubiquitously expressed across tissues however protein expression of RAGE is highest in bovine skeletal muscle tissue and its gene expression in skeletal muscle is the third highest amongst tissues studied [10]. Upon binding their ligands, RAGE initiates a self-perpetuating inflammatory cascade. The signal transduced by RAGE not only results in the induction of inflammatory cytokines but also RAGE itself via activation of Nuclear Factor Kappa B (NF-kB), which is able to bind to the promoter of RAGE and induce its transcription [9]. Activation of RAGE therefore results in a cyclical activation of RAGE and subsequent exacerbation of inflammatory signaling that contributes to tissue damage contributing to secondary diabetic complications. While the exact signaling cascade that is perpetuated by RAGE activation has not been fully elucidated, it is known that the cytosolic tail of RAGE is necessary for signal transduction.

Soluble versions of RAGE have been found in the circulation and are able to act as decoys for ligands such as AGEs by bind to AGEs without transducing downstream RAGE signaling [11]. Membrane bound RAGE has been demonstrated to be processed

by proteolytic cleavage via metalloproteases such as Matrix Metalloprotease-9 (MMP-9) and A Disintigrin And Metalloprotease 10 (ADAM10) resulting in a soluble cleaved (sRAGEc) isoform lacking the intracellular c-terminus. An alternative splice variant of RAGE, termed endogenous secretory RAGE (esRAGE), also exists and also lacks the transmembrane and cytosolic domains. Both of these isoforms maintain the ability to bind to RAGE ligands, however, because they lack the transmembrane and intracellular c-terminus domains of RAGE, these isoforms are unable to activate downstream signaling upon ligand binding. This makes these soluble isoforms of RAGE act as competitive inhibitors of the membrane-bound RAGE (mRAGE) receptors. However while mRAGE protein expression is exacerbated in T2DM, the protective sRAGE isoforms are decreased in individuals with T2DM [12-14] and therefore interventions targeting increased sRAGE production may be a viable therapeutic option for the treatment of diabetes-associated inflammation and complications.

Overwhelming evidence supports exercise as a potent method for prevention and treatment for T2DM and as such the American Diabetes Association and American College of Sports Medicine recommend that individuals with T2DM perform at least 150 minutes of moderate intensity aerobic exercise per week [15]. Both acute and chronic aerobic exercises are successful at sensitizing the skeletal muscle to insulin, as well as inducing anti-inflammatory and anti-oxidant defenses [16-18]. Skeletal muscle accounts for over 30 percent of body mass and approximately 80 percent of insulin stimulated glucose uptake making it extremely relevant in the pathogenesis and treatment of T2DM. However, the current literature on the effect of acute or chronic aerobic exercise on plasma sRAGE production is limited although most studies that exist in the current

literature suggest that exercise may be able to increase sRAGE production [19-22]. However, these studies are inconsistent in their methods making it difficult to draw definitive conclusions. Only one study has examined the effect of acute exercise on sRAGE production and no study has examined the effect of exercise intensity on sRAGE. Furthermore, there are no studies that examine the effect of exercise on both isoforms nor the mechanisms by which exercise may be modulating sRAGE production. Finally, only one study to date has examined the effect of exercise on sRAGE concentration in lean healthy individuals. Before research moves forward to study the effect of exercise RAGE biology in a diseased population, the normal physiology must be established in order to determine the degree to which diseases such as T2DM perturb sRAGE production and to what degree exercise may be able to rectify this disparity.

Skeletal muscle accounts for 30-40% of total body mass and therefore alteration of RAGE metabolism in this tissue via exercise may result in large contributions to sRAGE production potentially having significant systemic effects. However, no skeletal muscle or exercise-specific mechanisms have been studied, although cellular mechanisms for the production of sRAGEc and esRAGE, outside the context of exercise have been explored. ADAM10 seems to be the primary metalloprotease responsible for cleaving RAGE [23] and as such many mechanisms exploring sRAGEc production have focused on the modulation of its expression and activity. The NAD^+ dependent deacetylase SIRT1 has been previously shown to induce ADAM10 expression [24] and increases in cellular Ca^{2+} concentration has been shown to increase ADAM10 activity [25]. Exercise is known to modulate both NAD^+ and Ca^{2+}

concentration in the cytosol of skeletal muscle in an intensity dependent manner. Therefore, exercise may be able to increase ADAM10 expression and activity in an intensity dependent manner resulting in sRAGEc production to be enhanced following exercise. In regards to the alternatively spliced isoform of sRAGE (esRAGE) two splicing factors, hnRNPA1 and TRA2β-1, have recently been linked to mRAGE and esRAGE production respectively in vitro [26]. Exercise is known to drastically alter the spliceosome and therefore may also have an effect on esRAGE production by altering the ratio of these two splicing factors following exercise.

1.1 Purpose of the study

The purpose of this study was to determine the effect of exercise intensity on plasma sRAGE in lean healthy individuals and determine the mechanisms by which exercise modulated skeletal muscle sRAGE production. Blood samples were taken before and after exercise in order to measure sRAGEc and esRAGE production during three aerobic treadmill exercise bouts at 40%, 65%, and 80% of VO_{2Max}. The calories expended during the 40% and 80% trials were matched to ensure that any difference in sRAGE following each trial was due to the difference in intensity without being confounded by differences in total caloric expenditure. In addition, muscle biopsies were taken from the vastus lateralis 30 minutes before, 30 minutes after, and three hours after the 80% trial to investigate potential mechanisms of sRAGE production in the skeletal muscle.

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 exercise intensity on plasma sRAGE levels in*

lean healthy individuals. Our hypothesis for this aim is that higher intensity exercise will elicit greater plasma sRAGE levels than lower intensity exercise in lean healthy individuals. We will test this hypothesis by measuring plasma sRAGE levels 30 minutes before and immediately after acute bouts of aerobic exercise at 40% VO_{2max} for 1 hour and then at 65 VO_{2max} for 30 minutes and 80% of VO_{2max} for a time to achieve matched work (total kcals) achieved during 40% exercise bout.

Specific Aim 2: *Aim 2: Determine the mechanism of sRAGE production with exercise.* For this aim, our hypothesis is the skeletal muscle is a quantitatively important source of sRAGE production and that mechanisms related to skeletal muscle turnover and alternative splicing will be modulated by exercise to produce sRAGE. We will test this hypothesis by obtaining skeletal muscle biopsies at rest, immediately following (+0min) and 3 hours after (+3 hrs) cessation of exercise at 80% VO_{2max} and measuring the protein expression of RAGE, ADAM10, Tra2β, and hnRNPA1.

1.3 Problem Statement

Understanding the normal physiology of exercise-induced sRAGE is important for juxtaposition of diseased individuals in order to accurately gauge the degree of the pathology. In addition, optimal exercise prescription is necessary for targeting maximal sRAGE production for all populations making the effect of exercise intensity important knowledge to be had. Finally, the mechanisms by which exercise alters the production of sRAGEc and esRAGE are not currently known and are valuable for complete comprehension of the RAGE pathway. Intricate knowledge of mechanisms by which exercise improves sRAGE production will further implicate exercise as medicine and also may lead to the development of pharmacologic interventions that are ideal for

individuals who are contraindicated for exercise but would benefit from increasing their sRAGE concentration.

1.4 Significance of Problem

The frequency of T2DM has reached worldwide epidemic proportions and was responsible for 1.5 million deaths in 2012 [27]. T2DM is associated with chronic inflammation and oxidative stress that contribute to complications such as cardiovascular disease that are more likely to be fatal in individuals with T2DM. The AGE-RAGE mechanism plays a significant role in inflammation generation and thus exacerbation of diabetic complications and as such interventions targeting this pathway may be valuable in the treatment of T2DM. Soluble isoforms of RAGE have the capability to slow the progression of diabetic complications and exercise may be a viable way to induce their production. However, more needs to be elucidated about proper exercise dosing to optimally elicit this effect and the mechanisms by which exercise produces sRAGE. Understanding the normal physiology in this process is the first step towards better understanding this mechanism, which can later be adapted into disease model.

2. REVIEW OF THE LITERATURE

T2DM is characterized by insulin resistance, hyperglycemia, oxidative stress and chronic inflammation. This diabetic milieu if unchecked leads to secondary complications such as neuropathy, nephropathy, retinopathy and CVD. Although the genesis of these complications are complex and multifactorial, the accumulation of advanced glycation end products (AGEs) and their interaction with their receptor (RAGE) play a significant role in the underlying inflammation and oxidative stress that leads to cellular damage and eventually culminates in the manifestation of these secondary perturbations. However, soluble isoforms of RAGE are emerging as an interesting therapeutic target for diabetes and its complications due to their ability to interrupt AGE-RAGE interactions and downstream effects. Therefore, the purpose of this review is to: 1. provide background information on AGEs and RAGE and how they contribute to the diabetic milieu and genesis of complications, 2. explain the different isoforms of sRAGE, their role in diabetes and the mechanisms by which they are produced, and 3. explore exercise as an intervention that aims to increase sRAGE production.

2.1 What are AGEs?

AGEs are glycotoxins that are the result of protein, lipid, or nucleic-acid oxidation by glucose or glucose metabolites. AGEs are formed through a process known as the Maillard reaction. Named after its discoverer, Louis Camille Maillard, the reaction was first described in 1912 whereby glucose and amino acids underwent spontaneous interaction under heat, resulting in a physical change in the appearance of the

molecules [2]. Figure 1 describes the progression from a normal protein of native structure into a fully formed AGE having perturbed structure and function.

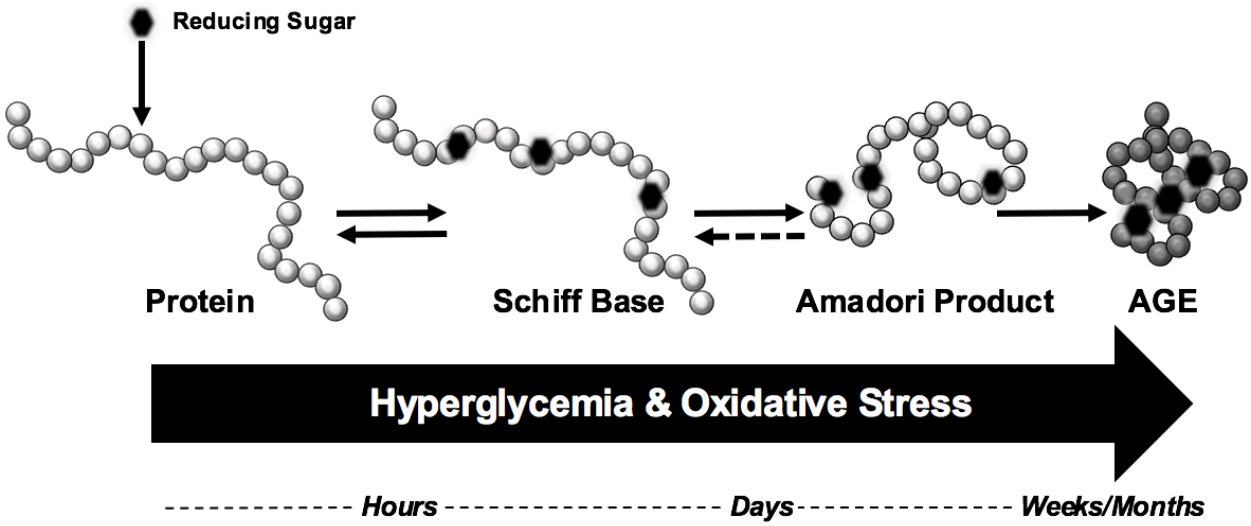

Figure 1. Progression from a native protein into an Advanced Glycation End Product (AGE). Reducing sugars are able to undergo a series of non-enzymatic processes that occur over time and eventually lead to the accumulation of AGEs which can alter a protein's structure and function by forming crosslinks. Hyperglycemia and oxidative stress accelerate this process by making sugar substrates and oxidizing agents more available.

These glucose modifications affect the structural and functional integrity of macromolecules and can cause them to become damaging [28]. Furthermore, these structural changes to proteins cause them to become resistant to normal proteolysis, allowing them to circumvent the cellular processes that would normally dispose of these agents [29], [3]. Although AGEs typically form slowly as a result of normal metabolism, certain disease states produce an environment that increases the rate of AGE

formation. The chronic low-grade inflammation and hyperglycemia in diabetes is an example of a disease that promotes acceleration of AGE formation. The combination of increased AGE formation and decreased proteolytic disposal causes accumulation of AGEs in circulation and various tissues, which confers susceptibility to AGE-mediated damage.

