THE EFFECT OF EXERCISE INTENSITY ON GHRELIN RELEASE

A Dissertation
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APPROVAL OF THE DISSERTATION

This dissertation, "The Effect of Exercise Intensity on Ghrelin Release," has been approved by the Graduate Faculty of the School of Education and Human Development in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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DEDICATION

This dissertation is dedicated to my Dad, Steve; Mom, Pam; brother, Ryan; and my Nana, Pauline. Their support throughout this journey helped me achieve this degree and I am thankful for them, always.

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ABSTRACT

Background: Ghrelin is a gut hormone and exists in a des-acyl form (DAG ~78% of total ghrelin(TG)), and in an acylated form (AG ~22% of total ghrelin)[7] *:* Ghrelin has numerous physiological effects, including the regulation of energy balance, insulin sensitivity, vascular health, and body composition. Exercise is a therapeutic option that can potentially optimize ghrelin levels, and sex and obesity may affect the ghrelin response to exercise. Exercise intensity may have an important role on altering not only TG, but may also have a differential effect on AG and DAG. *Purpose:* To identify the optimal dose of exercise to modulate ghrelin levels, and also explore the role of sex and obesity on the response to exercise. *Methods:* Peak oxygen consumption (VO_{2peak}) and lactate threshold (LT) were determined via an incremental test on a cycle ergometer. Subjects had their body composition assessed via dual energy x-ray absorptiometry (DEXA) to measure total body fat percentage (BF%) and an abdominal CT scan to measure abdominal visceral fat (AVF). The testing period consisted of three randomized visits: Control (CON, no exercise), Moderate intensity exercise (MOD, power output at LT), and high intensity exercise (HIGH, power output associated with 75% of the difference between LT and peak). The caloric expenditure was kept consistent within each subject for the exercise conditions. AG, TG, DAG, and lactate were measured at the following timepoints: 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 minutes. Appetite ratings (hunger, satisfaction, fullness and desire to eat) were measured via 100 mm visual analogue scale (VAS) at timepoints 0, 60, 90, 120, 150, and 180 minutes. Brachial flow mediated dilation (FMD) was measured at baseline, 30, 60, 90, and 120 minutes post-exercise. Subjects were split using previously determined cutoffs where females with a BF% >37.1% and males with a BF% > 25.8%

were placed in the obese group (OG). We examined area under the curve and data at each individual timepoint for both TG, AG, DAG, FMD lactate, hunger, and appetite. *Results:* Eight males (age: 42.25 ± 11.0y; BMI: 26.6 ± 5.7; VO_{2peak}: 30.65 ± 8.7 mL/kg/min) and eight females (age: 35.25 ± 11.1 y; BMI: 24.25 ± 4.7 kg/m2; VO_{2peak}: 27.65 ± 5.7 mL/kg/min) completed all measures for the study. HIGH significantly decreased all ghrelin isoforms compared to CON (all; $p < 0.05$). Females had a reduction of TG and DAG ($p<0.05$), while both males and females had a reduction of AG in HIGH compared to CON ($p<0.05$). OG had significantly decreased TG and DAG in HIGH compared to CON ($p<0.05$), and both LG and OG had suppressed AG in HIGH compared to CON ($p<0.05$). There was a significant inverse, relationship between AG/DAG and lactate ($p<0.05$). Appetite was significantly suppressed in HIGH compared to CON ($p<0.05$), and all forms of ghrelin were positively associated with appetite ($p<0.05$). HIGH had a higher %FMD than CON, and MOD had a higher %FMD compared to CON (both; $p<0.0001$). No ghrelin isoform was significantly related to FMD (all; $p>0.05$). *Discussion:* High intensity exercise significantly lowers plasma ghrelin levels. All isoforms of ghrelin may be associated with perception of appetite; however more work is needed to determine if the strength of such relationship differs by isoform. Our findings also suggest lactate may be involved in exercise-induced ghrelin suppression. Isocaloric acute exercise of moderate and high intensity both improved FMD to a similar extent. Although all ghrelin isoforms were suppressed following high intensity exercise, changes in FMD were not associated with changes in ghrelin levels regardless of obesity status.

TABLE OF CONTENTS

SECTION I: FRONT MATTER

SECTION II: LITERATURE REVIEW AND DESCRIPTION OF AIMS

SECTION III: MANUSCRIPTS

LIST OF TABLES

LIST OF FIGURES

SECTION II:
LITERATURE REVIEW AND DESCRIPTION OF AIMS

Introduction

In the early 1980's, Bowers and colleagues published data on a group of synthetic opioid peptide secretagogues they developed (termed growth hormone releasing peptides – GHRPs), that promoted GH release independent from the growth hormone releasing hormone (GHRH) and somatostatin pathways[1–3]. Although a GH secretagogue receptor (GHRS1) was cloned in 1996, the endogenous ligand for this was not discovered until 1999 when Kojima and colleagues identified the agonist, purified from stomach extracts, that they named ghrelin[4]. Shortly thereafter, the role of ghrelin actions on the brain to regulate food intake, body weight, adiposity and glucose metabolism was reported[5]. Subsequent studies identified ghrelin and its analogues have roles in growth hormone secretion, glucose metabolism, food intake, body weight and adiposity, energy metabolism, gastric intestinal motility, sleep, learning and memory as well as a variety of other physiological effects[5,6].

Although much of the early work focused on total ghrelin, the identification of its constituent parts AG and DAG and the balance between them has stimulated recent research examining the independent, synergistic and antagonistic roles of these peptides. Ghrelin exists in the blood in a des-acyl form (DAG ~78% of TG), and in an acylated form (AG ~22% of TG)[7]. Although less abundant, AG has multiple actions that promote energy storage, including stimulation of appetite, inhibition of insulin release from the pancreas, and increased adiposity via the characterized growth hormone secretagogue receptor[8–10]. Conversely, DAG often opposes AG promoting negative energy balance (appetite suppression and reduced fat mass (FM)) and improved insulin sensitivity, acting through a receptor yet to be identified [10,11]. The optimal ratios of TG, DAG, and AG for metabolic

health are not clear. Likewise, there is a need for targeted approaches that can effectively manipulate these peptides.

Ghrelin and Appetite

Animal Studies:

In wild type mice provided with ad libitum food intake, an intracerebroventricular infusion of AG caused an increase in chow consumption, supporting its role as an appetite stimulant, whereas DAG infusion showed no increase and a tendency to decrease food intake[9,12]. To examine whether DAG has anorexigenic activity the authors then administered DAG both intracerebroventricularly and intraperitoneally to mice deprived of food. DAG produced significant inhibitory effects on feeding behavior and food intake, suggesting a role for DAG as an appetite suppressant[12]. A similar pattern was observed in transgenic mice overexpressing DAG , who exhibited decreased food intake, less weight gain, and smaller fat pad mass than their wild-type littermates[12]. In contrast, transgenic mice displaying increased AG levels had higher FM and decreased energy expenditure[13].

Human Studies:

The literature is much less expansive in humans, with exogenous TG administration shown to increase appetite and food intake in healthy humans[16,14–17]. Observational studies also show endogenous TG concentrations appear to rise prior to meal initiation and decrease after consumption, even in the absence of food related cues[18], supporting its role in appetite stimulation. Interestingly, this pattern mirrors that of insulin secretion[19], which may be required in the postprandial diminution of TG[20]. It is important to note that the majority of human studies apply a supraphysiological dose of ghrelin; the effects of ghrelin while utilizing a physiological dose are less clear. In addition, while the scope of this dissertation

focuses on ghrelin and its peripheral effects, ghrelin has been shown to have robust central effects, and exhibits crosstalk with the periphery through a variety of pathways[21].

Impact of Obesity and Sex

While data suggests obesity status influences ghrelin levels, it is important to note that prior work in obesity often measured only TG, where levels are decreased in obese compared to lean individuals[37]. However, when measuring the individual forms, data is contradictory. Some studies show evidence where there is a relative DAG deficiency and/or AG excess in obesity[38]. The decrease in DAG may be responsible for the overall decrease in TG reported in other work, as DAG is present in higher amounts. Endogenous AG levels in humans have been associated insulin resistance in T2DM[39], obese , postmenopausal women[40], and obese, metabolic syndrome[41] patients.

Sex also needs to be taken into account when examining ghrelin levels. Limited data suggests females have a higher DAG level than males, a relationship that persists even when comparing lean and obese individuals[45,46]. In a twin study, TG was also shown to be higher in females, however this difference was absent in the obese cohort[47]. Collectively, variables such as sex and weight status need to be examined to illustrate a more comprehensive relationship.

Ghrelin and Vascular Health

Animal Studies:

Normal vascular function is reliant upon the balance between vasoconstrictor and vasodilator signaling. The role of ghrelin in this process is fully elucidated, however,

GHSR-1a receptors have been found in the heart, arteries, and veins,[48] and an unidentified receptor has been discovered within the endothelium and cardiomyocytes where AG and DAG have can bind to[49]. Furthermore, animal and human data suggest ghrelin has protective effects on the vascular system, where both AG and DAG may have allied effects.

TG administration was found to be beneficial in reducing blood pressure in pulmonary hypertension and sepsis by decreasing the overexpression of endothelin -1 (ET-1, a potent vasoconstrictor)[50,51]. TG also increased the vasodilator nitric oxide (NO) in bovine endothelial cells[52], and the expression of NO-synthase protein in aortic ring samples from GH-deficient rats[53]. Most studies have only measured TG, which did not allow for the examination of the individual roles of AG and DAG. However, DAG may have an independent role in vascular function as prior data indicate DAG was associated with an increase in NO and vasodilation of mouse cerebral arteries, whereas AG had no effect[54].

Human Studies:

TG appears to increase vasodilation by increasing NO release and antagonizing ET-1 in human models *in-vitro*[55]. Unlike the antagonistic effects of AG and DAG on glucose metabolism and insulin resistance, limited data suggests both have potent vasodilatory properties, suggesting there may be optimal levels of each[56]. Uncomplicated obesity as well as obesity associated co-morbidities (e.g. metabolic syndrome, prediabetes, T2DM) are correlated with endothelial dysfunction and increased risk for cardiovascular disease[57]. The potential for ghrelin to mediate endothelial release of NO along with its actions on appetite and glucose metabolism make it an attractive potential therapeutic in these populations. The mechanism of ghrelin induced NO release appears to be primarily via the PI3K-AKt

pathway within the endothelium[58]. This suggests overlap with insulin signaling pathways, and it is possible in insulin resistant states, enhancing ghrelin concentrations (e.g. via exercise) could play a significant role in maintaining vascular health and function via different cellular receptors[54,58]. DAG infusion in individuals with metabolic syndrome resulted in a subsequent increase in NO-mediated vasodilation [59]. Similarly, infusion of AG in individuals with metabolic syndrome, improved vasodilation albeit via reduction of excessive vasoconstrictor tone[60]. Importantly, this was not observed in healthy, control subjects in either study. These data suggest ghrelin levels associated with obesity and prediabetes/T2DM may be implicated in vascular function and provide a potential mechanism by which altering circulating ghrelin may not only improve glucose metabolism, but also be vaso-protective in these groups at higher risk for cardiovascular disease.

Ghrelin and Exercise

There is an extraordinary amount of interindividual variability to a standardized dose of exercise[61]. There are reports in the literature of responders and non-responders to exercise, although those who do not respond likely did not receive an adequate exercise stimulus[61]. As such, it is critically important to characterize the dose response to acute exercise and how this varies by obesity and prediabetes phenotype, prior to using chronic exercise prescription as a precision medicine. This should allow for the optimization of TG, AG, and DAG responses to exercise training and the downstream effects on appetite, glucose metabolism, insulin sensitivity, and vascular function.

Animal Studies:

There is evidence that ghrelin secretion is altered in response to exercise, however results are conflicting. TG and AG increase after an acute bout of exercise in horses and mice[62,63], yet both decrease in rats following chronic exercise training[64,65]. Importantly, one study supported the role of an exercise intervention in ameliorating some of the deleterious effects of a high fat diet (HFD). Rats fed a HFD and then given 10 weeks of exercise training restored plasma AG levels to pre HFD levels[66]. There are no known animal studies investigating DAG in response to exercise.

Human Studies:

Acute exercise tends to impact circulating ghrelin levels, although optimal dose and intensity is unclear. Obesity, prediabetes status and sex may all modulate the ghrelin response to exercise. Data suggests that exercise leads to greater changes to ghrelin levels in females than males. In exercise interventions, AG levels increase in females in response to exercise induced-weight loss[47,48,49], while there is no effect in males[70]. The independent effect of exercise on sex without weight changes are less known, as most studies include only males. Regarding acute exercise, females have been shown to either exhibit a more robust response to AG levels compared to males, or have similar responses[71,72]. Only one known study measured DAG and looked at sex differences in response to exercise and found no difference[45].

In non-obese healthy subjects, bouts of low intensity exercise increase TG independent of exercise duration[73]whereas, moderate and high intensity exercise appear to specifically reduce AG levels in a dose dependent fashion[74]. The decrease in AG has further been associated with reduced insulin levels, sustained glucose levels, and reduced hunger in lean

and obese subjects, suggesting AG may be a mediator of the improvement to insulin sensitivity and suppression of hunger seen in response to exercise training[75–78]. Equivocal results have been reported for DAG's response to exercise. DAG levels increased after a moderate exercise bout in obese, normoglycemic females[79], and after a 6-month military training regimen in lean males[80]. However, in obese adults with pre-diabetes, DAG levels were unchanged after a training intervention that included moderate and high intensity exercise[81]. A meta-analysis that focused on overweight and obese individuals found that exercise suppressed AG; they also indicated that the magnitude of the effect of exercise on ghrelin levels was greater for those with a higher BMI, suggesting lean and obese individuals may not respond similarly[82]. In a recent meta-analysis published by our group, we also found that exercise suppressed pre-prandial ghrelin levels. Although we included TG, AG, and DAG, the majority of studies only sampled AG, therefore our power to detect differences by ghrelin form was limited[83]. We also found that exercise intensity moderated this relationship, with higher intensities further suppressing ghrelin levels. We did not find BMI to be a significant moderator, however the overall lack of trials that include a BMI above normal may contribute to the equivocal results between analyses.

Although the mechanisms to explain the relationship between ghrelin and exercise are not fully elucidated, several hypotheses have been proposed. Changes in blood flow distribution and increases in lactate production offer more insight. With increasing exercise intensity, blood flow to the digestive system is reduced and distributed to the working tissues[84]. Since ghrelin is released from the stomach, it may be transiently suppressed during and after an exercise bout[85,86]. This concept is supported by data following gut ischemic injuries and gut hypoxia, in which ghrelin levels are also decreased[87]. In addition, as skeletal

metabolism increases, oxygen demand by working tissues can outstrip supply leading to a greater contribution for anaerobic metabolism and the generation of lactate and hydrogen ions[88]. It is possible that this increase corresponds with reduced gut blood flow, but there may be an additional direct effect of lactate. Evidence has shown that the gastric mucosal cells in the stomach contain G-protein receptor 81 (GPR81), where lactate can bind to GPR81 and therefore inhibit ghrelin release[89].

Collectively, these results suggest, sex and obesity may affect the ghrelin response to exercise. As such, exercise intensity may have an important role on altering not only TG, but may also have a differential effect on AG and DAG. Therefore, this proposal seeks to identify the optimal dose of exercise to modulate ghrelin levels, and also explore the role of sex and obesity on the response to exercise. An equal distribution of males and females will allow us to examine sex as a biological variable. Subjects will be evaluated for lactate threshold (LT), VO_2 peak, adiposity (DEXA and CT Scan), and complete 3 testing visits: (1) no exercise; and calorically equivalent exercise (2) below and (3) above the LT. Associations between ghrelin levels and exercise induced changes to appetite and the vasculature will also be identified.

The following three manuscripts will investigate the aforementioned research questions:

1. The Effect of Acute Exercise on Pre-Prandial Ghrelin Levels in Healthy Adults: A Systematic Review and Meta-Analysis
    1. This analysis examined the current state of the literature and determined if there was an overall effect of exercise on pre-prandial ghrelin levels in

healthy adults. This paper also investigated moderators (i.e. exercise dose, age, BMI) to explain this relationship.

2. The Effect of Exercise Intensity on Ghrelin Levels and Appetite: Impact of Sex and Obesity Status
    1. This manuscript will explore the relationship between exercise intensity and ghrelin levels and how this corresponds to exercise induced changes to appetite. Participants will be placed in groups based on sex and obesity status.

3. The Effect of Exercise Intensity on Ghrelin Levels and Flow Mediated Dilation: Impact of Sex and Obesity Status
    1. This manuscript will explore the relationship between exercise intensity and ghrelin levels and how this corresponds to exercise induced changes to endothelial function. Participants will be placed in groups based on sex and obesity status.

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SECTION III:
MANUSCRIPT I

The Effect of Acute Exercise on Pre-prandial Ghrelin Levels in Healthy Adults: A Systematic Review and Meta-analysis

Abstract

Background: Ghrelin is a gut hormone with numerous physiological effects, including the regulation of energy balance, insulin sensitivity, vascular health, and body composition. Acylated (AG) and des-acylated (DAG) ghrelin constitute approximately 22 % and 78 % of total plasma ghrelin (TG), respectively. Alterations in the TG concentration and the AG/DAG ratio may be implicated in conditions involving energy imbalances and insulin resistant states (e.g., metabolic syndrome or Type 2 diabetes mellitus). Exercise is a therapeutic option that can potentially optimize ghrelin levels. Understanding the precise intensity and dose of exercise to optimize ghrelin levels may lead to targeted interventions to restore metabolic regulation in obesity and other clinical conditions. *Objective:* To perform a systematic review and meta-analysis on the effects of acute exercise on pre-prandial levels of TG, AG, and DAG in healthy adults and to determine if sample demographics or exercise doses moderate such effects. *Methods:* Electronic databases (PubMed, Medline, SPORTDiscus, Web of Science, and Google Scholar) were searched with articles published through August 2020. The following criteria was determined *a priori* for article inclusion: (i) the study was a randomized controlled trial (RCT),(ii) exercise was an acute bout, (iii) the exercise bout for the intervention group(s)/condition was structured, (iv) the control group/condition received no exercise, (v) participants were adults age 18 or older, (vi) ghrelin was sampled through blood, (vii) there was at least one baseline measure and one post-exercise measure of ghrelin, (viii) there were at least 3 timepoints where ghrelin was measured while participants were fasted to allow for pre-prandial total area-under-the-curve (AUCtotal) calculation, (ix) participants were healthy with no overt disease, (x) interventions were carried out without any environmental

manipulations. Standardized mean difference (SMD) with 95 % confidence intervals were calculated using the restricted maximum likelihood estimation Moderator analyses to determine whether the overall pooled effect was influenced by: sex, ghrelin form, method of ghrelin analysis, age, body mass index, body fat percentage, fitness, intensity of exercise bout, duration of exercise bout, energy expenditure, and length of AUCtotal data. *Results:* The analysis included 24 studies that consisted of 52 trials, n = 504 (age 27.0 (8.8) years, BMI 24.7 (2.7) kg/m2) and measured AG (n = 38 trials), DAG (n = 7), and TG (n = 7). The overall model indicated that exercise lowered ghrelin levels compared to control (no exercise); (SMD=-0.44, $p < 0.001$), and exercise intensity exhibited an inverse relationship with ghrelin levels (regression coefficient (ß)=-0.016, $p = 0.04$). There was no significant difference by ghrelin form ($p = 0.18$). *Discussion:* Acute exercise significantly lowers plasma ghrelin levels, with higher intensity exercise associated with greater ghrelin suppression. The majority of studies applied a moderate intensity exercise bout and measured AG, with limited data on DAG. This exercise dose may be clinically significant in individuals with metabolic dysregulation and energy imbalance as a therapy to optimize AG levels. More work is needed to compare moderate and high intensity exercise and the ghrelin response in clinical populations.

Introduction

In 1999, Kojima et al. discovered the hormone ghrelin, via its role as an endogenous ligand to the growth hormone secretagogue receptor 1a (GHSR1a) and the consequent stimulation of growth hormone (GH) release[1]. The biological effects of ghrelin were later found to be much more diverse, which include effects on energy balance, glucose regulation, cardiovascular function, sleep, memory, and the immune system[2,3]. Primarily secreted from the gastric fundus, ghrelin circulates in two major forms: acylated (AG) and desacylated (DAG). The majority of ghrelin exists as DAG (~78 % of total ghrelin (TG)). The less abundant form, AG (about 22 % of TG), exists via a post-translational modification catalyzed by ghrelin o-acyltransferease (GOAT)[4]. AG is the form of ghrelin that binds to GHSR1a. The identification of AG and DAG and the relative amounts of each has stimulated recent research examining the independent, synergistic and antagonistic roles of these peptides. AG is the most studied and has multiple actions that promote energy storage, including stimulation of appetite, inhibition of insulin release from the pancreas, and increased adiposity via GHSR1a[5-7]. Although the DAG receptor is unknown and earlier reports suggest it was inactive[2], recent work has shown that DAG can antagonize AG in a variety of tissues; specifically by inducing a negative energy balance, reducing fat mass and promoting insulin sensitivity[5, 7, 8]. Conversely, limited data suggests a potential allied effect of ghrelin on the vasculature, with both displaying potent vasodilatory properties, perhaps via different pathways, suggesting there may be an optimal level of each[9]. Human circulating ghrelin concentrations are altered in numerous clinical conditions[3]. Although TG has been shown to be decreased in obese compared to lean individuals; data on the individual forms is equivocal [10]. Specifically, levels of AG have been shown to be

decreased in diabetes mellitus (T2DM)[11], and are either increased or unchanged in obesity[11-13]. DAG may be decreased in obesity, which may contribute to the low TG seen in this population[11]. Importantly, the change in the ghrelin profile may also be implicated in increased insulin resistance and elevated fat mass seen in metabolic disease[11,14] . The physiological effects of ghrelin, coupled with discrepancies in blood concentrations and balance in clinical populations, demonstrates the need for targeted approaches that may effectively optimize these peptides for metabolic and vascular health. Exercise provides a unique therapeutic approach in the treatment of dysregulated ghrelin, obesity, metabolic syndrome, prediabetes, and T2DM[15-17]. Studies examining exercise and ghrelin release are mostly equivocal or only document TG and/or a single (e.g. AG) form of ghrelin[18-35]. The quantification of these peptides in response to exercise is critical to understanding the role of exercise on ghrelin release and ghrelin's exercise-induced influence on overall glucose regulation and energy balance. Two previous meta-analyses have investigated the effects of acute exercise on total area-under the curve (AUC_{total}) ghrelin data. In both, exercise was found to suppress AG (standardized mean difference (SMD): -0.20 and -0.34) [36,37]. It is important to note that both of these meta-analyses contained only studies that measured AG, and included pre-prandial and post-prandial exercise and ghrelin data. Due to the functional differences in AG and DAG, separate analyses of each form are critical, along with measuring both forms in individual studies. Additionally, as macronutrient content and meal timing impacts both ghrelin levels and fuel utilization during exercise, it is important to pool results where ghrelin is measured in the same feeding state[38-41]. Currently, studies of AG, DAG and TG and their response to exercise is understudied and no clear consensus has emerged from the data; studies have used a variety of exercise prescriptions

in their protocols. Therefore, we sought to add to the existing literature by differentiating by form in our analysis, and only including pre-prandial data. Further, including exercise dose and demographical variables in this analysis has allowed us to determine if there is an optimal exercise dose to elicit a response to levels of this hormone. Our results will help provide targets for future studies that will advance the literature surrounding not only how exercise alters ghrelin, but the physiological variables that regulate this response. Understanding how the two forms of ghrelin respond to exercise can help guide future therapies and develop exercise prescriptions tailored to optimize ghrelin levels in distinct clinical populations.

Objective

Our objective was to perform a systematic review and meta-analysis on the effects of exercise on pre-prandial levels of TG, AG, and DAG in healthy adults. We also sought to determine significant moderators of the ghrelin response such as sample demographics and/or exercise dose.

Methods

This meta-analysis and systematic review was performed in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [42]. This review was not registered.

Literature search

Electronic databases (PubMed, Medline, SPORTDiscus, Web of Science, and Google Scholar) were searched by two authors, KA and GZ, with articles published through August 2020 included. The search used the following terms: ((((adult) AND (physical activity OR

exercise)) AND ghrelin AND human) NOT (child OR children OR adolescent OR rat OR mouse OR animal))). Reference lists of all relevant studies along with reviews and book chapters were also examined. Articles were limited to randomized controlled trials (RCT) in the English language.

Article selection

For the purpose of this meta-analysis, the term 'article' is used synonymously with 'study', and 'trial' is the unit included in the meta-analysis. Articles often contained multiple eligible trials that comprised an intervention group and a comparable control. First, the titles and abstracts of the articles were screened for eligibility. The following criteria were determined *a priori* for article inclusion: (i) The study was a RCT,(ii) exercise was an acute bout, (iii) the exercise bout for the intervention group/conditions(s) was structured, (iv) the control group/condition received no exercise, (v) participants were adults age 18 or older, (vi) ghrelin was sampled through blood, (vii) there was at least one baseline measure and one post-exercise measure of ghrelin, (viii) there were at least 3 timepoints where ghrelin was measured while participants were fasted to allow for pre-prandial AUCtotal calculation, (ix) participants were healthy with no overt disease, (x) interventions were carried out without any environmental manipulations. Two authors (KA, GZ) independently completed the study selection.