2.2 How are AGEs Formed?

The Maillard reaction initiates with the condensation of a carbonyl or reducing sugar molecule and a free amino group of a protein forming a reversible Schiff base, a process that takes place over the timespan of hours to days. In acidic environments, Schiff bases are rearranged to form Amadori products. A clinically relevant amadori product is Hba1c (glycated hemoglobin), which is now used as a marker for long-term (~3 weeks) glycemic control. Formation of Amadori products on long-lived proteins such as hemoglobin is still reversible until they are oxidized resulting in the cross linking of proteins and irreversible formation of AGEs [30]. Glucose can also generate AGEs independent of the formation of Amadori products through an alternative pathway whereby glucose is oxidized and produces the highly reactive AGE precursor methylglyoxal, which reacts with arginine residues on proteins to form AGEs such as hydroimidazolone [31]. Exogenous sources of AGEs, like the ones discovered by Louis Maillard, are rapidly formed by preparing food using high heat, low moisture cooking methods such as searing and grilling and are evident by the browning of the food. Exogenous sources of AGEs are believed to contribute minimally to the accumulation of AGEs with approximately 10% of the exogenous AGEs that are consumed contributing to the total AGE pool [32]. However, some studies have shown positive effects of a low

AGE diet [33-36]. Diabetic individuals who followed an AGE restricted diet for 4 months significantly lowered circulating concentrations of AGEs and improved insulin sensitivity whereas diabetic individuals who continued their normal diets experienced increased circulating AGEs and further worsening insulin resistance [34].

Unlike exogenous formation, endogenous AGE formation is largely a result of normal metabolism and accumulation often occurs at a much slower pace. The accumulation of endogenous AGEs were first observed on long-lived proteins such as the lens of the eye and skin collagen [2, 4, 5]. Although not pathologically relevant to T2DM, these were ideal tissues to study AGEs in vivo due to their limited amount of turnover, which allowed for AGEs to accumulate on them over time.

However, as previously mentioned AGE formation is accelerated by oxidative and hyperglycemic environments and as a result, accumulate in diseases such as T2DM. Individuals with T2DM have higher concentrations of circulating AGEs than healthy individuals. The most common circulating AGEs, Nε–Carboxymethyllysine (CML) and AGE-modified albumin, have both been repeatedly demonstrated to be significantly more concentrated in the plasma of diabetic individuals compared to healthy individuals [37-39]. Circulating AGE concentration has been correlated to insulin resistance [40, 41], as well as diabetic complications such as retinopathy, nephropathy and vascular dysfunction [7]. Further, older adults with high plasma CML is associated with significantly increased arterial stiffness [42], poor grip strength [43] and increased risk of all-cause mortality [44]. AGE accretion has also been demonstrated in skeletal muscle of older individuals and was shown to contribute to impairment of skeletal muscle function in these subjects via inducing crosslinking of modified proteins [6].

These data implicate AGE formation as a consequence of diabetes as well as a driver of diabetes related complications.

2.3 Receptor for Advanced Glycation End products (RAGE)

While AGEs cause cellular damage by altering protein structure and function and accumulating in tissues, circulating AGEs exhibit their damaging effects largely through binding to the receptor for advanced glycation end products (RAGE). RAGE is a multi-ligand, type 1 transmembrane receptor that belongs to the immunoglobulin super-family of proteins [45]. RAGE consists of three extracellular domains; one (variable) V-type domain that binds to ligands, and two (constant) C-Type domains that stabilize the V-type domain. RAGE was first discovered in bovine lung tissue and was so named because its first discovered ligand was AGE-modified albumin. The transfection of human cells with bovine RAGE, resulted in the discovery of a ~55 kDa protein with 90% homology to the bovine RAGE protein in humans [45]. RAGE is most concentrated in the lung [10] perhaps due to its physiological role for lung development [46]. However, RAGE is expressed in many other tissues in humans at considerable levels such as heart [10], brain [10], liver [10], endothelial cells [47, 48], macrophages [9], and skeletal muscle [10] as well. Among tissues studied by Brett J *et al*., skeletal muscle had the third highest gene expression in human samples and the highest protein concentrations in bovine samples [10]. Being of the immunoglobulin superfamily of protein receptors, RAGE plays a key role in innate immunity and inflammation. As such, RAGE protein expression is exacerbated in conditions of inflammation such as T2DM.

Mouse models of diabetes have shown increased RAGE expression in coronary arterioles and aortas of diabetic mice along with concomitant increases in circulating

RAGE ligands [49, 50]. In humans, peripheral blood mononuclear cells (PBMCs) of diabetic individuals have significantly greater expression of membrane bound RAGE compared to healthy controls [13]. A recent study found that diabetic individuals without vascular complications have a four-fold increase in PBMC RAGE protein whereas diabetics with vascular complications have up to 12 fold greater expression compared to healthy controls whereas [51]. This suggests that more inflammation begets more RAGE and that RAGE accumulation may be playing a role in the progression of vascular complications.

Although the protein expression of RAGE in skeletal muscle has previously been shown to be highest among some other tissues, the accumulation of RAGE in skeletal muscle is not well studied. Chiu *et al*. 2016 demonstrated that skeletal muscle from one elderly diabetic patient had an accumulation of RAGE compared to an age matched healthy individual and another study showed plasma AGE accumulation and weight-gain positively correlated with RAGE concentration in skeletal muscle [52]. Collectively these data suggest that RAGE may be playing a role in the progression of diabetic complications. Further, data that correlated increased RAGE concentration to that of their ligands also suggests that RAGE may be able to up regulate their own expression upon activation by AGEs.

2.5 Complications with AGE-RAGE Signaling

Once activated by AGE or one of its other ligands, RAGE induces an inflammatory cascade culminating in the activation of nuclear factor kappa B (NF-kB) [53]. NF-kB is a key transcription factor for many inflammatory cytokines including interleukins (ILs), cellular adhesion molecules (CAMs), and death receptor ligands such

as Tumor Necrosis Factor alpha (TNF-α). Importantly NF-kB also binds to the promoter region of the RAGE gene and induces its transcription meaning that activation of RAGE induces further RAGE up regulation [53]. Although the mechanisms by which AGE-RAGE interaction induce activation of NF-kB some data suggest that ROS production, in part by the activation of NADPH Oxidase and thioredoxin interacting protein (TXNIP), may be playing a key role [54, 55]. NADPH Oxidase is a major contributor to cellular ROS production and TXNIP is an inhibitor of the ROS scavenger thioredoxin (TRX). In vitro RAGE-mediated ROS production increases inflammatory markers including CAMs, cyclooxygenase (COX), and NF-kB.

Consequences of AGE-RAGE signaling have been demonstrated in an array of tissues. RAGE activation has been shown to increase adhesion molecules [56], and promote permeability in endothelial cells [47], promote the expression of adhesion molecules on macrophages [57], increase calcification of vascular smooth muscle [58], promote luminal vascular lesions [59], and perturb insulin sensitivity [33] and promote atrophy in skeletal muscle [60].

Cassese *et al*. demonstrated that treatment of L6 myotubes with human glycated albumin induced a physical interaction between RAGE and Src leading to activation of protein kinase-C (PKC) alpha [33]. PKC alpha has been implicated in the attenuation of insulin mediated glucose uptake via inhibitory phosphorylation of IRS-1. In agreement with this mechanism, tibialis muscles of mice fed a high AGE diet had decreased insulin sensitivity, and a significant increase in PKC alpha activity [33].

Skeletal muscle accounts for more than one third of total body mass [61] and is responsible for more than 80% of the insulin stimulated glucose uptake [62]. Therefore,

the ability of AGE-RAGE interactions to perturb skeletal muscle insulin sensitivity and promote muscle atrophy are important mechanisms by which RAGE may be contributing to the progression of diabetes. More research is needed to determine the role of RAGE in skeletal muscle perturbations in diabetes and also to elucidate potential mechanisms by which RAGE action may be limited in this tissue. A promising therapeutic target that has recently emerged is the soluble isoforms of RAGE. These compete for RAGE ligands without transducing an inflammatory signaling cascade, appear to be a viable therapeutic target in perturbing RAGE signaling.

2.6 Soluble RAGE

The structure of RAGE confers specific functionality and is increasingly important in understanding RAGE regulation. Although the precise mechanism by which the binding of ligands to RAGE initiates intracellular signaling is largely unknown, it is known that the cytosolic c-terminus of RAGE is necessary to stimulate RAGE signal transduction [63]. There are two isoforms of RAGE that lack this cytosolic c-terminus, collectively termed soluble RAGE (sRAGE). These isoforms lack of the intracellular domain possessed by the membrane isoform, liberating them from the membrane and allowing them to appear in circulation. The two most common of these soluble isoforms are produced either by proteolytic cleavage of the extracellular domain or via alternative splicing of the RAGE gene.

These soluble isoforms retain their ability to bind to RAGE ligands to the same capacity of membrane bound RAGE however, because they lack the cytosolic domain they do not transduce the downstream signaling and are therefore believed to act as competitive inhibitors of membrane bound RAGE [53]. The ability of sRAGE to perturb

RAGE signaling has been repeatedly demonstrated in vitro. Bovine aortic endothelial cells treated with AGE-modified albumin and sRAGE significantly suppressed activation of NF-kB compared to cells treated with AGEs alone [64]. Kidneys of mice treated with AGE-modified albumin and sRAGE had an ablation of NF-kB activation [64]. Intramuscular injection of sRAGE into mice treated with the diabetes inducer streptozotocin (STZ) and fed a high fat diet, reduced markers of oxidative stress and the prevalence of diabetes development [8]. In a study performed by Cassesse *et al*., treating skeletal muscle cells with sRAGE ablated AGE stimulated PKC alpha activity [33]. In addition, sRAGE negatively correlates with membrane bound RAGE [13, 33], and 8-iso-PGF_2 (a marker of oxidative damage) [65].

In contrast, many cross sectional studies demonstrate that individuals with T2DM have elevated sRAGE levels and that sRAGE correlates with a number of oxidative stress markers such as TNF-alpha, high-sensitivity c-Reactive Protein (hs-CRP), and AGEs, and have therefore been suggested as a possible biomarker [66-77]. However, other studies suggest that Type 2 Diabetics possess lower circulating levels of sRAGE compared to healthy controls, and that these low levels correlate with poor health outcomes and inflammatory markers [12-14] (Table 1).

TABLE 1. Human sRAGE Literature

Study	Sample Size	Renal Function	Age (y)	BMI (kg/m2)	Outcome(s)
[78]	n = 337 (203 T2DM)	Excluded T2DM w/ Proteinuria	T2DM: 58 ± 11 Control: 57 ± 12	T2DM: 23.3 ± 3.5 Control: 23.0 ± 3.0	↓ esRAGE in T2DM esRAGE Neg. Corr. w/ Carotid and femoral IMT, HOMA, & BMI
[79]	n = 160 (84 T2DM)	T2DM W/O Renal Dysfunction	T2DM: 60 ± 7 Control: 45 ± 10	T2DM: 28.8 ± 3.5 Control: 28.0 ± 3.9	↓ sRAGE in T2DM sRAGE Neg. Corr. w/ CRP, HOMA, HbA1C & CVD risk
[71]	n = 468 (318 T2DM)	T2DM stratified by renal function	Proteinuria: 54 ± 10 MircoA: 53 ± 9 NormoA: 51 ± 7 Control: 51 ± 6	Proteinuria: 26.7 ± 4.5 MircoA: 26.4 ± 3.9 NormoA: 25.3 ± 3.9 Control: 24.6 ± 3.3	↑ sRAGE in T2DM w/ proteinuria sRAGE corr. w/ creatinine in T2DM
[67]	n = 110 (All T2DM)	24 h Albumin excretion	T2DM: 59 ± 7	T2DM: 33 ± 6	sRAGE Corr. w/ 24 h Albumin excretion
[68]	n = 86 (All T2DM)	Serum creatinine	T2DM: 68 ± 10	T2DM: 24.7 ± 4.1	sRAGE Corr. w/ MCP-1 and TNF-alpha
[69]	n = 150 (75 T2DM)	Serum creatinine	T2DM: 66 ± 10 Control: 66 ± 12	T2DM: 24.9 ± 4.2 Control: 23.3 ± 3.7	↑ sRAGE in T2DM compared to control sRAGE Corr. w/ HbA1c & Creatinine
[70]	n = 172 (86 T2DM)	Serum creatinine	T2DM: 68 ± 10 Control: 68 ± 9	T2DM: 24.7 ± 4.1 Control: 23.2 ± 3.2	↑ sRAGE & Creatinine in T2DM compared to control
[66]	n = 107 (All T2DM)	T2DM stratified by nephropathy status	W/O Neph: 62 ± 13 W/ Neph: 63 ± 12 Dial: 65 ± 11	W/O Neph: 24.7 ± 4.4 W/ Neph: 24.7 ± 4.0 Dial: 21.8 ± 3.4	↑ sRAGE in T2DM w/ nephropathy and hemodialysis sRAGE neg Corr. w/ BMI and positively Corr. w/ creatinine