Data extraction and bias assessment

For studies that met the inclusion criteria, the following data was extracted and tabulated: (i): author, publication year; (ii) continuous variables: sample size, age, ghrelin values, BMI, body fat percentage (BF %), fitness (peak oxygen uptake (*V*O2peak)), intensity of exercise bout (%VO2peak), duration of exercise bout, exercise energy expenditure (EE),

length of AUC time; (iii) categorical variables: sex, ghrelin form (AG, DAG, or TG), and method of ghrelin analysis. Ghrelin values were either entered as pre-prandial AUC data if reported, or each time point was extracted from relevant figures using ImageJ software[43] to allow for manual AUCtotal calculation using the linear trapezoidal method. Extraction was done independently by two authors (KA, GZ) who demonstrated an interclass correlation coefficient of 1.0. If pre-prandial ghrelin time points were not able to be accurately extracted or not reported, the study author was contacted. In the case where authors did not respond to follow up, and standard deviations were not able to be extracted, standard deviations were imputed using the reported baseline values. All values for ghrelin were recorded as pg/mL or converted to such if necessary. Study quality was assessed using the Cochrane risk-of-bias tool for randomized trials (RoB 2) which includes the following domains: randomization, deviations from interventions, missing outcome data, measurement of outcome data, and results[44]. In each domain are signaling questions, where the risk of bias calculated from each domain is generated from an algorithm. Each study is scored as either "low risk", "high risk" or "some concern" of bias based on the answers to the signaling questions[22]. Two authors (KA, GZ) independently answered the signaling questions.

Statistical analysis

The meta-analysis was performed using R Software Version 4.0.2, and the "metafor" and "ggplot2" packages version 2.4 and 3.3, respectively[45,46]. Significance levels for all hypothesis tests were set *a priori* at $p \leq 0.05$. Descriptive data are presented as Mean (Standard Deviation) unless otherwise noted. A three-level, random effects model with restricted maximum likelihood estimation was chosen to account for any dependence of

effect sizes of each trial within the same study, as studies contributed multiple data points[47]. This model accounts for sampling variance of the extracted effect sizes at the level of the subject (level 1), variance of the effect sizes within the same study (level 2), and variance of the extracted effect sizes between studies (level 3). The standardized mean difference (SMD) of the ghrelin AUC_{total} between the exercise and control groups of each trial were inputted into the model to determine the pooled effect. Due to the different relative amounts AG and DAG circulate in the body, the use of SMD was considered appropriate. The SMDs of all analyses are expressed as Hedges *g*, and are interpreted as follows: ≤0.2, 0.2, 0.5, and 0.8 are considered to represent trivial, small, moderate, and large effect sizes, respectively[48]. Subsequent to running overall effect meta-analysis we examined the robustness of the pooled results via publication bias and statistical heterogeneity. Sensitivity analysis was performed utilizing Cook's distance to determine potential influential studies, along with excluding one study at a time, and rerunning the pooled analysis to determine the robustness of the pooled effect. We conducted a second sensitivity analysis where any trial that measured TG was excluded. Due to evidence showing AG and DAG opposing each other in certain tissues, and AG levels being elevated in obesity and T2DM, we investigated the directionality of the two forms in another three-level, REML model. We inverted the sign of each AG SMD, and signs for DAG SMDs were not changed. Positive values would indicate that exercise had a "favorable effect" and negative values would indicate that exercise had a "unfavorable effect". Publication bias was adjudicated through visual inspection of Begg's funnel plot. Last, statistical heterogeneity of the overall model was assessed with Cochrane's Q. The $I2$ statistic was used to assess the amount of heterogeneity at each level of the model, with the following

interpretation: Values <25 % indicates low risk of heterogeneity, 25–75 % indicates moderate risk of heterogeneity, and >75 % indicates considerable risk of heterogeneity[49]. Separate moderator analyses to determine which nominal variables moderated the overall pooled effect were performed with the following subgroups: sex, ghrelin form, and method of ghrelin analysis. Meta- regressions were also used to determine if the following continuous variables impacted the pooled effect size: age, BMI, BF%, fitness, in- tensity of exercise bout (only studies that reported $\%\dot{V}O_{2peak}$ were used for this meta-regression), duration of exercise bout, EE, AUC_{total} length. The regression coefficient (ß) is reported along with 95 % confidence intervals (CI). We completed moderator analyses with the original model, which included TG, AG, and DAG, and also the model where TG trials were excluded.

Results

Literature search

The PRISMA flow diagram outlining this process is presented in Figure 1.1. The initial search identified 1693 articles found via database searches, with an additional 11 articles identified through reference list searching. After title and abstract screening, 1635 articles were excluded, leading to a full text review of 69 eligible articles. After full text review, 24 articles met all inclusion criteria, which contained 52 acceptable trials. The majority of samples contained a young adult population (27.0 (8.8) years), only males (n = 41; only females n = 9; both n = 2) and measured AG (n = 38; DAG: n = 7; TG: n = 7). The full characteristics of included trials are in Table 1.1.

3.2. Risk of Bias

Overall, studies were deemed to have a low risk of bias (Figure 1.2). Due to the nature of study designs, which compared exercise to a resting control, it is impossible for participants and researchers to be blinded to the treatment assignment. However, due to the analysis methods of the plasma samples (e.g., batch analysis with ELISAs) for ghrelin, the absence of blinding was not deemed high risk to bias results.

3.3. Pooled effect

The overall model indicated that exercise had a significant, small to approaching moderate, suppressive effect on ghrelin levels (SMD=-0.44, 95 % confidence interval (CI): -0.65 to -0.23, $p < 0.001$, Fig. 1.3). The overall model had significant heterogeneity (Cochrane's Q = 78.9, df = 51, p < 0.001) with further analysis revealing no within-study (level 2) heterogeneity ($I^2 = 0$ %, $p = 1.0$), and moderate between-study (level 3) heterogeneity ($I^2 = 41.27$ %, $p < 0.01$).

3.4. Cook's distance and publication bias

Cook's distance identified one study as an influential study (Figure 1.4). When this study was removed from the pooled analysis, the effect of exercise on ghrelin levels remained significant but the SMD became weaker (SMD= -0.33, p < 0.01), and the heterogeneity non- significant. It is unclear why this study had such an effect on the model, beyond the trials having the strongest effect sizes and the study being published most recently. All other studies removed during the sensitivity analysis had no substantial effect on the overall model. Visual inspection of the funnel plot revealed asymmetry (Figure 1.5.). The plot

shows that several studies with large effect sizes drive the asymmetry, with one of those studies detected as the influential study by Cook's Distance[50].

Subgroup analyses

Moderator data is listed in Table 1.2. The subgroup analyses revealed nonsignificant moderation by sex ($p = 0.37$), ghrelin form ($p = 0.18$), and ghrelin analysis method ($p = 0.13$). Concerning meta-regressions, exercise intensity was found to be a significant moderator with an in- verse relationship with ghrelin levels (ß=-0.016, $p = 0.04$, Fig. 1.6.). In addition, exercise intensity explained a portion of the heterogeneity in the overall model, decreasing the variance of effect sizes between studies (level 2, $I^2 = 35.6$ %, $p < 0.01$). When the influential study was removed from this meta-regression, the relationship remained significant (ß=- 0.017, $p = 0.01$)[50]. All other meta-regressions were nonsignificant: age ($p = 0.18$), BMI ($p = 0.69$), BF% ($p = 0.60$), fitness ($p = 0.33$), EE ($p = 0.14$), AUC_{total} length ($p = 0.15$), and exercise duration ($p = 0.77$).

Sensitivity analyses

In the model with only AG and DAG trials, exercise had a significant, moderate effect on ghrelin levels (SMD = 0.36, 95 % CI: 0.17 to 0.54, $p < 0.001$). When the influential study was removed from this model, the significance remained (SMD = 0.35, 95 % CI 0.19 to 0.51, $p < 0.001$). The moderator analysis using this model revealed ghrelin form as the only significant moderator (DAG SMD:-0.25, AG SMD = 0.46, $p = 0.003$). However, when the influential study was removed from this model, ghrelin form is no longer a significant sub-

group (DAG SMD: 0.05, AG SMD = 0.37, p = 0.14) while the meta- regression with exercise intensity became significant (ß = 0.015, p = 0.03).

Discussion

Overall

The purpose of this systematic review and meta-analysis was to examine changes in pre-prandial ghrelin levels in response to an acute bout of exercise in adults, and establish if these effects are moderated by sample demographic characteristics and/or exercise dose. Dysregulated ghrelin occurs in several clinical conditions, including metabolic syn- drome and T2DM. An understanding of how exercise may modify ghrelin levels is critical to guide potential future therapies. The results of this analysis suggest that overall exercise suppresses plasma ghrelin levels, and this effect is moderated by exercise intensity; with higher exercise intensities lowering ghrelin to a greater extent. Although there was no statistical difference in the effect of exercise by ghrelin form, it is important to note that the majority of studies measured only AG and as such the data to examine TG, AG, and DAG separately is limited.

Potential mechanisms to explain the suppression of ghrelin in response to exercise are not fully elucidated. However, several hypotheses have been proposed. It is likely that there is considerable overlap and/or redundancy between these potential mechanisms and their contributions may differ between ghrelin forms. One mechanism is that ghrelin levels in humans are known to decrease after intravenous and oral intake of FFA[51,52]. Given that long-chain FFA have been shown to increase post-exercise[53], this may provide a potential

explanation for a reduction in ghrelin following exercise. The ghrelin system has other key components that may contribute to the exercise response; however, they remain understudied. Liver-enriched antimicrobial peptide 2 (LEAP2), has been shown to be a GHSR-1a antagonist, blocking the action of AG upon binding[54]. Data further suggests that LEAP2 has the ability to attenuate ghrelin induced food intake and GH secretion in mice, and is sensitive to changes in body weight and feeding status, specifically being suppressed by fasting[55], In addition, during chronic calorie restriction in mice expressing LEAP2, ghrelin levels increased less compared to the control group[56], which may indicate LEAP2 but can also inhibit the secretion of ghrelin. Future work should identify how LEAP2 responds to exercise to further illuminate the ghrelin pathway; specifically, if this hormone is involved in the exercise induced suppression of ghrelin levels we have reported. To our knowledge no study has yet to investigate LEAP2 and its response to exercise. Collectively, current data highlights that there is likely an interaction of multiple hormones that regulate this response.

To address the issue of exercise intensity and ghrelin concentration, it is likely that changes in blood flow distribution and increases in lactate production offer more insight. With increasing exercise intensity, blood flow to the digestive system is progressively reduced, as it is distributed to the working tissues[57]. Since ghrelin is released from the stomach, it is logical that it may be suppressed during and after an exercise bout[58,59]. This concept is supported by data following gut ischemic injuries and gut hypoxia, in which ghrelin levels are also decreased[60]. Similarly, as skeletal metabolism increases, oxygen demand by working tissues can outstrip supply leading to a greater contribution for anaerobic metabolism and the generation of lactate and hydrogen ions[61]. It is possible that this

increase coincides with reduced gut blood flow, but there may be an additional direct effect of lactate. The gastric mucosal cells in the stomach, produce ghrelin contain G-protein receptor 81 (GPR81). Lactate can bind GPR81 and inhibit ghrelin release from the gastric mucosa[62].

Comparison with other studies

The results of this meta-analysis suggest that exercise suppresses ghrelin levels, in agreement with previously published work[36,37,63]. The pooled effect of exercise on ghrelin levels was small to approaching moderate; the strength of this effect (SMD= -0.44) was stronger than those seen in prior analyses (SMD -0.20 and -0.34)[36,37]. Without the identified influential study[50], our reported effect size (SMD= -0.33) was similar to one of the previous analyses[57]. A possible explanation for the difference in strength of effect sizes between studies is the inclusion of only pre-prandial data. In addition, although we did not report a moderating effect of ghrelin form, as subgroup analyses are observational in nature, it is still possible that our inclusion of TG, AG, and DAG in one model influenced our results. Lastly, the model chosen could have added to the difference in effect sizes between studies. While previous literature utilized random effect models, we chose a three-level nested model. Certain trials in our analysis had separate samples for their control and exercise conditions, while others employed a crossover design. Because the non-independence of data points in our analysis breached the assumptions of typical meta-analysis models, the use of the three-level model was appropriate. Importantly, we extend the literature by reporting exercise intensity as a significant moderator, a result that was not reported by the previous analyses.

Moderator interpretation

Due to the wide variety of study sample and exercise characteristics, along with heterogeneity being present, exploration through moderator analyses were warranted. Because a majority of these analyses were not significant and likely underpowered, we can only speculate on their interpretation. Hypothesis testing revealed no significant moderation by age, sex, ghrelin form, BMI, BF%, fitness, exercise duration, EE, AUCtotal length, or ghrelin analysis method. It is known that AG levels exhibit a decline in older adults. However, data on how exercise affects the different ghrelin forms across adult age groups is scarce[64]. The trials included in this analysis largely sampled adults younger than 30 years old, where only two studies sampled an average age above that[30,65]. Due to ghrelin's beneficial role in inflammation, bone mass, and sarcopenia, future studies targeting ghrelin through exercise in middle aged and older adults is needed[66-68].

In addition, the majority of studies in our analysis comprised of males, which suggests that sex as a moderator was underpowered. Limited data has shown that males and females exhibit a sexual dimorphism concerning plasma ghrelin levels, highlighting the importance of exploring sex as a variable in response to exercise interventions[11,65]. We report that the effect of exercise on ghrelin levels were stronger in males (SMD= -0.50) than females (SMD=-0.32), although not reaching statistical significance. These studies varied widely in exercise dose and sample characteristics such as BMI, which adds difficulty in drawing conclusions.