Study	Sample Size	Renal Function	Age (y)	BMI (kg/m2)	Outcome(s)
[80]	n = 73 (T2DM 45)	Urinary Albumin Excretion	T2DM w/ Complications: 60 ± 2 T2DM w/o Complications: 60 ± 1 Control: 52 ± 1	T2DM w/ Complications: 27.2 ± 1.2 T2DM w/o Complications: 26.2 ± 0.9 Control: 22.0 ± 2.1	↓ sRAGE in T2DM w/ retinopathy and microalbuminuria
[72]	n = 265 (All T2DM)	T2DM stratified by renal function	ESRD: 68 Manifest DN: 65 Incipitent DN: 70 NormoA: 56	Not Reported	↑ sRAGE with worsening neph. sRAGE neg Corr. w/ GFR
[81]	n = 245 (All T2DM)	Serum creatinine	T2DM: 63 ± 7	T2DM: 24.6 ± 2.8	sRAGE neg Corr. w/ hsCRP
[73]	n = 551 (All T2DM)	GFR	T2DM: 61	T2DM: 29	Highest sRAGE tertile has highest hazard ratio to develop CVD
[75]	n = 130 (T2DM 100)	Serum creatinine	Control: 52 ± 9 T2DM: 59 ± 11	Control: 25.6 ± 1.2 T2DM: 29.4 ± 2.4	↑ sRAGE in T2DM with PDR
[77]	n = 82 (T2DM 41)	GFR	Control: 53 ± 9 T2DM: 57 ± 9	Control: 22.8 ± 3.5 T2DM: 24.4 ± 3.4	esRAGE & sRAGE neg Corr. w/ ROS and fasting plasma insulin. sRAGE neg Corr. w/ HbA1c
[74]	n = 276 (All T2DM)	Serum creatinine	T2DM: 60 ± 13	T2DM: 23.6 ± 3.6	Highest quartile of sRAGE associated w/ highest hazard ratio for developing CVD after controlling for GFR
[76]	n = 606 (T2DM 371)	GFR	Control: 57 ± 4 No DR 59 ± 10 DR: 57 ± 10 NPDR: 59 ± 10 PDR: 53 ± 9	Control: 25.6 ± 4.8 No DR: 27.2 ± 4.4 DR: 26.3 ± 5.0 NPDR: 26.2 ± 4.5 PDR: 26.6 ± 5.9	↑ sRAGE in T2DM with retinopathy. sRAGE neg Corr. w/ SOD and glutathione peroxidase
[82]	n = 61 (All T2DM)	GFR	T2DM w/ vascular plaques: 65 ± 9 T2DM w/o vascular plaques: 58 ± 8	T2DM w/ vascular plaques: 27.9 ± 4.4 T2DM w/o vascular plaques: 28.4 ± 4.4	↓ esRAGE in T2DM w/ vascular plaques. esRAGE neg Corr. w/ fasting plasma glucose and BMI

Table 1. Human literature examining the concentration of soluble RAGE in Type 2 Diabetic individuals. Majority of the literature suggests that sRAGE is increased in Type 2 Diabetics. However, this observation may be due to renal dysfunction in these individuals. Abbreviations: Neg. Corr.: Negative Correlation, IMT: Intermedia Thickness, MicroA: Microalbuminuria, NormoA: Normoalbuminuria, Neph: Nephropathy, Dial: Hemodialysis, DN: Diabetic Nephropathy, ESRD: End Stage Renal Disease, GFR: Glomerular Filtration Rate, DR: Diabetic Nephropathy, NPDR: Nonproliferative Diabetic Retinopathy, PDR: Proliferative Diabetic Retinopathy, SOD: Super Oxide Dismutase.

A possible explanation for the discrepancy of whether sRAGE is elevated or reduced in diabetic individuals recently offered in a review by Schmidt *et al*. was that even a modest amount of kidney dysfunction in diabetic patients may contribute to the observed high levels of sRAGE [11]. This hypothesis is given in light of a multitude of evidence that consistently finds low circulating sRAGE in other disease and inflammatory models other than T2DM. Single nucleotide polymorphisms (SNPs) of the AGER gene that encodes RAGE has also been examined in an attempt to explain these discrepancies [83]. Maruthur *et al*. found that specific a SNP (rs2070600) accounted for up to 26% of the sRAGE variation but were not able to correlate this genetic determinant of sRAGE to mortality or diabetes risk [83].

In support of sRAGE as a protective protein against RAGE stimulation and diabetic complications, a study on over 1,200 people found that low levels of circulating sRAGE was associated with an increased risk of diabetes and death following ~18 year follow up [84]. Further, Di Pino *et al*. demonstrated lower esRAGE levels in pre-diabetic compared to healthy controls, and that HbA1c, and hs-CRP were also inversely associated with esRAGE [85]. esRAGE negatively correlated with carotid intima-media thickness in individuals with T2DM [86], which is a clinical measure of atherosclerosis progression. A cross sectional comparison of individuals across the insulin sensitivity continuum revealed circulating sRAGE and esRAGE were associated with reduced insulin sensitivity and increased markers of oxidative stress in pre-diabetic individuals [65].

Due to its therapeutic potential in T2DM and other chronic diseases, sRAGE has emerged as a promising target for treatment of these diseases. However, in order to

progress towards exploiting this pathway as a therapy, a deeper understanding of the mechanisms regulating sRAGE production are needed.

2.7 sRAGE production via Proteolytic Processing

Soluble cleaved RAGE (sRAGEc) is produced by cleavage of the extracellular domain of membrane bound RAGE from the cell surface. This is achieved primarily by a disintegrin and metalloproteinase 10 (ADAM10) [23] and secondarily by soluble matrix metalloproteinases (MMPs) [87]. ADAM10 is a member of an extensive family of zinc-dependent proteinases. Since ADAM10 is the primary proteinase responsible for cleavage of membrane-bound RAGE, understanding how its activity and protein expression are regulated will give insight into how sRAGEc production can be modulated.

Following translation, ADAM10 possesses a prodomain, the removal of which is necessary for ADAM10 to become constitutively active. The removal of the prodomain is achieved by proprotein convertases as ADAM10 progresses through the secretory pathway and migrates to the Golgi apparatus where the prodomain is cleaved [88].

ADAM10's activity can be further modulated by increasing intracellular calcium concentrations [25]. Treatment of mouse and human cell models with ionomycin, which induces calcium influx, stimulates RAGE shedding and subsequent sRAGEc production [25, 89-91]. For example, knock out of ADAM10 in HEK cells treated with an adenylate cyclase agonist reduced sRAGEc production by 5-fold in HEK cells [91]. In addition, inhibition of PKC and CAMKII, two calcium sensitive kinases, reduced RAGE shedding in HEK cells treated with the same adenylate cyclase agonist [91]. Additionally, Galichet *et al*. demonstrated that inhibition of Ca^{2+}-ATPase on the sarcolplasmic

reticulum increased intracellular Ca^{2+} concentration and stimulated sRAGEc production[89]. Skeletal muscle contractions are known to open ryanodine receptors (RyR) on the sarcoplasmic reticulum (SR) increasing cytosolic calcium concentration thus implicating exercise as a regulator of ADAM10 activity and proteolytic cleavage of RAGE.

In addition to modulating ADAM10 activity, increasing the expression of ADAM10 may also be a mechanism by which exercise induces sRAGEc production. The transcription factor Retinoid X Receptor (RXR), has been shown to dimerize and bind to the promoter of ADAM10 and induce its transcription [92]. RXR binds to DNA by forming homo- or heterodimers with other transcription factors such as RARβ. Importantly, RARβ is deacetylated and activated by the silent mating type information regulator 2 homolog 1 (SIRT1) allowing RARβ to dimerize with RXR and induce ADAM10 transcription [24]. SIRT1 is an NAD^+ dependent deacetylase, becoming active when the cell is in a low energy state and abundant with AMP, both of which occur during exercise. Therefore, exercise may also be modulating ADAM10 expression through activation of SIRT1 subsequent activation of transcription factors that enhance ADAM10 transcription.

2.8 Splicing Mechanisms Responsible for esRAGE Production

In addition to proteolytic mechanisms of sRAGE production, alternative splicing of the RAGE gene has also been proven to contribute to the sRAGE pool. Processing of pre-mRNA into mature mRNA involves removal of its introns and alternative splice variants of the pre-mRNA are produced by removing or including different exons in the final mRNA product ultimately resulting in a product that differs from the nascent

protein. Approximately 95% of multi-exonic genes in the human genome undergo alternative splicing allowing for single genes to code for multiple proteins [93]. AGER (the gene encoding RAGE) undergoes alternative splicing and produces many variants of the RAGE protein. RAGE_V1 also referred to as endogenous secretory RAGE (esRAGE) is the second most abundant splice product of AGER after the full length membrane-bound version [94].

The AGER gene contains 11 exons, esRAGE is produced by the utilization of an alternative splice site on intron 9 resulting in the inclusion of the 5' region of intron 9 and skipping of exon 10. Exon 10 codes for the transmembrane domain of RAGE, therefore this alternative splicing results in a 50 kDa soluble RAGE protein lacking the transmembrane domain but retaining the extracellular domain as well as the ability to bind to RAGE ligands [95]. Although the exact mechanisms regulating alternative splicing of RAGE into esRAGE are unknown this process may be regulated by two splicing proteins, heterogeneous nuclear ribonuclear protein A-1 hnRNPA-1 and Transformer 2β-1 (Tra2β-1).

The hnRNP family is one of the largest splicing protein families consisting of at least 20 different members named A through U with hnRNPA1 being one of the most abundant nuclear proteins with nuclear concentrations comparable to that of histones [96]. hnRNPs possess RNA Recognition Motifs (RRMs) that allow them to bind to RNA as well RGG boxes (arg-gly-gly repeats) that allow hnRNPs to interact with proteins in order to alter spliceosome formation [97]. Specifically, hnRNPs inhibit splicing events by binding to splicing silencers forming a multimeric complex on RNA to block RNA binding

sites for other splicing factors. hnRNPs are the direct antagonists of the serine-arginine splicing factors (SRSF).

Tra2β-1 is a member of the SRSF family, which are so named for their recognition of serine-arginine (SR) rich regions on RNA and are critical for enhancing gene splicing [98]. SR proteins, like hnRNPs, also possess RRMs that allow them to bind to RNA. Once bound to RNA, SR proteins bind to exon splicing enhancers (ESEs) via their RS domain. The ability of Tra2β-1 and other SR proteins to enhance splicing events is inversely proportional to the concentration of hnRNPs suggesting that the ratio of these two splicing factors determines the occurrence of a splicing event [97].

Another hnRNP isoform, hnRNP H, has been directly shown to bind to RAGE RNA [99] and has also been implicated in AMPK mediated glucose uptake in muscle cells [100]. Recent work in vitro and in post mortem brain specimens of individuals with Alzheimer's disease has implicated an imbalance in Tra2β-1 and hnRNPA1 in the regulation of alternate splicing of the RAGE gene [26]. Data by Liu *et al*. suggest that an increase in hnRNPA1:Tra2β-1 ratio initiated by altered glucose metabolism, results in imbalanced splicing of the RAGE gene in favor of the production of the membrane bound isoform [26]. Exercise may also be able to increase esRAGE production due to evidence that suggests the drastic alteration of the spliceosome with exercise via activation of AMPK [101]. Exercise may therefore be a viable method for inducing sRAGEc and esRAGE production (Figure 2). These mechanisms may play significant roles in the insulin sensitizing and anti-inflammatory effects of acute and chronic exercise by blunting RAGE stimulation and expression (Figure 2).

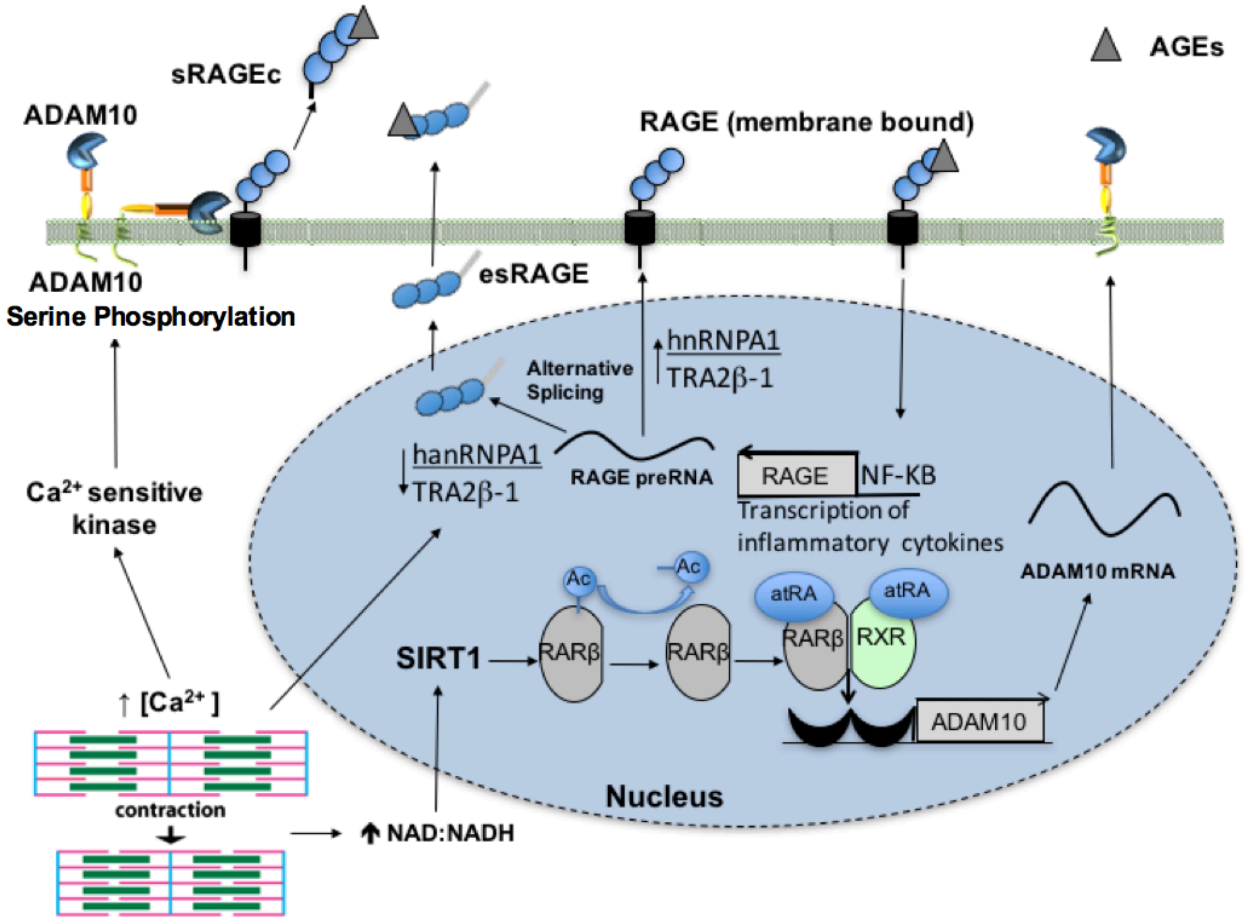

Figure 2. Schematic depicting the activation of RAGE, and the role exercise may produce soluble isoforms of RAGE. Binding of AGEs to membrane bound RAGE activates NF-kB which regulates transcription of RAGE and other inflammatory cytokines. Muscle contraction is proposed to increase sRAGEc production via modulation of ADAM10 activity and ADAM10 transcription. Muscle contraction increases intracellular calcium, which may activate calcium sensitive kinases resulting in phosphorylation and increased activity of ADAM10. Exercise also increases the NAD:NADH ratio, activating SIRT1 allowing for transcription factors to bind to the promoter site and induced the transcription of ADAM10. Finally, exercise may also alter the ratio of splicing factors that regulate splicing of RAGE pre RNA into esRAGE.

2.9 Exercise Intervention and sRAGE

Lifestyle modifications such as exercise are recommended by the American Diabetes Association and American College of Sports Medicine to be included in the treatment of diabetes [15]. Despite these recommendations and strong evidence to support exercise' efficacy in the treatment of diabetes, there are only four studies to date that have been published examining aerobic exercise's effects on circulating sRAGE [19-22]. While two of these studies did show an improvement in sRAGE or esRAGE with exercise training [21, 22], one showed a decrease in sRAGE with training [20] and another showed no change following an acute bout of exercise [19]. Unfortunately, these studies are heterogeneous in their designs and populations of interest making comparisons and definitive conclusions difficult.

Two of these studies consisted of a non-supervised intervention whereby individuals in the treatment group were prescribed a certain amount of physical activity or exercise and the control was instructed to retain their current lifestyle habits [20, 21]. Additionally, only one study used indirect calorimetry to monitor the intensity of the exercise performed [22]. In the aforementioned study, subjects began their aerobic exercise training performing 45 minute bouts of cycling exercise at 55% of their VO_{2Max}, and gradually worked their way up to performing exercise 55 minute bouts at 75% of VO_{2Max} [22]. Choi *et al*. was the only group to examine exercise's ability to modulate sRAGE levels in T2DM while the other studies populations of interest were generally older, overweight, and sedentary individuals [21]. Moreover, none of these studies differentiated between and measured both sRAGEc and esRAGE nor did they explore

the mechanisms by which exercising may be modulating their concentrations. Finally, the normal physiologic effect of exercise intensity on sRAGE has yet to be established thus making comparisons to a diseased model such as T2DM difficult.

The gaps that exist in literature exploring exercise's effects on sRAGE level are as follows. 1) Neither acute nor training effects of aerobic exercise are definitively understood. 2) The effect of exercise intensity on sRAGE levels is not known and is important for proper exercise prescription. 3) The sRAGE responses to exercise are not known in healthy individuals. Knowing the normal response to exercise will be important for juxtaposition of responses in diseased individuals such as diabetics. The mechanisms by which sRAGE changes occur with exercise are unexplored and will be important to elucidate in an effort to develop pharmacologic interventions that target these mechanisms to exploit the positive effects of sRAGE production and ameliorate the detrimental effects of RAGE over-production. Finally, the response of the heterogeneous isoforms of sRAGE to exercise has yet to be investigated, these differences are important because the divergent pathways of sRAGE generation have the potential to have different responses to exercise or may even have an additive effect if both mechanisms are maximally stimulated.

With these literature gaps in mind our study aims to examine the effect of acute aerobic exercise intensity on plasma sRAGEc and esRAGE levels in lean healthy individuals. This research's second aim is to elucidate potential mechanisms by which exercise promotes the production of sRAGEc and esRAGE by the skeletal muscle. We believe that due to skeletal muscle's increased metabolic activity during exercise and skeletal muscle making up such a large proportion of body mass, sRAGEc and esRAGE

may be produced by the skeletal muscle during exercise and released into the circulation. Because sRAGEc and esRAGE are produced through different means, an effort will be made to elucidate exercise's effect on both pathways in skeletal muscle via analysis of samples taken before, immediately after and 3 hours after exercise via muscle biopsy. In order to study esRAGE regulation following exercise, protein and mRNA transcripts of Tra2β-1 and hnRNPA1 will be measure in skeletal muscle in all three biopsy time points. Analysis of SIRT1 concentration, and phosphorylation of ADAM10 will be measured in muscle as well in an effort to better understand exercise' role on ADAM10 regulation. Elucidation of these mechanisms in lean healthy individuals will provide insight into the normal physiological response to exercise in the context of sRAGE and esRAGE and will provide a precedent against which responses in diabetic individuals can be juxtaposed in future research endeavors.

We hypothesize that acute aerobic exercise will cause a significant increase the concentrations of sRAGEc and esRAGE in lean healthy individuals. Further, we expect that there will be a dose response of exercise intensity on plasma sRAGE levels whereby exercise intensities 40%, 65%, and 80% VO_{2Max} will elicit progressively greater increases in sRAGE. Finally, we hypothesize that metabolic changes in the skeletal muscle will play a mechanistic role in the production of esRAGE and sRAGEc following exercise via regulation of splicing factors and increased transcription and activation of ADAM10 respectively.

3. METHODS

3.1 Participants

Fifteen healthy adults, eight males and seven females, 25 ± 4 (mean ± SD) years old volunteered to participate in the study. Prior to their first visit, 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). Subjects were excluded for history if they were current smokers or if they quit smoking with in the past year, 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. However menstural status was not recorded for all female subjects. Criteria for inclusion were the participants had to be 18-35 years of age, and have a BMI 18-26 kg/m^2. Baseline characteristics for all participants are presented in Table 2.

TABLE 2. Subject Baseline Characteristics

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

Table 2. Subject Baseline Characteristics. Data are depicted as mean ± SD.

3.2 Study Design

The study consisted of four non-randomized visits, the procedures of which are depicted in Figure 3. During the first visit all procedures and potential risks involved with the study were explained to the partcipants. 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 xray absorptiometry (DXA), and maximal aerobic capacity (VO_{2Max}) were assessed. Visits 2, 3, and 4

consisted of treadmill exercise at 40, 65, and 80 percent of VO_{2Max} respectively. The duration of exercise during visit 2 was 60 minutes and the duration of the exercise during visit 3 was 30 minutes. The exercise during visit 4 was performed until subjects matched the calories they expended during visit 2. 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 for each subsequent visit. Subjects were also instructed not to perform vigorous exercise or consume alcohol 48 hours before each visit, to abstain from caffeine consumption 24 hours before each visit and to arrive at each visit euhydrated and having fasted for at least 12 hours.

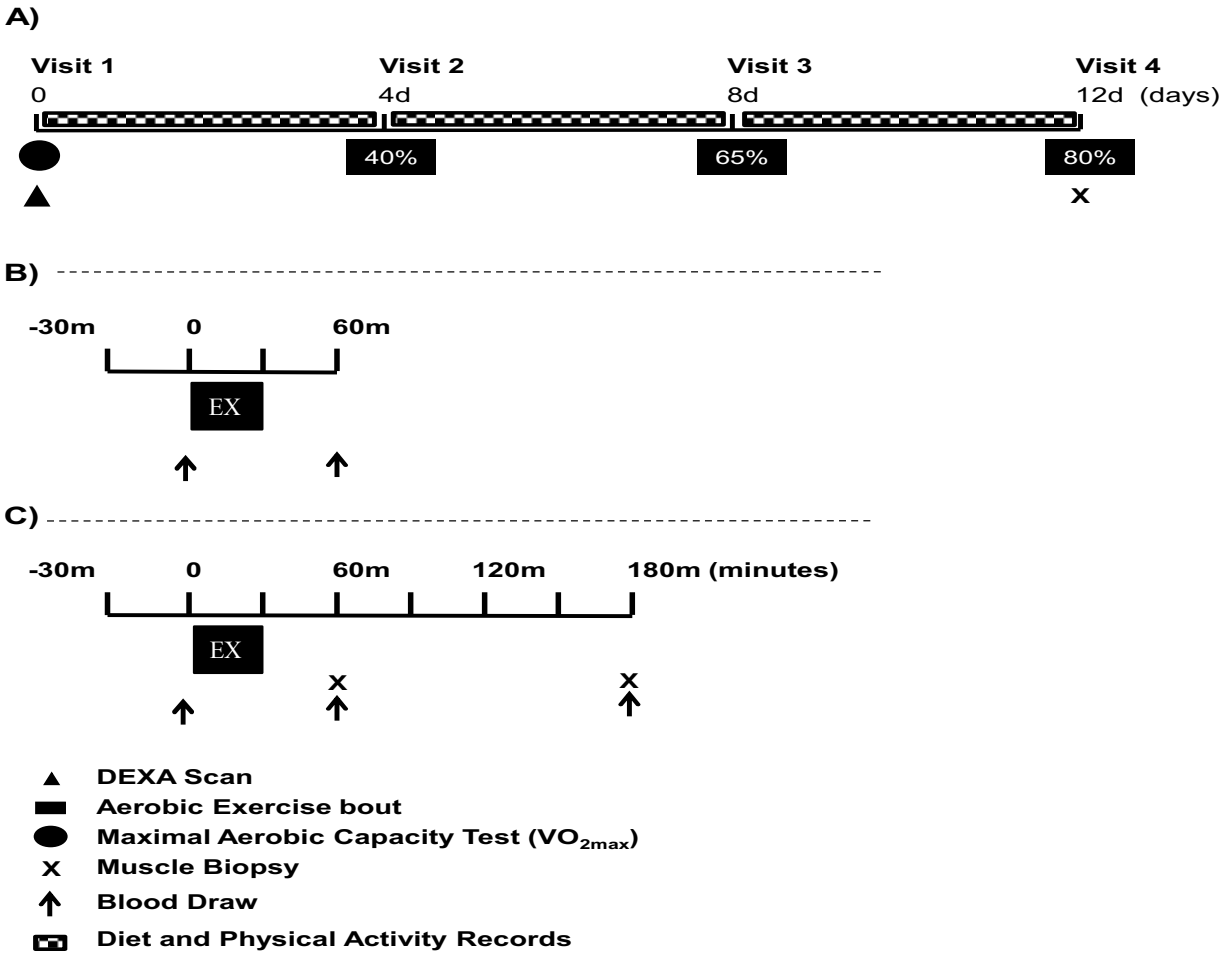

Figure 3. Schematic depicting study timeline. **A)** Subjects came into the lab on four separate occasions each separated by at least four days with diet and physical activity logs being completed three days prior to each visit. The first visit consisted of a DEXA scan, and a VO_{2Max} test. Visits 2-4 consisted of treadmill exercise at 40% of VO_{2Max} for 60 minutes, 65% of VO_{2Max} for 30 minutes, and 80% of VO_{2Max} until calories were matched to visit 2. **B)** Blood sampling for the first three visits was conducted immediately before and 30 minutes after exercise. **C)** Blood and biopsy samples for the final visit were taken immediately before, 30 minutes after, and three hours after exercise.