Data suggest that weight status and obesity can influence ghrelin levels[33], yet how exercise specifically alters responses in lean versus overweight/obese individuals is uncertain. The

average BMI in this analysis was 24.7 (2.7) kg/m^2, which indicates a need for more studies to sample individuals with BMIs above normal weight; only seven studies in this analysis did so[25,30-32, 65, 69, 70]. The meta-analysis by Douglas et al. found BMI to be a significant moderator; when lean individuals were included in the model, the effect size of the pooled data decreased[37]. We did not observe BMI as a significant moderator, consistent with the meta-analysis by Schubert et al. [36]. We also did not find BF% to significantly moderate ghrelin levels, however many studies did not measure this variable, and those that did most often utilized skinfolds[20,21,28,29,34,35,69,71], an unreliable measure compared to computerized topography, hydrostatic weighing, and dual energy x-ray absorptiometry scans[72,72]. Skin folds do not differentiate between the types of adipose tissue, and prior data indicates TG levels are associated with visceral but not subcutaneous fat[74] the need to further examine adipose tissue type and ghrelin is warranted. Animal models suggesting AG acts to increase fat mass, while DAG has been shown promote the opposite, also necessitates the measurement of both forms individually when looking at body composition[75,76].

Most studies (71 %) in this analysis only measured AG. It is interesting to note the effect sizes of each ghrelin form, which did not reach statistical significance (SMD: TG -0.23, AG -0.52, DAG -0.15, Fig. 2). We did not feel it was appropriate to report weighted effect sizes, due to the biological ratio of AG:DAG and the relative amounts of each. However, we did calculate a percent difference between the exercise and control AUC_{total} data, where we report AG decreased by 22.8 % on average, DAG decreased by 0.04 %, and TG decreased by 1.2 % compared to the control condition (Table 1.1.). The difference between effect sizes and percent differences of AG and DAG underpins the importance of measuring the forms individually. As DAG is prevalent in higher amounts compared to

AG, it is unsurprising that the effect size of TG may be more closely aligned with this form. As the biological functions of the two forms can oppose each other, a decrease in AG levels can have a different implication than a decrease in DAG. This is further illustrated in obesity, where AG levels have been found to be increased and DAG levels are decreased compared to lean individuals[11,14]. Therefore, a beneficial effect of exercise in this population would be a decrease in AG and an increase in DAG. To address the disparate directionality of AG and DAG, we conducted a sensitivity analysis by running another model with just AG and DAG trials, where the direction of AG SMDs was inverted (ESM Appendix S3). This model strengthened the robustness of our results, indicating that exercise has a favorable effect on ghrelin levels (SMD = 0.36).

Overall, most studies that solely measured AG reported a decrease following exercise. GOAT has been found to be downregulated in certain conditions such as during a positive energy balance and feedback from ghrelin itself [78,79], whether exercise can also induce this effect is an attractive, albeit unstudied, theory that may help explain how exercise induces a suppression of this form. Only three studies measured DAG and reported a decrease[50,65] or no change[70]; whether DAG has a blunted response to exercise compared to AG is unknown. Lastly, TG responses have varied widely, with studies reporting no change[20], a decrease[33,80], or an increase post-exercise[24]. Although we did not find ghrelin analysis method a significant moderator, if blood samples are not treated with a protease inhibitor, AG will degrade and the in- dividual forms cannot be measured accurately[4]. In addition, acidification of the sample before storing aids in stability when frozen[4]. Studies in this analysis reported a variety of blood collection methods, therefore we were unable to add this variable as a moderator and cannot rule out the possibility of collection methods

affecting results. Even with the inconsistency in prior data, it is clear that each form of ghrelin may not respond similarly when given an identical exercise dose within studies.

The variety of exercise doses utilized between studies adds to the difficulty in establishing a consensus on ghrelin and exercise data. We report no moderation by exercise duration, a result that is corroborated by the two previous meta-analyses[36,37]. Conversely, we report that exercise intensity significantly moderated ghrelin's relationship with exercise; the higher the intensity, the stronger the suppression of ghrelin. This was a finding that was not significant in the previous meta-analyses[26,27]. Possible explanations include fewer number of studies in their reviews, including post-prandial data, or only focusing on AG in their models. Ghrelin has been shown to stimulate fatty acid oxidation in skeletal muscle[81], and as high-intensity exercise is typically fueled by muscle and liver glycogen over lipids[82], our finding that ghrelin is suppressed more during higher intensities suggests it may aid in energy utilization during exercise. In addition, as previously discussed, lactate and FFA are increasingly produced during high intensity exercise may inhibit ghrelin release[62]. However, more work is needed to understand how intensity alters the ghrelin pathway. The majority of studies utilized a moderate intensity exercise bout and report that ghrelin suppression is achievable at this level. Therefore, more studies that compare high and moderate intensity exercise are needed to demonstrate whether high intensity leads to larger suppression of ghrelin. As intensity was the only significant moderator that reduced but did not eliminate the heterogeneity within our data, there are likely other factors we did not explore and/or that are not reported in the literature that contribute to the relationship.

Both ghrelin and exercise have been shown to be involved in energy balance, with prior work focused on determining if alterations in ghrelin from exercise are due to energy expenditure and intake. We report that energy expenditure is not a significant moderator of exercise-induced ghrelin suppression, in line with the meta-analysis by Schubert et al.[36] Other results indicate the ghrelin is not associated with subsequent relative[83] or ad libitum energy intake post-exercise[25,28]. Although we only included pre-prandial data in our analysis, the majority of studies calculated AUC for the entire visit, which often included both pre- and post-prandial time points. Even with a RCT design, macronutrient content and timing of meals can influence ghrelin levels[38] , exercise performance[84], and post-exercise physiological processes[85] which can confound interpretations. Future studies that are designed with exercise and meals should consider calculating separate pre-prandial and post-prandial AUC calculations to help differentiate diet- and/or exercise- induced effects. Overall, data suggests that exercise energy expenditure does not mediate changes to plasma ghrelin, rather this hormone may be more sensitive to meal-induced changes to energy balance.

4.4. Implications

Our findings strengthen previous conclusions about the suppressive effect of exercise on ghrelin levels. The rationale for this response is likely multifaceted and complicated by ghrelin form. Decreased AG has been associated with reduced insulin levels, sustained glucose levels, and reduced hunger in lean and obese subjects, suggesting AG may be a mediator of the improvement to insulin sensitivity and suppression of hunger seen in response to exercise[22, 23, 71, 86, 87] . Although we did not report a significant moderator effect

by ghrelin form, it does high- light the need for more studies examining exercise and its effect on DAG, as the majority of studies measured just AG. Additionally, exercise mode is an understudied facet of the literature that we were underpowered to assess; the majority of studies in our analysis applied an aerobic exercise bout. Due to the opposing effects of AG and DAG on appetite, insulin sensitivity, and energy balance, pinpointing the best exercise prescription to restore or maintain the balance of the two is critical. AG has been found to be elevated in obesity and T2DM[11], and the results of this analysis suggest exercise may be an attractive modality to decrease, and therefore normalize, levels in these populations. Importantly, ghrelin levels have found to be disrupted in conditions beyond those that primarily exhibit energy imbalance and/or insulin resistance; such as heart failure, Parkinson's disease, multiple sclerosis, and chronic obstructive pulmonary disease[2,3] . Therefore, future work that continues to develop precise exercise doses to target different components of the ghrelin pathway specific to condition is warranted.

The novel finding of this analysis is that exercise intensity was a significant moderator; the higher the exercise intensity, the more ghrelin is suppressed. This has important implications for individualized medicine; optimizing the ghrelin response during exercise can have further downstream effects on insulin sensitivity, appetite, and vascular function[2,10]. However, more work needs to be completed to understand if high intensity exercise is the best prescription for different clinical populations, as we only included studies that sampled healthy adults. Lastly, more exercise training studies that employ a chronic high intensity stimulus is warranted to understand if the suppressive effects of exercise on ghrelin levels can be maintained over time.

4.5. Limitations

This analysis has several limitations. First, we limited our inclusion to studies published in the English language. In addition, the results of this review are restricted to healthy populations, as we excluded major diseases, and are not necessarily comparable to exercise training studies. We recognize that including multiple trials from one study may contribute to analytical issues such as "double counting," [88] however we feel our choice in applying a nested model helped reduce this effect. It should also be noted that half of the included trials in this analysis occurred from the same laboratory group[18-21, 28, 29, 34, 50, 65], however we did account for between study variance in our model. Finally, it is possible that many of our moderators were underpowered to detect significant effects, therefore our moderator analyses remain speculative in nature.

4.6. Conclusions

The results of this meta-analysis suggest high intensity exercise may be superior to low and moderate intensities when it pertains to ghrelin suppression, although there is a paucity of data in overweight/obese, female, and older adults. Future work should be concentrated on developing precise exercise prescriptions to best target both AG and DAG during acute exercise, which can later be applied to chronic training interventions. As many facets of ghrelin function remain ambiguous, research should also focus on establishing clear pathways for AG and DAG effects, which can help illuminate how exercise can be applied to lead to clinically meaningful changes to endogenous ghrelin levels.

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Table 1.1. Trial Characteristics

	Sample			Exercise			SMD	Ghrelin Percent Difference (Exercise vs Control AUC_{total})		
Trial	(Sex) Size	Age (years)	BMI (kg/m^2)	Mode	Intensity	Duration (min)		TG	AG	DAG
Alajmi et al. [1][18]	(F) 8	22.3 ± 2.5	22.3 ± 2.3	AEx	73.3 ±0.6 % VO_{2peak}	60.0 ± 0.0	-0.79	-	-37.0%	-
Alajmi et al. [2][18]	(M) 8	22.6± 3.8	23.1 ± 2.1	AEx	70.9 ± 1.4 % VO_{2peak}	60.0 ± 0.0	-0.93	-	-50.5%	-
Balaguera-Cortes et al. [1][77]	(M) 10	21.3 ±1.4	23.7 ±2.0	REx	3 sets of 12 repetitions (or to failure)	45.0 ± 0.0	-0.54	-	-19.4%	-
Balaguera-Cortes et al. [2][77]	(M) 10	21.3 ±1.4	23.7 ±2.0	AEx	71.0 ± 7.0 % VO_{2peak}	45.0 ± 0.0	0.078	-	3.02%	-
Bishop et al. [19]	(M) 9	24 ± 2.0	22.8 ± 1.9	AEx	73.1 ± 3.7% VO_{2peak}	60.0 ± 0.0	-1.7	-	-64.4%	-
Broom et al.[a] [22]	(M) 9	21.2 ± 0.7	22.2±0.7	AEx	72.0 ± 2.0 % VO_{2peak}	60.0 ± 0.0	-0.24	-	-46.7 %	-
Broom et al. [b,1][23]	(M) 11	21.1 ± 0.3	23.1 ± 0.4	REx	80% of 12 $repetition_{max}$	90.0 ± 0.0	-0.19	-	-19.2%	-
Broom et al. [b,2][23]	(M) 11	21.1 ± 0.3	23.1 ± 0.4	AEx	69.0 ± 2.0 % VO_{2peak}	60.0 ± 0.0	-0.30	-	-29.7%	-
Broom et al. [c,1][21]	(M) 8	21.7±1.7	24.5 ± 2.4	AEx	52.0 ± 3.0 % VO_{2peak}	55.0 ± 7.0	-0.30	-	-12.2%	-
Broom et al. [c,2] [21]	(M) 8	21.7±1.7	24.5 ± 2.4	AEx	75.0 ± 4.0 % VO_{2peak}	36.0 ± 5.0	-0.16	-	-15.8%	-
Broom et al. [c,3] [21]	(M) 9	23.2±2.1	22.7 ± 1.5	AEx	70.0 ± 2.0 % VO_{2peak}	45.0 ± 0.0	-0.30	-	-26.8%	-
Broom et al. [c,4] [21]	(M) 9	23.2±2.1	22.7 ± 1.5	AEx	70.0 ± 2.0 % VO_{2peak}	90.0 ± 0.0	-0.42	-	-38.5%	-
Burns et al. [1][20]	(M) 9	24.5+1.3	25.1+1.2	AEx	71.5± 2.5% VO_{2peak}	60.0 ± 0.0	-0.32	-8.3%	-	-
Burns et al. [2][20]	(F) 9	23.4+1.0	22.5+0.8	AEx	75.5 ± 3.0% VO_{2peak}	60.0 ± 0.0	-0.94	-12.8%	-	-
Crabtree et al. [1][80]	(M) 15	22.5 ± 3.1	24.2 ± 2.4	AEx	70.0 ± 0.0 % VO_{2peak}	60.0 ± 0.0	-0.49	-17.1%	-	-
Crabtree et al. [2][80]	(M) 15	22.5 ± 3.1	24.2 ± 2.4	AEx	70.0 ± 0.0 % VO_{2peak}	60.0 ± 0.0	-0.87	-	-44.4%	-
Dorling et al. [1] [50]	(M) 12	20.9 ± 3.5	23.5 ± 2.7	AEx	71.0 ± 2.0% VO_{2peak}	60.0 ± 0.0	-0.76	-	-	-18.7%
Dorling et al. [2] [50]	(M) 12	21.3 ± 3.6	23.5 ± 2.3	AEx	70.0 ± 2.0% VO_{2peak}	60.0 ± 0.0	-1.2	-	-	-33.1%
Dorling et al. [3] [50]	(M) 12	20.9 ± 3.5	23.5 ± 2.7	AEx	71.0 ± 2.0% VO_{2peak}	60.0 ± 0.0	-2.4	-	-60.0%	-