3.3 VO_{2Max} and Treadmill Testing

VO_{2Max} was determined using a treadmill ramp protocol where by subjects ran at a consistent speed and the grade of the treadmill was increased by two percent every two minutes. The treadmill speed was determined by asking subjects what speed would ellicit a vigorous 10-12 minute run. Indirect calorimetry was used to collect expired air for the duration of the test using a PARVO Medics metabolic cart (Salt Lake City, Utah). Subjects were also fitted with a Polar heart rate monitor throughout the test and RPE was collected from the Borg scale of 6-20 at the end of each two minute stage. VO_{2Max} was confirmed if subjects accomplished 3 of the 4 following criteria: an increase in workload and no change in VO_2 > 200mL of O_2, an RPE ≥ 18, RER > 1.1 and a heart rate greater than 85% age predicted heart rate. Data from the VO_{2Max} testing

Once the subject's VO_{2Max} was determined, 40, 65, and 80% of their VO_{2Max} were calculated and the approximate speed and grade to ellicit these intensities were determined using the appropriate ACSM metabolic equations. Subjects came to the lab on three additional days which were seperated by at least 4 days to allow for the completion of the 3-day diet and physical activity logs, and to prevent a training effect. Indirect calorimetry was performed for the duration of exercise during each of the visits to confirm that subjects were exercising at the appropriate intensity. If the speed and grade calculated from the metabolic equations did not yield the target VO_2, adjustments to speed and grade were made during the exercise to ellicit the correct work rate.

3.4 Phlebotemy

Blood samples were collected before and after the exercise bout of each visit as well as 3 hours after the final visit's exercise bout. Prior to all blood draws, subjects sat quietly for 30 minutes to allow for plasma volume to equilibrate in an attempt to prevent fluid shifts as a result of activity and postural changes to influence our results. After 30 minutes of quiet sitting 5 mL of blood was collected from the antecubital vein, into an EDTA collection tube. The blood samples were immediately centrifuged at 4°C for 10 minutes at 3000 rpm to separate the plasma from whole blood. Plasma was then aliquoted into a labeled cryovial and stored at -80^{o} C until further analysis.

3.5 sRAGE Quantification

Plasma samples from a subgroup of the first eight individuals completed were analyzed for total soluble RAGE (sRAGE) (R&D Systems; Minneapolis, MN, USA) and endogenous secretory RAGE (esRAGE) (BBridge International; Santa Clara, CA, USA) via commercially available ELISA kits per manufacterer's protocol. Data collection on plasma sRAGE was concluded after eight subjects for finacial reasons. A spectrophotometer emitting at 450nm was used to quantify the optical density of a standard curve to which the optical densities of our samples were fitted to determin sRAGE and esRAGE concentrations in our specimens. Samples and standard curve were run in triplicate. Values from the esRAGE ELISA yielded an intra-assay and inter-assay variability of 2.9 – 15.4% and 6.2% respectively and the sRAGE ELISA had an intra-assay variability of 8.8 % and an inter-assay variability of 3.1 – 21.1%. Plasma concentrations of soluble cleaved RAGE isoforms were calculated by subtraction of esRAGE from sRAGE.

3.6 Muscle Biopsy

Muscle biopsies were taken from the medius vastus lateralus before, after and 3 hours after exercise on the final visit. Briefly, a small area of skin was anesthesized via injection of a local anesthetic (1% lidocaine HCL) at the biopsy site. A small incision (~0.5 cm) was then made at the biopsy site. A Bergstrom needle was inserted into the incision and with suction applied ~100 mg of muscle tissue was extracted. A portion of each sample was immediately flash frozen in liquid nitrogen and then stored at -80°C for later western blotting and immunoprecipitation analyses to be performed. The other portion of muscle sample was immediately placed in RNAlater to stabilize the RNA and was then stored at -20°C until extraction of the RNA and gene analysis was performed.

3.7 Western Blotting and Immunoprecipitation

Approximately 10 mg of muscle tissue was homogenized in 20 volumes of 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 β-glycerophosphate, 1mM Na_3PO_4, 1 μg/mL leupeptin; Cell Signaling Technology, Danvers, MA, USA) supplemented with 5X MS-Safe protease and phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) to a final concentration of 1:500. Samples were homogenized using Lysing Matrix D and a Fast prep 24 homogenizer per manufacterer's reccomendations (MP Biomedicals, Santa Ana, California, USA). Following homogenization, the sample and beads were placed on a perforated filter contained within a microcentrifuge tube. Samples were then centrifuged to collect the cell homogenate in the microcentrifuge tube and isolate the homogenization beads which were subsequently discarded.

Protein sample concentrations were then determined using a commercially available BCA assay.

For Western blotting experiments, laemmli buffer (65.8 mM Tris-HCl, pH 6.8, 26.3% (w/v) glycerol, 2.1% SDS, 0.01% bromophenol blue; BioRad, Hercules, CA, USA) supplemented with β-mercaptoethanol (Sigma-Aldrich, St. Louis,MO, USA) was added to aliquots of muscle homogenates. Samples were then seperated by 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were then incubated overnight at 4°C with primary antibodies to probe for RAGE (1:500; Abcam, rabbit monoclonal; ab172473), hnRNPA1 (1:250; Cell Signaling Technology, rabbit monoclonal; 8443), Tra2β-1 (1:250; Santa Cruz Biotechnology, mouse monoclonal; sc-166769), ADAM10 (1:500; Santa Cruz Biotechnology, mouse monoclonal; sc-28358), and GAPDH (1:1000; Cell Signaling Technology, rabbit monoclonal; 2118). Following incubation in primary antibody, membranes were washed with 1X TBST supplemented with blocking buffer (0.3% BSA) and incubated with appropriate secondary antibodies conjugated with flouresent dyes for use with Li-Cor's Odyssey imaging system (Li-Cor, Lincoln Nebraska, USA) for one hour at room temperature. Following incubation in secondary antibody membranes were scanned still wet and quantified on the Odyssey CLx near-infrared imaging system (Li-Cor).

3.8 Statistical Analysis

All baseline data are presented as mean ± standard deviation and all protein data are presented as the mean ± standard error of the mean. Statistical analysis was carried out using SPSS statistical software version 23. Comparisons of changes in total sRAGE, sRAGEc, and esRAGE concentrations following exercises across the three

different exercise intesities were analyzed via two-way, time by intensity, repeated measures ANOVA. Protein concentrations across the three biopsy times were also analyzed via one-way repeated measures ANOVA and correlative analysis using Pearson's r was performed to analyze the relationships between outcome measures. Significance was set at $\alpha = 0.05$. Outlliers were defined as being outside of 2 standard deviations and excluded from analysis. sRAGE concentrations and protein results were normally distributed as confirmed by Shapiro-Wilk test. Non-normally distributed data included VO_{2Max} and average VO_2 during exercise at 65% of VO_{2Max} for 30 minutes (visit 3). These data were only analyzed by correlatation (Pearson's r), which does not assume normal disitribution. In addition, VO_{2Max} was log transformed before correlative analysis.

4. RESULTS

4.1 Effect of Acute Exercise Intensity on sRAGE Production

Baseline characteristics for the subset of participants analyzed for plasma sRAGE is presented in Table 3. Metabolic parameters during each visit are presented in Table 4. With the exception of visits 40% and 80% trials, which were matched for work, the calories expended and across all visits were significantly different ($p< 0.05$). Average heart rate differed for each visit except when comparing the 40% and 65% trials and average VO_2 was different between all trials ($p< 0.05$) (average VO_2 during the max was not icluded in that anaylsis).

There were no significant changes in total plasma sRAGE, sRAGEc, nor esRAGE following any of the visits (Figure 4) ($p> 0.05$). Additionally, the change in all sRAGE isoforms was not significantly different across visits of varrying exercise intensity ($p> 0.05$). However, energy expenditure during visit 3 (30 minutes of running at 65% VO_{2Max}) significantly correlated to the change in total sRAGE during visit 3 ($p< 0.05$) (Figure 5). In addtion, there was a trend toward significant correlation between average RER during this exercise bout and change in sRAGEc ($r = 0.661$, $p = 0.074$, $n = 8$).

TABLE 3. Characteristics of Subjects Used for Analysis of sRAGE.

N	8
Age (y)	26 ± 4.2
Gender (%F)	50%
Weight (kg)	67.7 ± 13.0
BMI (kg/m^2)	22.3 ± 2.5
BF%	22.6 ± 4.1
VO_{2Max} (mL/kg/min)	50.2 ± 8.1

Table 3. Baseline Charactersitics for Subset of Subjects Used for Analysis of Plasma sRAGE. Values are mean ± SEM.

Table 4. Criteria for $VO_{2Max.}$

Subject	M/F	VO_{2Max} (mL/kg/min)	Age (y)	Age Predicted Max HR	Max HR	% of MAX HR	Max RER
1	M	58.5	29	191	Missed	Missed	1.14
2	M	56.7	20	200	192	96	1.15
3	F	40.0	27	193	204	106	1.15
4	F	41.3	33	187	198	106	1.13
5	F	50.2	24	196	196	100	1.09
6	F	41.0	27	193	Missed	Missed	1.09
7	M	56.7	25	195	179	92	1.13
8	M	56.8	22	198	182	92	1.10
9	M	41.9	23	197	Missed	Missed	1.19
10	F	39.9	22	198	175	88	1.14
11	M	51.2	23	197	207	105	1.15
12	F	42.4	25	195	186	95	1.11
13	M	54.3	33	187	187	100	1.14
14	F	38.8	23	197	194	98	1.12
15	M	46.2	26	194	190	98	1.19

Table 4. Criteria for determining max effort during VO_{2Max}. Some data were missed due to technical difficulty of heart rate monitor.

TABLE 5. Exercise Dynamics

	VO_{2Max}	40% VO_{2Max} 60 Mins	65% VO_{2Max} 30 Mins	80% VO_{2Max} Match 40% kcals
VO_2 (mL/kg/min)	47.7 ± 1.9	18.5 ± 2.9	31.4 ± 1.6	36.4 ± 1.3
Goal VO_2	N/A	18 ± 1	31 ± 2	38 ± 2
Kcals	157 ± 13	372 ± 40	307 ± 22	372 ± 29
Goal Kcals	N/A	N/A	N/A	371.9 ± 39.7
AVG RER	0.925 ± 0.01	0.825 ± 0.01	0.830 ± 0.01	0.916 ± 0.01
AVG HR	148 ± 4	114 ± 4	152 ± 2	168 ± 2

Table 5. Exercise Dynamics for all study visits. Metabolic parameters during exercise bouts of varying intensities VO_2 for VO_{2Max} is the average of the maxes achieved by all subjects. All data are Mean ± SEM.

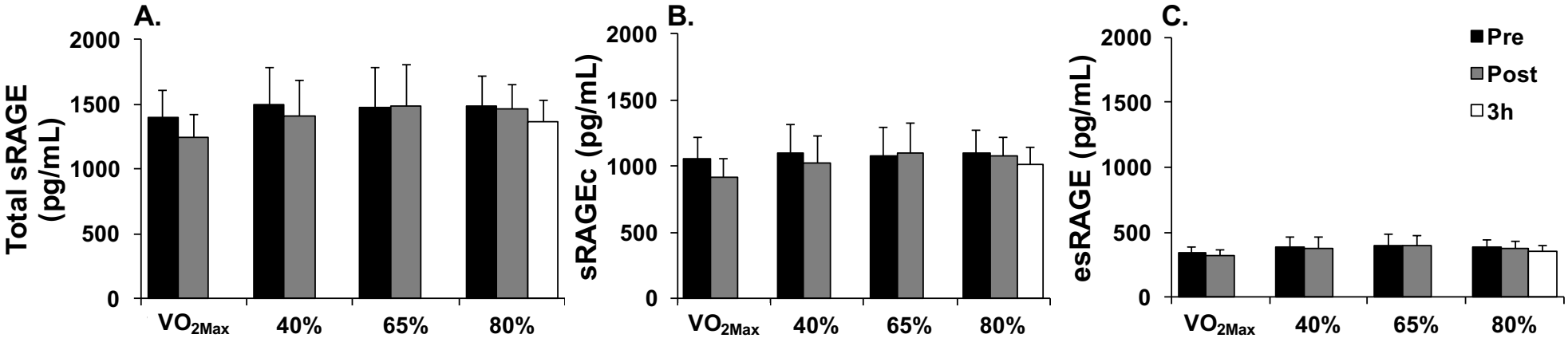

Figure 4. Baseline and post exercise sRAGE concentrations in lean healthy participants. Black, gray, and white bars represent concentrations before, 30 minutes after, and 3 hours after exercise respectively. **A)** Plasma esRAGE levels before and after exercise of varying intensity. **B)** Plasma sRAGEc levels before and after exercise of varying intensity. **C)** Total plasma sRAGE levels before and after exercise of varying intensity. Values are mean ± SEM, n = 8.

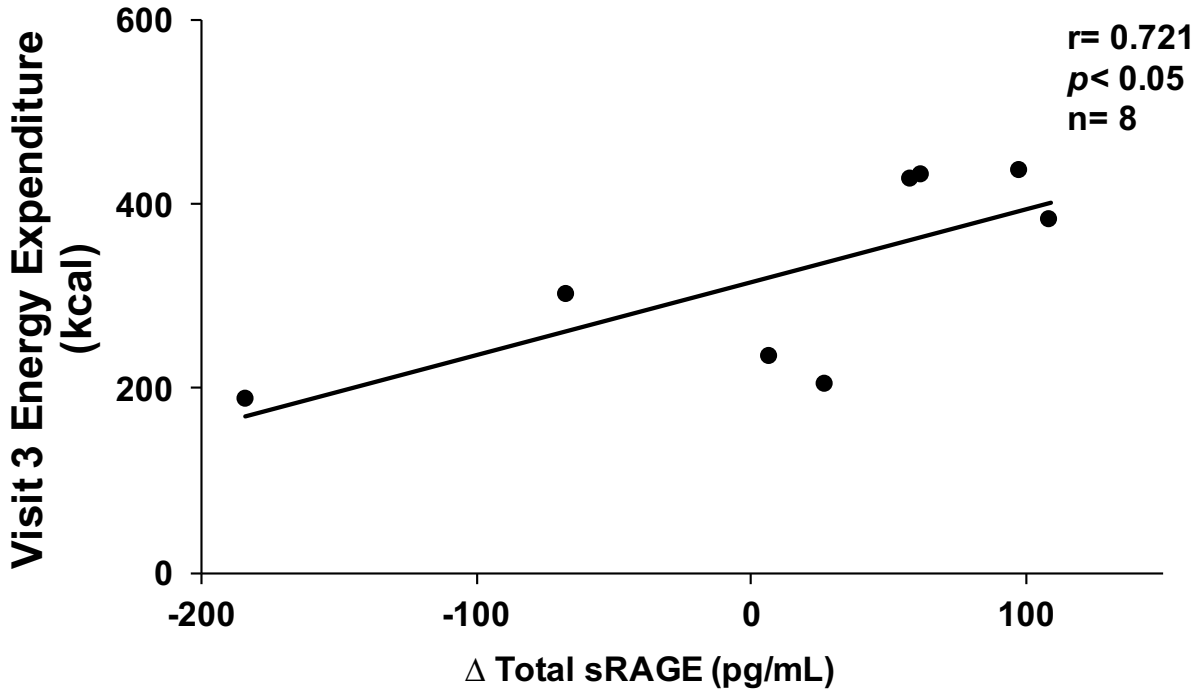

Figure 5. Energy Expenditure During Exercise Correlates With Change in Total sRAGE During exercise at 65% for 30 minutes. Energy expenditure did not significantly correlate with independent isoforms that make up total sRAGE (sRAGEc or esRAGE) and this correlation was not present for any of the other study visits. n = 8.

4.2 Effect of Acute Exercise on RAGE Expression in Skeletal Muscle

There was no change in skeletal muscle expression of mRAGE following acute exercise at 80% of VO_{2Max} (Figure 6). However, percent change in skeletal muscle mRAGE three hours following exercise at 80% of VO_{2Max}, did significantly correlate with the same percent changes in total sRAGE and sRAGEc (Figure 7).

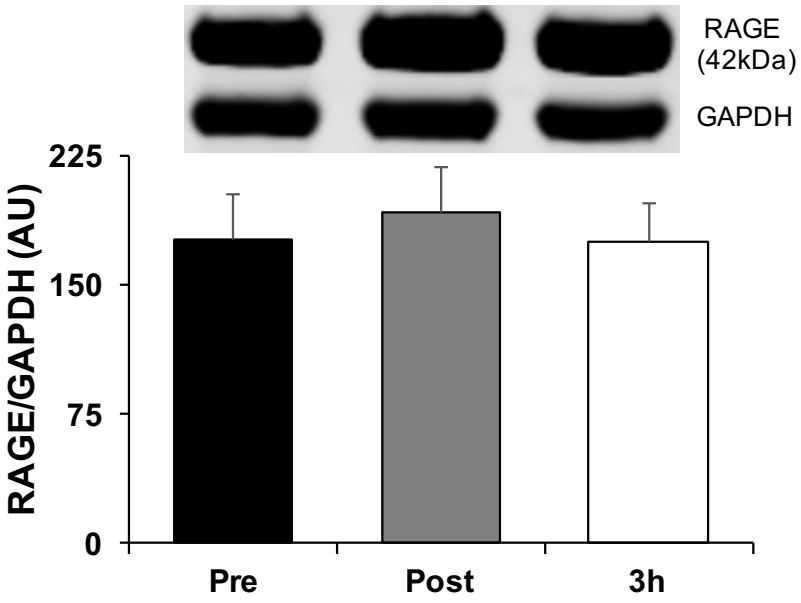

Figure 6. Effect of Exercise on Skeletal Muscle Expression of RAGE. RAGE expression did not change 30 minutes nor 3 hours following exercise at 80% VO_{2Max}. Data is presented as a percentage of GAPDH.

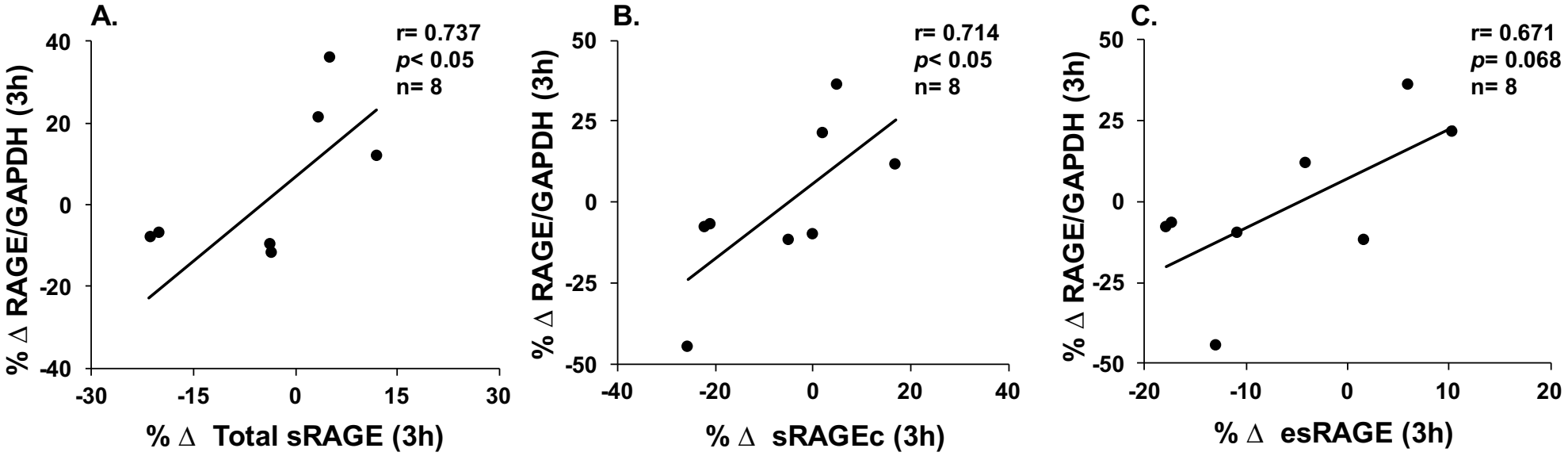

Figure 7. Relationships Between % Changes in mRAGE and sRAGE Three Hours Following Exercise. Correlations between % Change in mRAGE and: **A)** % Change in Total sRAGE, **B)** % Change in sRAGEc, and **C)** % Change in esRAGE (non-significant) three hours following exercise at 80% of VO_{2Max}. n = 8 for all correlations.

4.3 Effect of Acute Exercise on ADAM10 Expression in Skeletal Muscle

The antibody used to detect ADAM10 via western blotting was able to reveal two distinct bands at 90 and 60 kDa, which are believed to represent the inactive pro-protein and the constitutively active version lacking the prodomain respectively. Following acute aerobic exercise at 80% of VO_{2Max} there was no significant change in either isoform of the ADAM10 proteins nor their ratio following acute exercise (Figure 8).

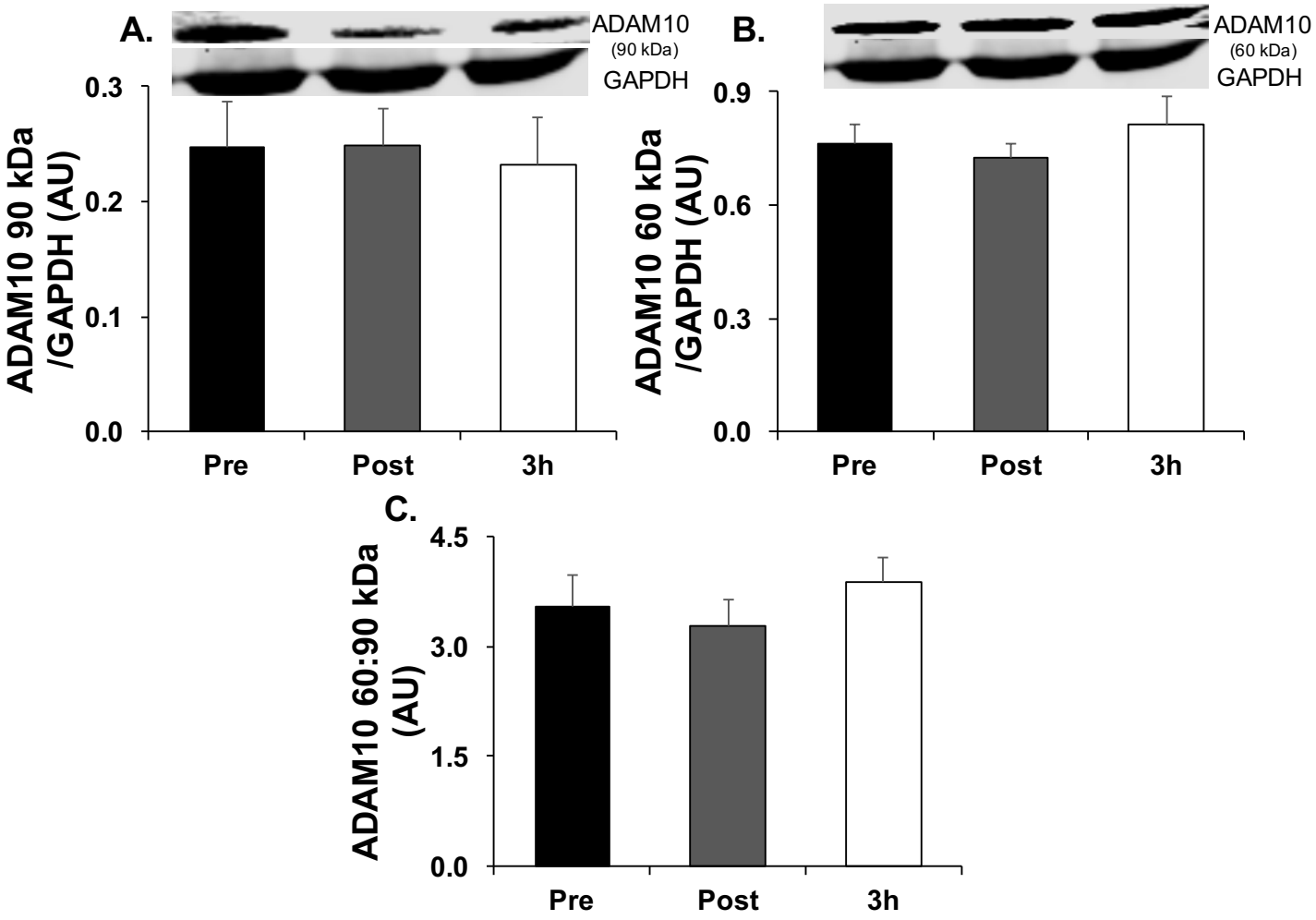

Figure 8. Effect of acute aerobic exercise intensity on muscle ADAM10 expression. **A)** 90 kDa band representing inactive form of ADAM10. **B)** 60 kDa band representing active form of ADAM10. **C)** Ratio of active to inactive ADAM10. ADAM10 protein expression is presented as percent of GAPDH. All values are presented as Mean ± SEM. n = 15.