Dorling et al. [4] [50]	(M) 12	21.3 ± 3.6	23.5 ± 2.3	AEx	70.0 ± 2.0% VO_{2peak}	60.0 ± 0.0	-2.3	-	-58.6%	-
Douglas et al. [1][65]	(F) 10	38.1± 16.7	21.8 ± 1.6	AEx	49.0 ±26.0% VO_{2peak}	60.0 ± 0.0	0.17	-	-	8.1%
Douglas et al. [2][65]	(F) 11	45.5± 13.2	28.7±2.8	AEx	57.0 ± 4.3% VO_{2peak}	60.0 ± 0.0	0.20	-	-	28.7%
Douglas et al. [3][65]	(M) 10	33.8± 13.1	22.9 ± 1.4	AEx	59.3 ± 2.9% VO_{2peak}	60.0 ± 0.0	-0.055	-	-	-3.3%
Douglas et al. [4][65]	(M) 12	44.9± 13.2	29.3 ±3.1	AEx	57.9 ± 2.2 % VO_{2peak}	60.0 ± 0.0	-0.091	-	-	-8.9%
Douglas et al. [5][65]	(F) 10	38.1± 16.7	21.8 ± 1.6	AEx	49.0± 26.0% VO_{2peak}	60.0 ± 0.0	-0.023	-	-1.1%	-
Douglas et al. [6][65]	(F) 11	45.5± 13.2	28.7±2.8	AEx	57.0 ± 4.3 % VO_{2peak}	60.0 ± 0.0	0.15	-	14.0%	-
Douglas et al. [7][65]	(M) 10	33.8± 13.1	22.9 ± 1.4	AEx	59.3 ± 2.9% VO_{2peak}	60.0 ± 0.0	-0.24	-	-10.7%	-
Douglas et al. [8][65]	(M) 12	44.9± 13.2	29.3 ±3.1	AEx	57.9 ± 2.2 % VO_{2peak}	60.0 ± 0.0	-0.29	-	-29.0%	-
Erdmann et al. [1][24]	(M)2, (F) 5	24.4 ± 0.6	21.4 ± 0.8	AEx	Cycling at 50W	30.0 ± 0.0	0.64	33.2%	-	-
Erdmann et al. [2][24]	(M)2, (F) 5	24.4 ± 0.6	21.4 ± 0.8	AEx	Cycling at 100W	30.0 ± 0.0	0.30	18.3%	-	-
Gholipour et al. [69]	(M) 9	20.6±1.4	32.7±2.5	AEx	59.9 ± 0.0 % VO_{2peak} †	27.0 ± 0.0	-2.3	-	-37.8%	-
Hagobian et al.[1][25]	(M) 11	22.0 ±2 .0	26.0 ±4.0	AEx	70.0 % VO_{2peak}	82.0 ±13.0	-0.079	-	-4.0%	-
Hagobian et al. [2][25]	(F)10	21.0 ±2 .0	24.0 ±2 .0	AEx	70.0 % VO_{2peak}	84.0 ±17.0	-0.14	-	-8.3%	-
Kawano et al. [1][26]	(M) 15	24.4 ± 1.7	22.1 ± 2.0	REx	64.8 ± 6.9 % VO_{2peak}	30.0 ± 0.0	-0.42	-	-27.5%	-
Kawano et al. [2][26]	(M) 15	24.4 ± 1.7	22.1 ± 2.0	AEx	63.9 ± 7.5% VO_{2peak}	30.0 ± 0.0	-0.46	-	-30.7%	-
Kelly et al. [27]	(M) 10	21.4 ± 1.3	23.9 ± 2.1	AEx	70.0 ± 0.0 % VO_{2peak}	45.0 ± 0.0	0.16	-	8.9%	-
King et al. [a][28]	(M) 9	22.2 ± 0.8	23.6 ± 0.4	AEx	68.8 ± 0.8% VO_{2peak}	90.0 ± 0.0	-1.1	-	-49.6%	-
King et al. [b][29]	(M) 14	21.9 ± 0.5	23.4 ± 0.6	AEx	45.2 ± 2.0 % VO_{2peak}	60.0 ± 0.0	0.0015	-	0.09%	-
Larsen et al. [1][30]	(M) 12	48.0 ± 5.0	29.9 ± 1.9	AEx	75.0 ±1.0 % VO_{2peak}	50.0 ± 0.0	-0.40	-	-20.6%	-
Larsen et al. [2][30]	(M) 12	48.0 ± 5.0	29.9 ± 1.9	REx	27.0±1.0 % VO_{2peak}	30.0 ± 0.0	-0.099	-	-4.9%	-

Larsen et al. [3][30]	(M) 12	48.0 ± 5.0	29.9 ± 1.9	CEx	REx:29.0±1.0, Aex: 74.0 ± 1.0 % VO_{2peak}	40.0 ± 0.0	0.031	-	1.6%	-
Mattin et al. [1][31]	(M) 12	26.0 ± 5.0	25.5 ± 3.5	AEx	40.0 ±0.0 % VO_{2peak}	60.0 ± 0.0	0.35	-	13.4%	-
Mattin et al. [2][31]	(M) 12	26.0 ± 5.0	25.5 ± 3.5	AEx	70.0 ±0.0 % VO_{2peak}	60.0 ± 0.0	-0.82	-	-28.3%	-
Metcalfe et al. [1][32]	(M) 8	21.0 ± 2.0	25.0 ± 4.0	AEx	Cycling at 60W plus 2 all out sprints against a resistance of 7.5% of bodyweight	10.0 ± 0.0	-0.88	-	-51.9%	-
Metcalfe et al. [2][32]	(M) 8	21.0 ± 2.0	25.0 ± 4.0	AEx	53.0 ± 5.0 % VO_{2peak}	30.0 ± 0.0	-0.059	-	-3.5%	-
Stokes et al. [1][33]	(M) 7	26.0 ± 3.0	24.9 ± 5.0	AEx	Cycling for 4 min at 60 W, 30 s at 80 W, 30 s at 100 W then a 5-minute rest period. Ending with a 30 sec sprint at 7.0% of bodyweight	5.5 ± 0.0	-0.44	-12.8%	-	-
Stokes et al. [2][33]	(M) 7	26.0 ± 3.0	24.9 ± 5.0	AEx	Cycling for 4 min at 60 W, 30 s at 80 W, 30 s at 100 W, then a 5-minute rest period. Ending with a 30 sec sprint at 9.0% of bodyweight	5.5 ± 0.0	-0.31	-9.0%	-	-
Tiryaki-Sonmez et al. [1][70]	(F) 9	22.8±1.4	28.3 ±1.8	AEx	53.1 ±3.31 % VO_{2peak}	60.0 ± 0.0	-0.39	-	-8.1%	-
Tiryaki-Sonmez et al. [2][70]	(F) 9	22.8±1.4	28.3 ±1.8	AEx	53.1 ±3.31 % VO_{2peak}	60.0 ± 0.0	0.027	-	-	0.45%
Wasse et al. [1] [34]	(M) 11	22.7 ± 2.3	23.4 ± 2.4	AEx	71.7±2.5 % VO_{2peak}	60.0 ± 0.0	-0.44	-	-30.2%	-
Wasse et al. [2][34]	(M) 11	22.7 ± 2.3	23.4 ± 2.4	AEx	70.3±4.0 % VO_{2peak}	60.0 ± 0.0	-0.51	-	-32.8%	-
Vatansever-Ozen et al. [35]	(M) 10	20.1 ± 0.17	22.0± 0.44	AEx	54.6 ± 3.5 % VO_{2peak} †	120.0 ± 0.0	-0.34	-	-5.9%	-

Aerobic Exercise (AEx), Combined Exercise (CEx), Resistance Exercise (REx)

† Exercise Intensity was calculated using a time-weighted average if several intensities were used in a single exercise bout

Table 1.2. Moderator Analysis Data

Moderator Variable	p value	Comparison
Age	0.18	Meta-regression (ß = -0.017, 95% CI= -0.008 to 0.043)
Sex	0.37	Male (ES= -0.50, 95% CI= -0.60 to 0.22)
		Female (ES= -0.32, 95% CI= -0.72 to 0.09)
Ghrelin Form	0.18	TG (ES= -0.23, 95% CI= -0.78 to 0.63)
		AG (ES= -0.52, 95% CI= -0.82 to 0.08)
		DAG (ES= -0.15, 95% CI= -0.62 to 0.32)
BMI	0.69	Meta-regression (ß = -0.013, 95% CI= -0.076 to 0.051)
BF%	0.60	Meta-regression (ß = -0.014, 95% CI= -0.067 to 0.039)
Fitness	0.33	Meta-regression (ß = -0.008, 95% CI= -0.024 to 0.008)
Exercise Intensity (% VO_{2peak})	0.04	Meta-regression (ß = -0.016, 95% CI= -0.032 to -0.001)
Exercise Duration	0.77	Meta-regression (ß = -0.001, 95% CI= -0.01 to 0.007)
EE	0.14	Meta-regression (ß = -0.0001, 95% CI= -0.0002 to 0.00003)
AUC_{total} length	0.15	Meta-regression (ß = -0.21, 95% CI= -0.50 to 0.08)
Ghrelin Analysis Method	0.13	ELISA (ES= -0.56, 95% CI= -0.98 to 0.12)
		RIA (ES= 0.04, 95% CI= -0.73 to 1.08)
		xMAP (ES= -0.13, 95% CI= -0.62 to 0.37)