4.4 Effect of Acute Aerobic Exercise on esRAGE Splicing Factors

In cell culture, esRAGE has been demonstrated to be dependent on the ratio of the antagonistic splicing factors hnRNPA1 and Tra2β whereby a greater hnRNPA1:Tra2β ratio was related to a greater RAGE:esRAGE ratio [26]. The same study demonstrated that peripheral blood mononuclear cells of patients with Alzheimer's disease had significantly greater RAGE expression, lower esRAGE expression, and increased hnRNPA1:Tra2β ratio compared to healthy controls. Skeletal muscle metabolism is greatly altered during exercise and is able to alter spliceosome function via increase in AMPK activity [101]. Therefore, a sub-aim of our second specific aim was to uncover the effect of acute exercise on hnRNPA1 and Tra2β expression and to determine if there is a relationship between their expression and the production of esRAGE and RAGE. Our hypothesis was that exercise would lower hnRNPA1:Tra2β ratios and that this ratio would negatively correlate with esRAGE concentrations. To test this hypothesis, skeletal muscle biopsies taken from participants' vastus lateralis muscles before, 30 minutes after, and 180 minutes after aerobic treadmill exercise at 80% of VO_{2Max} were probed via western blotting for Tra2β and hnRANPA1. Skeletal muscle samples were also analyzed for expression of membrane bound RAGE via western blotting and blood samples taken at corresponding time points were analyzed for esRAGE via ELISA. Following acute aerobic exercise, there were no significant changes in the expression of Tra2β, hnRNPA1 or the ratio of hnRNPA1:Tra2β (Figure 9).

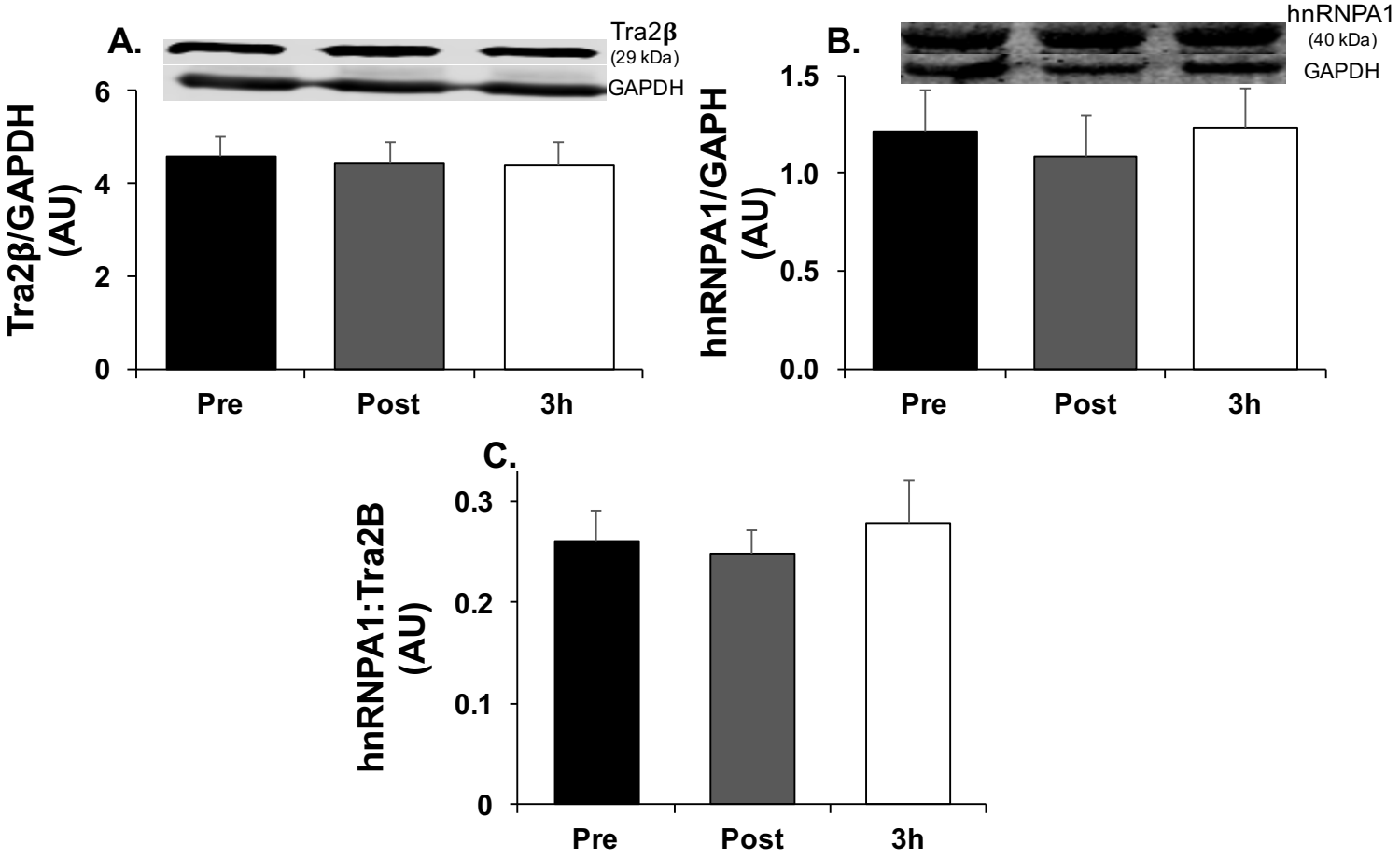

Figure 9. The Effect of Acute Aerobic Exercise on hnRNPA1 and Tra2β Expression. **A)** Tra2β skeletal muscle protein expression. **B)** hnRNPA1 skeletal muscle protein expression. **C)** Ratio of hnRNPA1:Tra2β skeletal muscle protein expression. Tra2β and hnRNPA1 protein expression are presented as percent of GAPDH. All values are presented as Mean ± SEM. n = 15.

In addition, changes in esRAGE following exercise at 80% of VO_{2Max} did not significantly correlate to changes in protein expression of the splicing factors measured or to changes in their ratio. However, there was a significant negative correlation between baseline Tra2β and change in esRAGE (Figure 10) and a trend toward a significant positive correlation between baseline levels of esRAGE and Tra2β protein expression (r = 0.692, *p* = 0.057, n= 8).

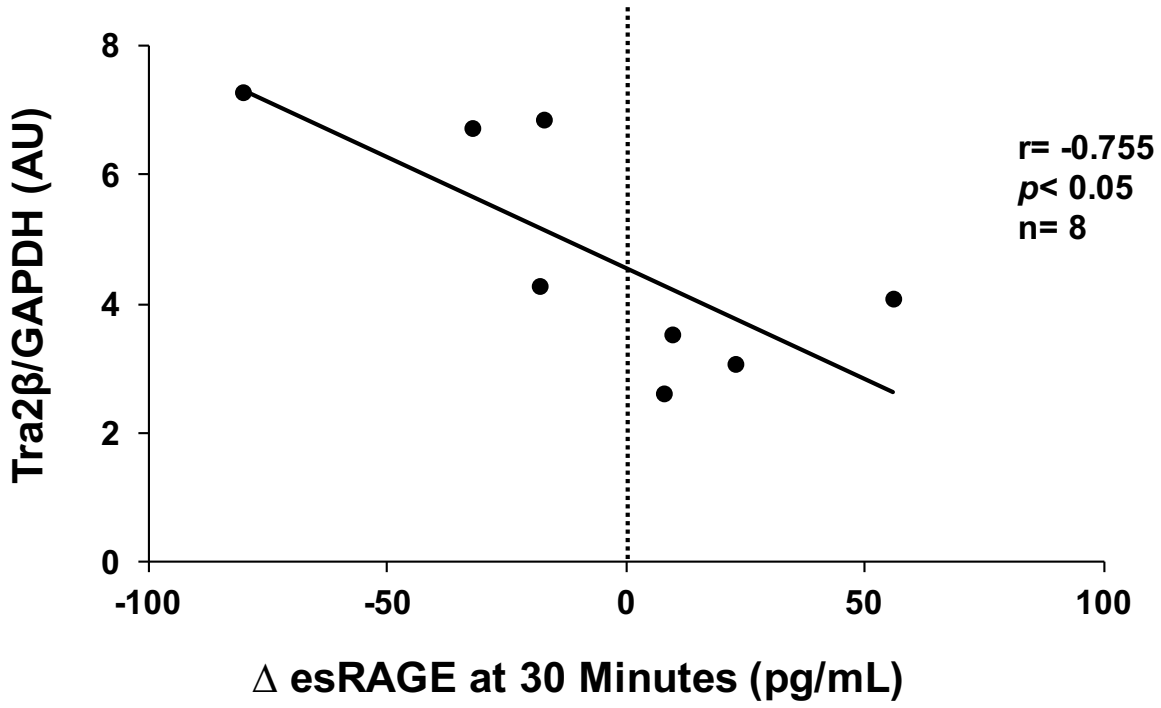

Figure 10. Correlation Analysis of Baseline Tra2β Expression to Change esRAGE Concentration. Delta esRAGE is the difference between esRAGE concentrations before and 30 minutes after exercise at 80% of VO_{2Max}. n = 8.

5. DISCUSSION

The present study set out to determine the effect of acute aerobic exercise intensity on sRAGEc and esRAGE production in lean healthy individuals. In addition, this study also examined if acute aerobic exercise modulated mechanisms that play a role in sRAGE production in skeletal muscle. Our hypotheses were that acute exercise would increase sRAGEc and esRAGE concentration in the plasma in an intensity dependent manner and that acute exercise would alter mechanisms of sRAGE production in skeletal muscle, potentially contributing to the circulating sRAGE pool.

5.1 Exercise and sRAGE Production in Lean Healthy Individuals

In order to test the first hypothesis, participants performed treadmill exercise at 40, 65, and 80% of their VO_{2Max} during three separate study visits. Blood samples were collected before and after each exercise bout and the plasma was analyzed for sRAGE isoforms. In order to attribute any potential differences in sRAGE production across the separate study visits to intensity alone, the exercise bouts at 40 and 80% of VO_{2Max} were matched for energy expenditure. However, contrary to our hypothesis, acute exercise did not elicit a significant change in plasma sRAGE of either isoform.

This lack of an increase in sRAGE could be in part due to the high baseline sRAGE concentration, and low expression of membrane bound RAGE in our lean, healthy cohort. Previous data from our lab has demonstrated a disparity in the expression of RAGE in skeletal muscle in healthy compared to diseased individuals whereby the latter group possesses significantly more mRAGE (not published) and baseline total sRAGE concentrations for our subjects are almost double what was

reported for the baseline concentrations in T2DM individuals studied by Choi *et al.* (1400 pg/mL vs. 726 pg/mL) [21]. sRAGEc is the predominant contributor to the total sRAGE pool having a concentration of approximately three times that of esRAGE (data not shown). Therefore, as mRAGE is the substrate for sRAGEc production, basal mRAGE is likely a determinant of the ability to generate sRAGE. However, attempts to correlate baseline skeletal muscle RAGE concentrations to changes in sRAGE isoforms following exercise. We also went on to explore relationships between changes in mRAGE with changes in sRAGE isoforms. This analysis revealed a significant positive correlation between the percent change of both parameters three hours following exercise at 80% of VO_{2Max}. This suggest that mRAGE in the skeletal muscle is contributing to the circulating sRAGE pool. In addition, it is evident by these correlations that individuals with a negative change in mRAGE also had a negative change in sRAGE suggesting not only that these two are proportional to each other, but also our lean healthy subjects may be at the optimal proportion of these two proteins. In the future it will be important to study a cohort of individuals who have a disproportionate amount of mRAGE:sRAGE such as in T2DM and determine if acute aerobic exercise is able to rescue that ratio to levels more similar to our healthy cohort.