Figure 1.1 PRISMA Diagram

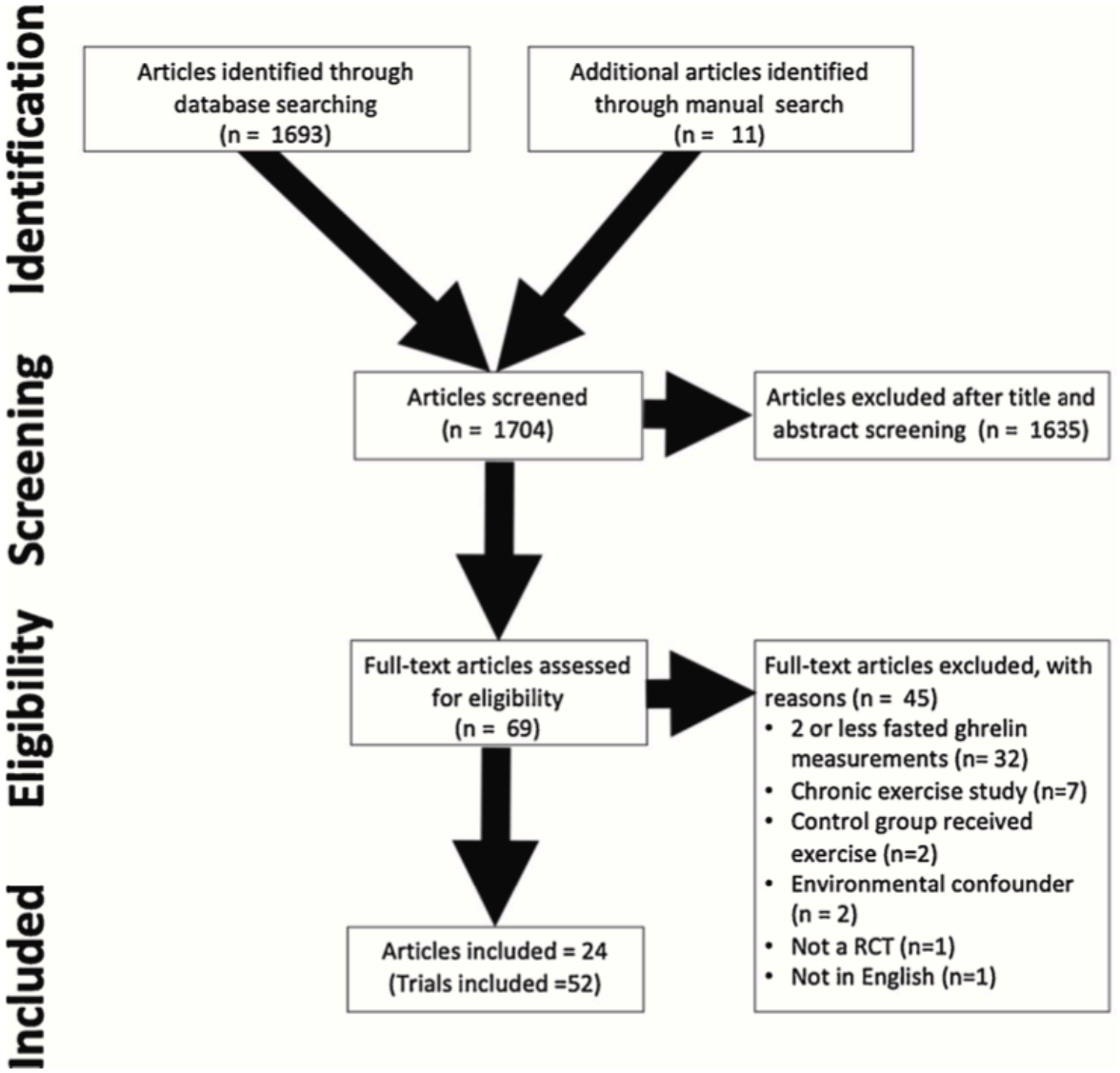


Figure 1.2. RoB2 Risk of Bias Assessment

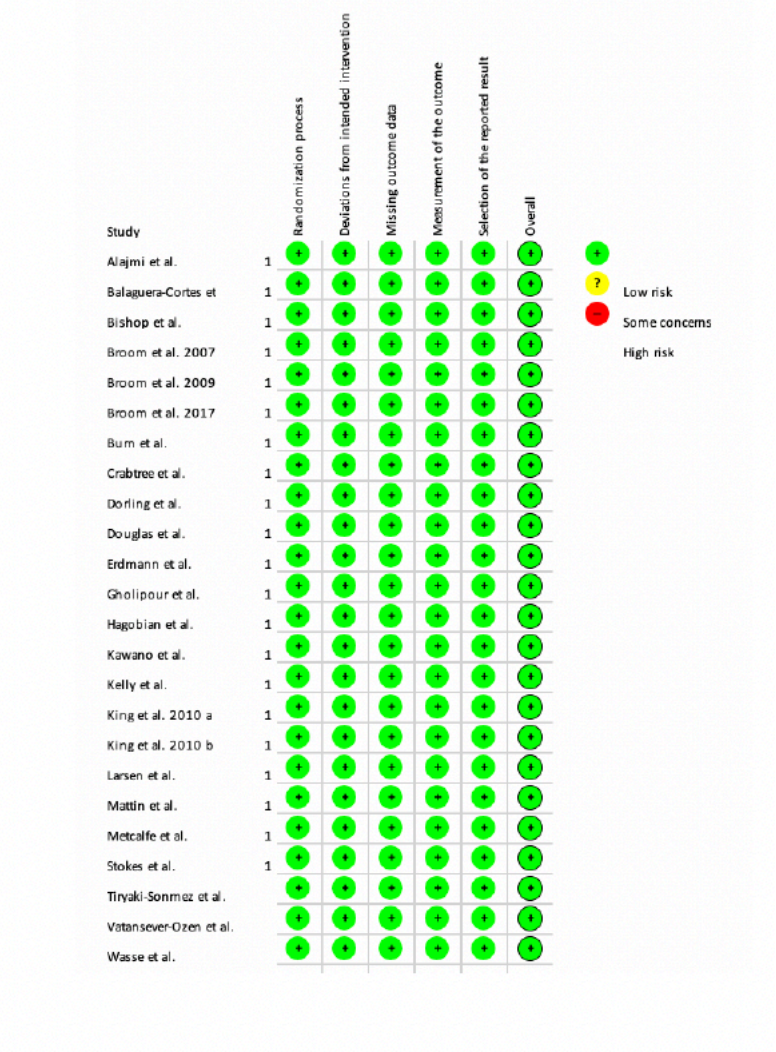
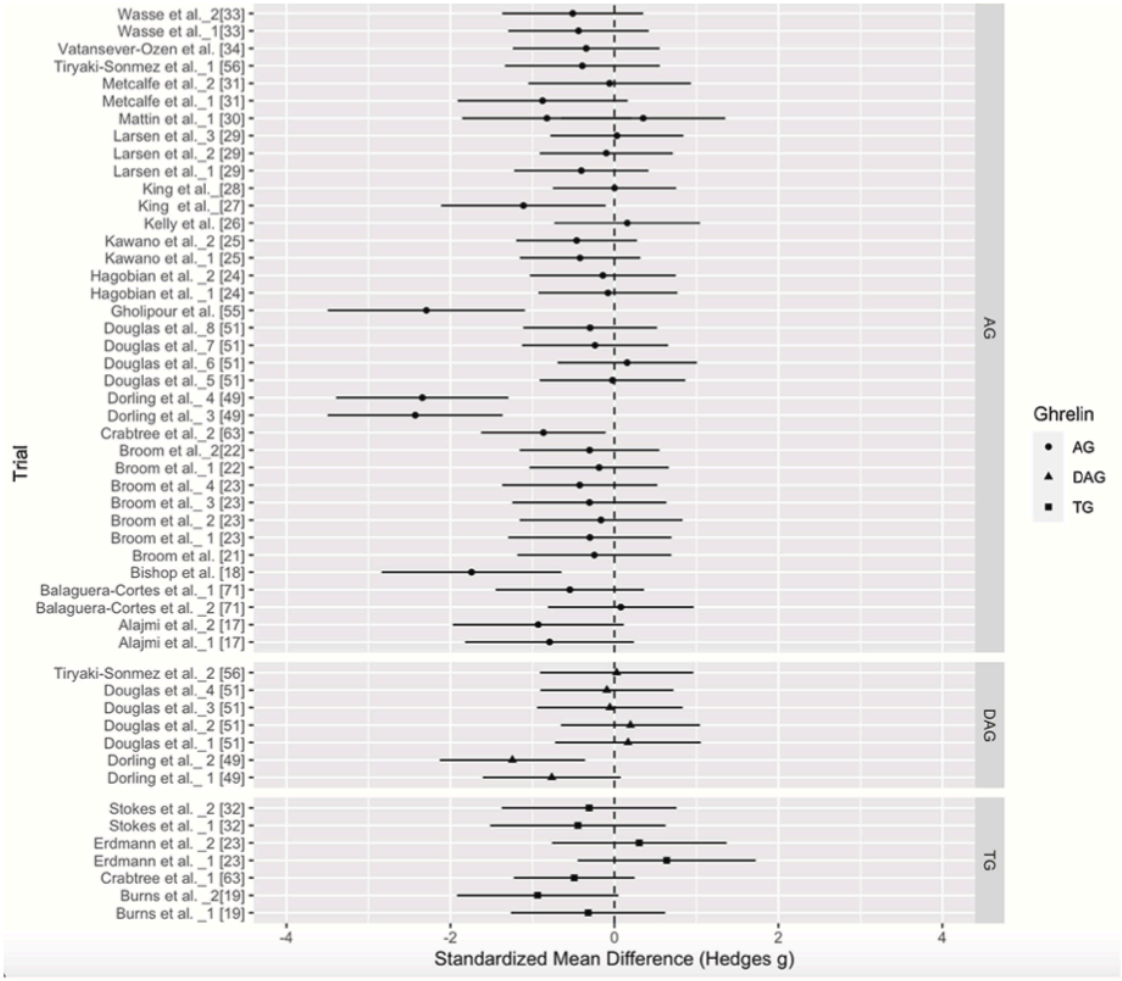


Figure 1.3. Forest Plot of the overall model sorted by ghrelin form (AG, DAG, TG)



Overall (-0.44, 95% CI: -0.65 to -0.23, p<0.01)

Figure 1.4. Cooke's Distance Plot

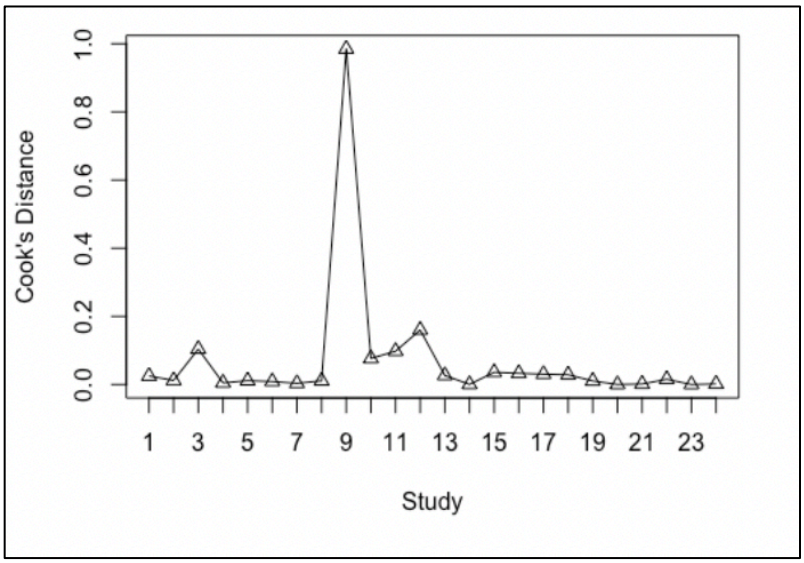


Figure 1.5. Funnel Plot of Publication Bias

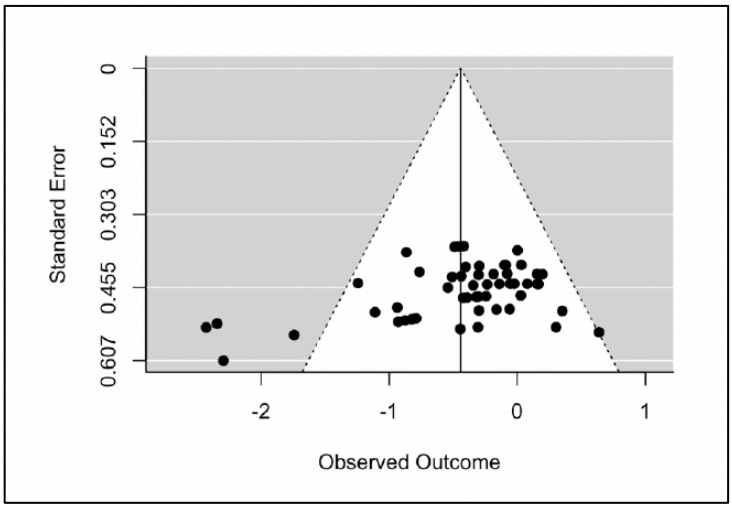
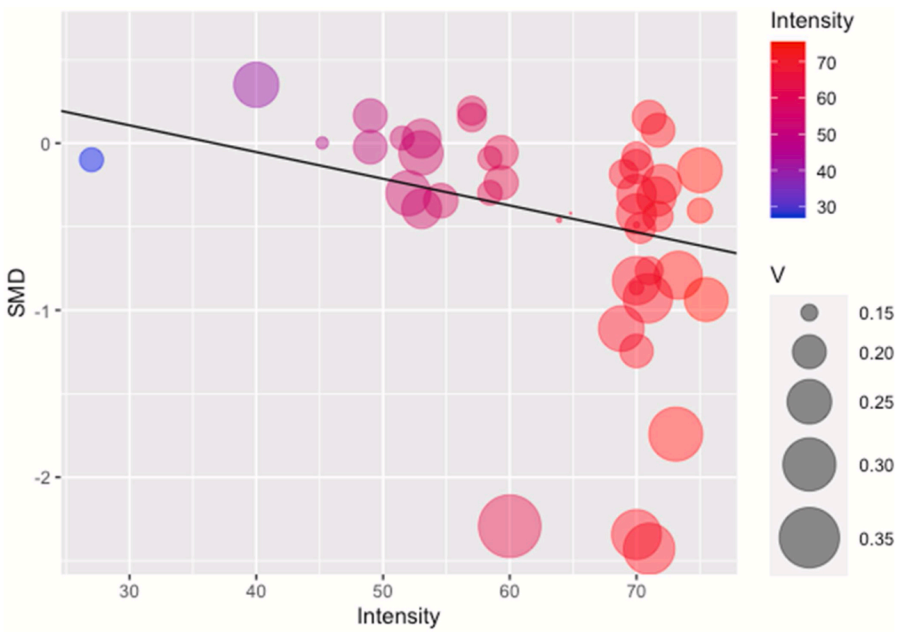


Figure 1.6. Bubble Plot of the Meta Regression of Exercise Intensity and SMD



Intensity = %VO2peak; V= Size of trial variance

SECTION III:

MANUSCRIPT II

The Effect of Exercise Intensity on Ghrelin Levels and Appetite in Individuals with Varying Levels of Body Fat

Abstract:

Background: AG has been shown to stimulate appetite in human and animal models, while DAG has been shown to either have no effect or to suppress appetite. This particular effect of ghrelin is of interest in exercise studies, as acute exercise of appropriate intensity can suppress appetite in healthy and clinical populations. However, literature on the link between exercise, appetite, and ghrelin levels is unclear. *Purpose:* to investigate the effects of exercise intensity on appetite and ghrelin levels between two distinct groups: males and females, and obese and lean individuals. *Methods:* Peak oxygen consumption (VO_{2peak}) and lactate threshold (LT) were determined via an incremental test on a cycle ergometer. Subjects had their body composition assessed via dual energy x-ray absorptiometry (DEXA) to measure total body fat percentage (BF%) and an abdominal CT scan to measure abdominal visceral fat (AVF). The testing period consisted of three randomized visits: Control (CON, no exercise), Moderate intensity exercise (MOD, power output at LT), and high intensity exercise (HIGH, power output associated with 75% of the difference between LT and peak). The caloric expenditure was kept consistent within each subject for the exercise conditions. AG, TG, DAG, and lactate were measured at the following timepoints: 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 minutes. Appetite ratings (hunger, satisfaction, fullness and desire to eat) were measured via 100 mm visual analogue scale (VAS) at timepoints 0, 60, 90, 120, 150, and 180 minutes. Subjects were split using previously determined cutoffs where females with a BF% >37.1% and males with a BF% > 25.8% were placed in the obese group (OG). We examined area under the curve and data at each individual timepoint for both TG, AG, DAG, lactate, hunger, and appetite. *Results:* Eight males (age: 42.25 ± 11.0y; BMI: 26.6 ± 5.7; VO_{2peak}: 30.65 ± 8.7 mL/kg/min) and eight females (age: 35.25 ± 11.1 y; BMI: 24.25 ± 4.7 kg/m2; VO_{2peak}:

27.65 ± 5.7 mL/kg/min) completed all measures for the study. HIGH significantly decreased all ghrelin isoforms compared to CON (all; $p < 0.05$). Females had a reduction of TG and DAG ($p<0.05$), while both males and females had a reduction of AG in HIGH compared to CON ($p<0.05$). OG had significantly decreased TG and DAG in HIGH compared to CON ($p<0.05$), and both LG and OG had suppressed AG in HIGH compared to CON ($p<0.05$). There was a significant inverse, relationship between AG/DAG and lactate ($p<0.05$). Appetite was significantly suppressed in HIGH compared to CON ($p<0.05$), and all forms of ghrelin were positively associated with appetite ($p<0.05$). *Discussion:* High intensity exercise significantly lowers plasma ghrelin levels. All isoforms of ghrelin may be associated with perception of appetite; however more work is needed to determine if the strength of such relationship differs by isoform. Our findings also suggest lactate may be involved in exercise-induced ghrelin suppression.

Introduction

Ghrelin was discovered in 1999 by Kojima and Kangawa as an endogenous ligand to the growth hormone secretagogue receptor 1a (GHSR1a)[1]. Although early work focused on ghrelin's ability to stimulate growth hormone release, ghrelin was later shown to have wide ranging biological effects in a variety of areas, such as energy balance, appetite, glucose homeostasis, immune function, sleep, and memory[2].

Ghrelin exists in two forms: acylated (AG) and deacylated (DAG), where the majority circulates as DAG (~78% of total ghrelin (TG)). The less abundant AG (~22% of TG) is the form that binds to GHSR1a, and is catalyzed by ghrelin 0-acyltransferase (GOAT)[2]. DAG, once thought to be inactive, binds to an unidentified receptor. Differentiation of the two forms is critical due to the ability of AG and DAG to act independently, antagonistically, or synergistically within the body[2].

Ghrelin and its effect on appetite highlight the divergent effects of the two forms. While AG has been shown to stimulate appetite in human and animal models[3–5], DAG has been shown to either have no effect or to suppress appetite[6,7]. This particular effect of ghrelin is of interest in exercise studies, as acute exercise of appropriate intensity can suppress appetite in healthy and clinical populations[8,9]. However, literature on the link between exercise, appetite, and ghrelin levels is unclear. This may be due several factors including; a variety of exercise doses being utilized, only AG being measured, obesity status, and different feeding states. Additionally, the populations sampled typically contain healthy, young adult individuals[10,11].

Identifying the ideal exercise dose for appetite suppression in clinical populations, such as obesity, is critical for disease management. A study by Vanderheyden et al. used sodium bicarbonate supplementation to investigate lactate's role in exercise induced ghrelin suppression. They found that the condition with higher blood lactate levels (via sodium bicarbonate) had suppressed AG levels and also decreased perception of appetite, suggesting lactate is involved in the suppression of ghrelin[12]. Therefore, exercise intensity may be key, as elevated levels of blood lactate seen during high intensities may suppress AG and appetite post exercise. A recent meta-analysis published by our group determined that exercise suppresses ghrelin levels, and that exercise intensity moderates that relationship, however most studies included in the meta-analysis utilized a moderate intensity exercise bout and included healthy populations with only males sampled[13]. In addition, there is evidence that suggests the GH and appetite response to exercise may be blunted/absent in obesity[14] and that ghrelin concentrations differ between lean and obese individuals as well as between males and females[15].

Therefore, the purpose of this study was to investigate the effects of exercise intensity on appetite and ghrelin levels between two distinct groups: males and females, and obese and lean individuals. We hypothesize that high intensity exercise will lead to the greatest alterations in ghrelin levels (i.e., decrease in AG levels) and suppression of appetite, and that obesity will blunt the ghrelin response to exercise.

Methods

Participants

Individuals between the ages of 18-55 years were recruited for this study. They were selected for screening if they were untrained, non-smoking, and weight stable (<3 kg over 3 months) and met the BMI criteria's of between 18.5-25 or and 30-40 kg/m^2. Criteria for exclusion included: history of T2DM, pregnancy/fertility treatments, disorders of the endocrine and gastrointestinal system, and/or any medications/treatments that effected the ability to safely exercise or measure hormones. Individuals arrived at the University of Virginia Clinical Research Unit (CRU) between 7 and 9 am after an overnight fast for all visits. Subjects were asked to refrain from exercise and alcohol for 24 hours, and tobacco products for 12 hours prior to each CRU admission. The study was conducted in accordance with the Declaration of Helsinki, the protocol was approved by the University of Virginia Institutional Review Board (IRB-HSR # 200241), and all subjects provided written informed consent.

Screening Period

Peak oxygen consumption (VO_{2peak}) and lactate threshold (LT) were determined via an incremental test on a cycle ergometer(Lode Model 960900). Subjects began at an initial power output of 50 W and power output was increased by 25 W every 3 minutes until volitional fatigue. Indirect calorimetry using standard open circuit spirometry (Vyntus, Viasys, Yorba Linda, CA) was used to measure oxygen consumption and carbon dioxide production (as well as to provide min-by-min kcal in order to equate caloric expenditure between the two exercise bouts). Blood drawn from an indwelling catheter placed in an antecubital vein, was sampled at the end of each stage and assayed for lactate (YSI Instruments 2900, Yellow Springs, OH, USA). The LT was determined as the power output just prior to the curvilinear increase in blood lactate and the VO_2 at this power output was chosen as VO_2 LT. The highest 1 min segment VO_2 attained was chosen as VO_{2peak}. During

the screening period, subjects had their body composition assessed via dual energy x-ray absorptiometry (DEXA, Hologic Horizon) to measure total body fat percentage (BF%) and an abdominal CT scan to measure abdominal visceral fat (AVF). AVF was measured using a low-dose scan (1 mSV or sub-mSV levels) optimized to reduce image noise using PixelShine (AlgoMedica, Inc.), a novel machine learning based algorithm, performed on a Siemens CT scanner (Siemens Somatom Force Dual Source CT, Erlagen, Germany). AVF was quantified using a machine learning-based method to segment images into regions of interest (i.e, the abdominal cavity).

Testing Period

The testing period consisted of three randomized visits: Control (CON, no exercise), Moderate intensity exercise (MOD, power output at LT), and high intensity exercise (HIGH, power output associated with 75% of the difference between LT and peak). Females were tested during the early follicular phase of the menstrual cycle, and there was a minimum of 72 hours between exercise sessions. In the 24 hours before each visit, participants were asked to standardize their diet. They filled out a diet log before the first experimental visit and were instructed to follow that log each day prior for the remaining two visits.

The caloric expenditure was kept consistent within each subject for the exercise conditions. At each visit, subjects were observed for three hours (Figure 2.1.). An indwelling catheter was inserted into the antecubital vein and blood was sampled at baseline, every 10 mins for the first hour, and then every 30 mins for the remaining two hours (Figure 2.1.) to measure TG, AG, DAG, and lactate. The aforementioned biomarkers were measured at the following timepoints: 0, 10, 20,

30, 40, 50, 60, 90, 120, 150, and 180 minutes. Appetite ratings (hunger, satisfaction, fullness and desire to eat) were measured via 100 mm visual analogue scale (VAS) at timepoints illustrated in Figure 1 (0, 60, 90, 120, 150, 180 minutes). The VAS is composed of a series of identical lines with anchors on the end of each line (i.e. 'I have never been more hungry'/ 'I am not hungry at all'). Subjects make a mark along the line depending on how close they are to each anchor to quantify their feelings. A score for each rating (hunger, satisfaction, fullness, and desire to eat) is calculated by measuring the distance from the left end of the line to the mark[16].

Biochemical Analyses

Blood lactate was immediately analyzed (YSI Instruments 2900). Blood to measure TG, AG, and DAG was collected in EDTA vacutainers containing protease inhibitor AEBSF and were centrifuged for 10 minutes at 3,000 rpm at 4ºC. Hydrochloric acid 1N was added to the plasma aliquots immediately after centrifugation. Plasma ghrelin was stored at -80ºC for later analysis. Ghrelin was analyzed using Bertin Pharma ELISA kits by University of Virginia Center for Research in Reproduction, Ligand Assay and Analysis Core. All timepoints were run in duplicate. Area under the curve (AUC) was calculated for TG, AG, DAG, and lactate (LAC) from each testing visit via the trapezoidal method.

Statistics

Based on previous literature[17] , assuming a power of 80% for an ANOVA with significance of $\alpha = 0.05$, an adequate sample size of n=8 per group was determined *a priori* to assess group differences between acute exercise and ghrelin levels. Power calculations were made with G*Power version 3.1. Data was analyzed via R (Version 4.0.2). To examine percent body fat (BF%- obese vs lean)

subjects were split using previously determined cutoffs from Macek et al., where females with a BF% >37.1% and males with a BF% > 25.8% were 2-4 higher times more likely to develop a cardiovascular risk factor[18] (Obese Group, OG).

Baseline comparisons were evaluated using independent sample t-tests, and normality was assessed using Q-Q plots and the Shapiro-Wilk tests. We examined area under the curve and data at each individual timepoint for both TG, AG, DAG, lactate, and appetite. A total appetite score at each timepoint was calculated with the following formula:

APP = Desire to eat + Hunger + (100 − Fullness) +(100-Satisfaction))/4

Total appetite scores (APP_{AUC}) and individual hunger (HUN_{AUC}) scores were then used to calculate AUC using the trapezoidal method. Several linear mixed models were used examine the ghrelin response to exercise. For each timepoint ($LAC_{TIMEPOINT}$, $APP_{TIMEPOINT}$, $TG_{TIMEPOINT}$, $AG_{TIMEPOINT}$, $DAG_{TIMEPOINT}$) the following models were examined: Subject as a random factor and sex (male or female), condition, time, and body fat (BF) group ("Lean" or "Obese") were fixed factors. For AUC (LAC_{AUC}, APP_{AUC}, TG_{AUC}, AG_{AUC}, DAG_{AUC}) data: Subject was a random factor and sex, condition, and BF group were fixed factors. Satterwaite's approximation was utilized to determine significance. F tests of nested models were used to determine differences in fixed effects. Estimated marginal means (EMM) were utilized to estimate the means that were adjusted for the factors in each model. Associations were determined using spearman rank correlations if data was not normal and/or relationship between variables of interest was not linear, and Pearson product

moment correlations were utilized in normal and linearly related data. Significance was set a-priori as $p < 0.05$. Data are reported as mean ± SD unless otherwise noted.

Results

Eight males (age: 42.25 ± 11.0y; BMI: 26.6 ± 5.7; VO_{2peak}: 30.65 ± 8.7 mL/kg/min) and eight females (age: 35.25 ± 11.1 y; BMI: 24.25 ± 4.7 kg/m2; VO_{2peak}: 27.65 ± 5.7 mL/kg/min) completed all measures for the study. Baseline differences between sex are shown in Table 2.1**.** There were significant differences in AG (p=0.001), DAG (p=0.001), and TG (p=0.002), with females having higher levels of all isoforms compared to males. Males in HIGH had a higher energy expenditure (p=0.045) and AVF (p=0.006) than females in HIGH. Baseline differences between BF% group are shown in Table 2.2. There were significant differences in BMI, BF%, and VO_{2peak} between Lean (LG) and Obese (OG). OG had lower VO_{2peak} (p=0.02), but higher BF% (p=0.03) and BMI (p=0.03). There were no differences observed for baseline TG, AG, DAG, HUN, APP, or LAC between experimental conditions.

Total Ghrelin

Due to supply chain issues for the ELISA kits, results for TG and DAG include 10 subjects (4 males, 6 females). The TG_{AUC} model revealed a significant main effect for condition (p=0.005), and a significant interaction effect for condition and sex (p=0.04, Figure 2.2.). HIGH had lower TG levels than MOD and CON (p<0.01). The EMM contrasts for the interaction showed that the females had lower TG levels in HIGH compared to MOD and CON (both; p<0.05). There was a trending difference between the CON levels of TG between males and females (p=0.06).

The model for $TG_{TIMEPOINT}$ showed a significant main effect for condition ($p<0.0001$) and time ($p=0.007$), and significant interaction effects for condition and sex, and condition and BF% (both; $P<0.0001$, Figure 2.3.). For the main effect of condition, TG levels was 47.3 pg/mL lower in HIGH than CON, and 60.7pg/mL lower in HIGH than MOD (both; $p<0.0001$). Regardless of condition, TG levels were elevated at 10 minutes compared to 90 minutes ($p<0.05$). With the condition and sex interaction, TG levels decreased in HIGH compared to CON and MOD in females (both; $p<0.0001$). For males , TG levels were decreased in CON compared to MOD ($p<0.05$), and increased in MOD compared to HIGH ($p=0.01$). Concerning the condition and BF% comparison, those in OG had significantly lower TG levels in HIGH compared to CON, and HIGH compared to MOD (both; $p<0.0001$). There were no differences in conditions within the Lean group.