Our hypothesis that sRAGE production would be dependent on exercise intensity was in part based on evidence that ADAM10 is the major contributor to membrane shedding of RAGE [23] and that calcium is a key regulator of RAGE cleavage and ADAM10 activity [25, 89, 91]. In addition, spikes in calcium concentration in skeletal muscle cause activation of CAMKII which has previously been shown to play a role in sRAGE production *in vitro* [91]. Calcium is released by the sarcoplasmic reticulum upon

neural stimulation and subsequent depolarization of the skeletal muscle and is directly related to muscle force production. ADAM10 transcription is also under the control of the NAD^+ deacetylase SIRT1 [24]. Acute exercise is known to increase SIRT1 activity by increasing cellular concentration of NAD^+ [102, 103], which is produced in an intensity dependent manner as a byproduct of cellular metabolism. Thus, increased exercise intensity may result in greater ADAM10 activity and expression resulting in increased sRAGE production.

Although not significant, each of the exercise bouts resulted in a negative delta value for sRAGE with the exception of the exercise bout performed at 65% of VO_{2Max} for 30 minutes, which may point toward 65% of VO_{2Max} as the optimal exercise intensity for the modulation of RAGE metabolism. While there is considerable evidence that would logically link greater intracellular calcium concentrations to greater sRAGE production, calcium also interacts with many molecules and signals to generate innumerable downstream effects. Therefore, where too low of an exercise intensity may not be robust enough of a stimulus to cause optimal calcium mediated sRAGE production, too great of an exercise intensity may produce cross-talk to other calcium dependent signaling mechanisms preventing an appreciable change in sRAGE production.

Interestingly, energy expenditure during the exercise bout at 65% of VO_{2Max} significantly correlated with the change in total sRAGE and there was also a trend toward significance when correlating the average RER of this exercise bout to the change in sRAGEc ($r = 0.661$, $p = 0.074$, $n = 8$). Collectively these data suggest that energy expenditure as well as the mechanisms responsible for modifying fuel utilization at this intensity for at least 30 minutes may be related to RAGE metabolism.

5.2 Exercise and Mechanisms of sRAGEc Production

To better understand why we did not observe changes in sRAGE production following exercise, we probed skeletal muscle samples taken from our participants before, 30-minutes post, and 180-minutes following exercise at 80% of VO_{2Max}. In agreement with our finding no significant change in plasma sRAGE concentrations following exercise, there was also no significant change in mRAGE protein in skeletal muscle of our participants following exercise.

ADAM10 is the primary protease responsible for cleavage of mRAGE and production of sRAGEc [23]. The antibody we used to measure ADAM10 expression, produced a band at 60 and 90 kDa representing the constitutively active isoform of ADAM10 and the inactive ADAM10 proprotein, respectively. There was also no significant change in either isoform of ADAM10 following exercise, nor was there a change in the ratio of the constitutively active to inactive ADAM10 protein.

Although no changes were observed in ADAM10 protein expression following exercise, ADAM10 activity may still be able to be modified by acute exercise. As previously mentioned, inhibition of CAMKII was previously shown to reduce ADAM10 activity suggesting that ADAM10 activity may be altered by CAMKII dependent phosphorylation [23]. It is possible that exercise was able to alter ADAM10 activity and that the lack of sRAGE change was independent of ADAM10. In addition, ADAM10 has many substrates beyond RAGE, the proteolysis of which may result in different cellular consequences. Therefore, there may be an optimal protein expression and activity of ADAM10 which our healthy cohort has already achieved.

To this point, lean individuals may experience an effect of exercise on ADAM10 and RAGE metabolism that differs from metabolically impaired individuals. For example, Alzheimer's disease patients have been shown to have lower levels of ADAM10 expression compared to healthy controls [104]. Therefore, future studies examining the effect of exercise on ADAM10 activity and protein expression in a diseased cohort would give a more complete insight into how mechanisms responsible for sRAGEc production are altered in response to exercise.

5.3 Exercise and Alternative Splicing of RAGE

The alternative splice variant of mRAGE (esRAGE) also contributes to the total sRAGE pool and is governed by independent and parallel mechanisms. hnRNPA1 and Tra2β influence the splicing of RAGE pre-mRNA to produce mRAGE or esRAGE respectively [26]. Exercise is known to generically alter gene splicing, so to determine if acute exercise could alter the regulation of hnRNPA1 and Tra2β splicing proteins skeletal muscle homogenates were analyzed for changes in the protein expressions of these two splicing factors. Acute aerobic exercise did not alter protein expression of hnRNPA1, Tra2β or the hnRNPA1:Tra2β ratio. However, baseline Tra2β trended toward a significant and positive correlation to baseline esRAGE concentrations ($r= 0.692$, $p= 0.057$, $n= 8$). This is in line with the results produced *in vitro* by Liu *et al*. demonstrating Tra2β as a positive factor for esRAGE production. Interestingly, baseline Tra2β expression significantly and negatively correlated with change in esRAGE following exercise during visit 4. This analysis seems counterintuitive at first, however, it is in line with observations made by Liu *et al*. *in vivo* who observed that although healthy individuals present with lower levels of both Tra2β and hnRNPA1, they maintain a lower

hnRNPA1:Tra2β ratio compared to individuals with Alzheimer's disease. However, hnRNPA1:Tra2β ratio did not significantly change with exercise in our cohort. Therefore there may be other regulators at play that we are not yet aware of and this could potentially be unique to our subjects with such high levels of fitness (VO_{2Max} 47.7 ± 7.4 mL/kg/min, mean ± SD).

5.4 Limitations and Future Directions

This study is the first to examine the effect of acute aerobic exercise and exercise intensity on plasma sRAGEc and esRAGE concentrations in lean healthy individuals. Furthermore, this study's level of control is superior to previous studies that examined the effect of exercise on sRAGE. The current study was the only of its kind to utilize a combination dietary logs and physical activity logs prior to each visit and indirect calorimetry during to each visit. The current study is also only the third to use supervised exercise for each visit. The two other studies that explored the effects of exercise on sRAGE used physical activity type interventions or exercise prescription.

Although this study was extremely well controlled it is not without its limitations. One of the limitations of this study was the our relatively small sample size with only an n of 8 for sRAGE analysis, and 15 for analysis of protein expression of sRAGE-producing mechanisms. We also did not record menstruation status for our female participants which may be a confounding variable as previous research has demonstrated that estrogen has an effect on sRAGE concentrations in post-menopausal women undergoing hormone replacement therapy [105]. Another limitation of this study was the high level of fitness of our healthy cohort. While is important to understand the normal physiology of RAGE metabolism in healthy individuals, this

cohort bordered on being considered athletes rather than normal healthy individuals. Another limitation of this study was that calories were not matched across all exercise intensities and study visits were not randomized. Matching calories allows potential differences of sRAGE production across study visits to be attributed to exercise intensity rather that total work. Also, in an attempt to limit participant burden, muscle biopsies were only taken during the final study visit, therefore the effect of exercise intensity on RAGE metabolism mechanisms in skeletal muscle were not able to be deduced.

Although no changes in protein expression were observed in any of the mechanistic proteins of interest, there may be underlying regulation of protein activity via posttranslational modification as well as transcriptional regulation that was not explored in this study. In particular, ADAM10's activity is able to be altered regardless in changes of protein expression and may be due to phosphorylation by CAMKII, other calcium sensitive kinases, or other unknown mechanisms. Finally, esRAGE mRNA levels are of interest to determine if exercise is able to elicit changes in splicing patterns at the transcriptional level. This will help better inform our protein results and give insight into esRAGE regulatory mechanisms.

5.5 Conclusion

In conclusion, acute aerobic exercise had no effect on sRAGE production in lean healthy individuals and this observation was not intensity dependent. There was however a significant correlation between energy expenditure and change in sRAGE concentration following exercise performed at 65% of VO_{2Max} for 30 minutes. We also observed a correlation that approached significance between RER and change in sRAGEc following the same exercise bout. Suggesting that this time and intensity could

be the ideal exercise stimulus to elicit changes in sRAGE in lean healthy individuals and may be worth investigating in future studies with diabetic individuals as well. Also, acute aerobic exercise did not have an effect on protein expression of ADAM10, hnRNPA1, or Tra2β in skeletal muscle. However, a significant correlation was found between baseline protein expression of Tra2β and baseline esRAGE following exercise, indicating that it may be playing some regulatory role at least at rest. Future studies should be carried out in diseased populations to determine if exercise is a viable option for altering RAGE expression in skeletal muscle, subsequently altering plasma sRAGE concentrations. Finally, future study in a diseased cohort may uncover mechanisms of RAGE metabolism that may be exploited as an alternative therapy to be used in combination with exercise to improve efficacy of prevention and treatment of diabetes and its complications.

APPENDICES

APPENDIX A
TELEPHONE ELIGIBILITY CHECKLIST

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

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/m^2) → must be greater than 18 kg/m^2 and less than 26 kg/m^2.*

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

APPENDIX B (continued)

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.

APPENDIX B (continued)

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.

APPENDIX B (continued)

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-

APPENDIX B (continued)

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?

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 B (continued)

Signature of Subject

I have read (or someone has read to me) the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research. I will be given a copy of this signed and dated form.

_____ _____

Signature Date

_____

Printed Name

Statement of Person Conducting Informed Consent Discussion

I have discussed the information contained in this document with the participant and it is my opinion that the participant understands the risks, benefits, alternatives and procedures involved with this research study.

_____ _____

Signature of Person Obtaining Consent Date (must be same as subject's)

_____

Printed Name of Person Obtaining Consent

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"

Beverages
Record in fluid ounces or measuring cup sizes.

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

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.

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

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

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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Education

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Professional Certifications

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Protecting Human Research Participants-NIH, *Current*
CPR, First Aid, AED- Red Cross, *Current*
Health Fitness Specialist- American College of Sports Medicine, *Current*

Professional Memberships

American Physiological Society

University and Departmental Committees

Exercise Science Program Advisory Committee, Salisbury University 2012-2014
Student Representative, USARA Grants Committee, Salisbury University, 2011-2014

Honors & Awards

APS Minority Travel Fellowship Opportunity Award
Spring 2016

Central Society for Clinical and Translational Research (CSCTR) Travel Award
Spring 2016

NCUR Travel Grant Award
Spring 2014

Dean's List, Salisbury University
Spring 2012, Fall 2012, Spring 2013, Spring 2014

Salisbury University Scholar Holler Award
March 2012

Scientific Publications

Collins, B., R. Sapp, J. LaManca , C. Wolff , **E.R. Miranda** , V. Gutierrez , S. Mazzetti. Influence of Xpand Nitric Oxide Reactor, L-Arginine Alpha-Ketoglutarate, and Caffeine Supplementation on Calf Muscle Re-Oxygenation During and After Acute Resistance Exercise. *Journal of Nutritional Therapeutics. 2012.*

Current Research Projects

Effects of Acute Aerobic Exercise Intensity on Plasma sRAGE in Lean Healthy Individuals. **Miranda, E.R.**, J.T. Mey, B.K. Blackburn, V. Meyers, E. Velic, J.M. Haus.

Scientific Presentations

Production of Soluble Receptor for Advanced Glycation End-Products Following Acute Aerobic Exercise is Gender Specific. **Miranda, E.R.**, J.T. Mey, B.K. Blackburn, S.S. Farabi, L. Quinn, JM Haus. *Experimental Biology, San Diego, CA. April,* 2016.

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Production of Soluble Receptor for Advanced Glycation End-Products Following Acute Aerobic Exercise is Gender Specific. **Miranda, E.R.**, J.T. Mey, B.K. Blackburn, S.S. Farabi, L. Quinn, JM Haus. *CSCTR/MWAFMR Combined Annual Meeting. Chicago, IL. April,* 2016.

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Educational/Lay Presentations

RAGE, A Novel Contributor to Diabetes and its Complications. American Diabetes Association Expo. **Miranda, E.R.,** J.T. Mey, A. Chaves, J.M. Haus. *Chicago, IL. April, 2016.*

The Salisbury University Laboratory for Human Performance: A Model for Exemplary Undergraduate Research. Innovative Showcase, Salisbury University Research Day. Meyers, V., **E.R. Miranda**, J. Watso, W.M. Castor, P. Ferrara, J. Bock, and S. Mazzetti. *Salisbury, MD. September,* 2013.

Explanation and Demonstration of Tendo and Myotest Accelerometers. **Miranda, E.R.**, P. Ferrara, A. Bushong. *Salisbury, MD. May*, 2012.

Nutrition for Performance Enhancement in Volleyball. **Miranda, E.R.**, R. Sapp, C. Rivera-Pratts, *Salisbury, MD. April,* 2012.

Nutrition for Sports Performance. **Miranda, E.R.**, R. Sapp, and S. Mazzetti. *Democratic Republic of Congo SportsUnited, Salisbury, MD. January,* 2012.

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