Acylated Ghrelin

The model for AG_{AUC} (Figure 2.4.) revealed a significant main effect for condition ($p<0.0001$), and a significant interaction for condition and sex ($p<0.05$). EMM contrasts showed that AG in CON and MOD were greater than AG in HIGH (both; $p<0.0001$). Regarding the interaction within females, AG in HIGH was lower than CON and MOD (both; $p<0.0001$). No condition was significantly different within males.

$AG_{TIMEPOINT}$ (Figure 2.5.) had a significant main effect for condition and time. Significant interactions included condition and time, condition and sex, and condition and BF% group. For condition, AG in HIGH was less than CON and MOD (both; $p<0.0001$). Overall, baseline AG was higher than the 40, 50, and 90-minute timepoints regardless of condition (all; $p<0.01$). The

condition and time interaction showed AG in HIGH being significantly lower than CON at 30, 40, 50, and 60 minutes, and lower than MOD at 20, 30, 40, 50, and 60 minutes (all; $p<0.01$). AG levels in females during HIGH was significantly lower than MOD and CON (both, $p<0.0001$). For males, the AG HIGH was also significantly lower than MOD and CON (both; $p=0.01$). The condition by BF% group showed that OG and LG had higher AG levels in the MOD and CON than HIGH (all; $p<0.0001$).

Deacylated Ghrelin

The DAG_{AUC} exhibited a significant main effect for condition ($p<0.0001$, Figure 2.6.). EMM contrasts showed that DAG in CON ($p<0.01$) and MOD ($p<0.05$) were greater than HIGH.

The $DAG_{TIMEPOINT}$ had a significant main effect for condition and time. Significant interactions include condition and time, condition and sex, and condition and BF% (Figure 2.7.). For condition, DAG in HIGH was less CON and MOD (both; $p<0.0001$). Overall, DAG levels at 10 minutes were higher than the 60 and 90-minute timepoints ($p<0.05$). For the condition and time interaction, DAG levels in HIGH were significantly lower than MOD at 50 and 60 minutes ($p<0.05$). With the condition and sex interaction, females during HIGH had DAG levels less than in the CON and MOD conditions (both; $p<0.0001$). Males during CON exhibited DAG levels less than MOD, and DAG levels less in HIGH than MOD (both; $p=0.05$). Lastly, OG significantly lower DAG levels in HIGH compared to CON and MOD (both; $p<0.0001$). There were no differences within LG by condition.

Appetite

The model for APP_{AUC} had no significant main or interaction effect (p=0.18-0.85). The $APP_{TIMEPOINT}$ model had significant main effects for condition (p=0.0007), and time (p<0.0001). $APP_{TIMEPOINT}$ (Figure 2.8.) in HIGH was lower than MOD and CON (p<0.05). Baseline APP levels were significantly lower than at 90, 120 (p<0.0001), 150 ,and 180 minutes (all; p<0.0001). There was a trending interaction of condition and sex (p=0.06).

Hunger

The HUN_{AUC} model had no significant main or interaction effects (all; p>0.05). The $HUN_{TIMEPOINT}$ (Figure 2.9.) model had a significant main effect for condition and time (both; p<0.0001). HIGH had hunger scores that were lower than CON and MOD (both; p<0.0001). Hunger scores were higher at all timepoints compared to baseline regardless of condition (all; p<0.005). There was also a significant condition and sex (p=0.001) and BF% and time (p=0.01) interactions. Females had significantly lower hunger scores in HIGH compared to MOD and CON (both; p<0.0001). There were no significant differences across any condition within males. Those in the LG group had significantly higher scores at 90 (p=0.001), 120, and 150 (p<0.001) minutes compared to baseline (all;p<0.01). Individuals in the OG group had significantly higher hunger scores at 120, 150, and 180 minutes compared to baseline (all; p<0.0001).

Lactate

There was a significant main effect for condition in the LAC_{AUC} model, where HIGH LAC_{AUC} was higher than MOD and CON (both; p<0.0001).The $LAC_{TIMEPOINT}$ model (Figure 2.10.) revealed significant main effects for condition and time, and significant interactions for condition and time,

and condition and BF% group (all; $p<0.0001$). For the condition main effect, HIGH>MOD>CON (all; $p<0.0001$). For the time main effect, baseline values were significantly lower than timepoints 10-60 minutes (all; $p<0.0001$). For the interactions, HIGH had greater lactate levels compared to CON from timepoints 10-90 minutes (all; $p<0.0001$). MOD had lower lactate levels compared to HIGH at timepoints 20-40 and 90 minutes (all; $p<0.05$), and MOD had higher lactate levels compared to CON at timepoints 20-40 (all;$p<0.01$). The condition by BF% group showed that those in OG and LG groups had higher lactate levels in HIGH compared to CON and MOD (all;$p<0.0001$). There was also a significant difference between the BF% group in HIGH, where OG had lower lactate levels than LG ($p<0.05$).

Correlations

Correlations for AUC data are in Table 2.3. APP_{AUC} was associated with AG_{AUC}, DAG_{AUC}, but not TG_{AUC} (Figure 2.11., $p<0.05$). HUN_{AUC} was associated with AG_{AUC} only ($p=0.001$, Figure 2.12.). There was a trending inverse association between AG_{AUC} and LAC_{AUC} ($p=0.07$, Figure 2.13), while there were significant inverse associations between LAC and DAG_{AUC} and TG_{AUC} ($p<0.05$). Correlations for timepoint data are in Table 2.4. APP and HUN were associated with all isoforms of ghrelin ($p<0.05$). LAC was also inversely associated with all forms of ghrelin ($p<0.05$).

Discussion

The major findings of the present study were that exercise intensity, sex, and obesity level modulate plasma ghrelin levels and affect appetite and hunger. In line with our hypothesis, high intensity exercise transiently suppressed TG, AG, and DAG plasma levels. The finding that ghrelin

is transiently suppressed by exercise is reinforced by numerous other studies, however most applied a moderate intensity exercise stimulus and measured AG[19]. In contrast, we found that moderate intensity exercise did not alter ghrelin levels compared to the control condition. As our protocol utilized blood lactate to determine exercise intensities, these findings suggest that exercise above the lactate threshold may be necessary to illicit a suppression in ghrelin. This is supported by prior work which has shown highly enriched lactate receptors within the gastric fundus[20] and the ability of these receptors to block ghrelin secretion from the cells within the stomach via g-coupled receptor GPR81[21]. It is important to note that both TG and AG have been shown to be reduced following lactate-mediated GPR81 signaling[21], which may suggest a reduction in acylation; more work is needed to explore the activity of GOAT within this mechanism. However, it does not appear that lactate is the primary driver of suppressed ghrelin as we report a modest inverse correlation between DAG and LAC, and a small inverse correlation between AG and LAC.

We also report a difference in the ghrelin response to exercise between males and females. Only females had a reduction of TG and DAG with high intensity exercise, however both sexes had a reduction in AG. Although there was a difference in sample size when measuring TG/DAG and AG, this may suggest that there are sex differences in ghrelin release and/or degradation. A sexual dimorphism has been previously reported regarding ghrelin levels, with females having higher DAG and AG levels than males, regardless of obesity status[17,22]. We found that females had higher baseline levels of TG, AG, and DAG compared to their male counterparts, with overall BMI and age being similar between groups. Studies have shown that estrogen can alter circulating ghrelin levels, however results are conflicting. Estrogen has been shown to either upregulate ghrelin levels, decrease TG levels in post-menopausal women undergoing estrogen replacement therapy, or

decrease AG following ovariectomy in rats[23]. Moreover, literature suggests females have larger changes to energy-related hormones in response to exercise than men, although data specifically on ghrelin is scarce and mostly measures AG[22]. Although the mechanism behind this sex difference is currently unclear, the present data strengthen the need for more work on the sex differences between gut hormones in response to exercise.

Ghrelin has been suggested to modulate body weight control via a negative feedback loop[24], and AG levels have been found to be increased, while DAG and TG plasma levels are reduced in individuals with obesity[15,25]. Although there was no difference in baseline ghrelin levels between BF% groups (Table 2.2.) we did find a differential response to the exercise conditions. Only those in the OG had suppressed TG and DAG within the high intensity condition, while both OG and LG had a suppression of AG following high intensity exercise. An explanation for our results is unclear. Differences in fitness cannot be ruled out, as that was significantly different between groups. In addition, we used total BF% to separate subjects into each group. Visceral adiposity, which has been negatively associated with TG[26] and DAG levels[27], may have provide a partial explanation. Although, there was not a significant difference in AVF between our lean and obese groups, r males had significantly elevated levels of AVF compared to females. We found a significant, negative, correlation between AVF and TG_{AUC}, and a negative trending relationship with DAG_{AUC}. In contrast, we report a trending, positive correlation between AVF and AG_{AUC}. The present data indicate that only females (with lower AVF) had a suppression of TG and DAG in response to high intensity exercise. The present results are in partial agreement with data showing a significant, positive relationship between DAG and AVF or WC[28] and no relationship between AG and AVF[27]. Differences in results may be due to body composition methodology, sample

characteristics, and/or sample size, where we characterized AVF using CT Scans, and the other studies used bioelectrical impedance analysis in individuals with T2DM or waist circumference measurements [27,28].

Exercise intensity altered the subjective perception of appetite in our cohort, with no sex difference. However, when examining hunger, we did find that only females had a significant decrease in their hunger score during the high intensity condition. This is in disagreement with other literature suggesting that there are no differences in the perception of appetite/hunger between males and females[29–31]. Data also suggests exercise differentially effects appetite based on obesity status, where the decrease in appetite/hunger is blunted compared to lean individuals [9,32]. A possible explanation for our findings between sex may be due to males having higher levels of AVF in our sample, as AVF has been associated with the perception of hunger[33]. Furthermore, our obese group did not have a significantly different amount of AVF than our lean group, which may have contributed to the lack of difference in hunger/appetite perception.

Ghrelin has been found to exhibit a diurnal variation, peaking before mealtimes and falling after eating[34]. Additionally, hunger and ghrelin were first linked when one of the first studies that investigated a TG infusion in humans reported hunger as a side effect, and many studies replicated this result[34–36]. Together, these data support the relationship between ghrelin and appetite. We found appetite to be moderately associated with all isoforms of ghrelin. The relationship between hunger and ghrelin levels in our study were in the same direction as appetite, but weaker across all forms. This suggests ghrelin may be involved in both satiety and hunger signals, due to our appetite measure using both domains within the calculation. GHSR1a receptors, which are utilized by AG,

are found in the hypothalamus , yet there is no known evidence of DAG receptors. Therefore, AG may be the only isoform that interacts with the appetite center in the brain. However, limited evidence suggests that DAG may influence hunger by blocking AG-induced hypothalamic neuronal activity involved in appetite and food intake[38,39]. Our results mirror other exercise studies showing an increase in both TG and AG along with appetite, yet weak or non-significant correlations[40–42]. There are likely multiple mechanisms that regulate appetite and ghrelin levels that are not captured in the present study.

There are limitations of this study. We did not match for sex within our obese and lean groups; therefore, we cannot rule out the influence of sex differences on our results within each BF group. Additionally, our ability to assess DAG and TG may be underpowered, due to our sample size of ten subjects. As our protocol included an overnight fast for each visit, real world application is weakened as most individuals consume mixed meals before and/or after exercise. Therefore, future work should examine the impact of meal content on each ghrelin isoform in response to exercise. Lastly, although our appetite and hunger measures were not different between groups at baseline, 44% of our sample had poor intra-rater reliability when answering the VAS, which could have impacted our results.

Collectively, the results of the present study strengthen data on the role of high intensity exercise in the reduction of appetite. All isoforms of ghrelin may be associated with perception of appetite; however more work is needed to determine if the strength of such relationship differs by isoform. Our findings also suggest lactate may be involved in exercise-induced ghrelin suppression. This response may differ based on sex and AVF, with females not only having higher baseline ghrelin

levels and lower AVF, but responding differently to exercise based on isoform. Sex needs to be taken into account when designing an exercise intervention, along with measuring all ghrelin isoforms to provide a more comprehensive physiological response. Future work should focus on whether a chronic training program at differing exercise intensities mirror these results, to allow for the development of precision exercise prescription designed to aid in reducing and/or preventing obesity and its related complications.

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Table 2.1. Demographics of study sample between males and females.

	Males		Females		P-value
N	8		8		-
Age (years)	42.25 ± 10.9		35.25 ± 11.1		0.2
BMI (kg/m2)	26.6 ± 5.7		24.25 ± 4.7		0.4
BF (%)	28.4 ± 7.8		34.9 ± 4.4		0.06
AVF (cm^2) *	417.15 ± 85.6		349.4 ± 117.2		0.006
VO_{2peak} (ml/kg/min)	30.65 ± 8.2		27.65 ± 5.7		0.4
Baseline TG (pg/mL)*	130.6 ± 32.3		231.6 ± 97.6		0.001
Baseline AG (pg/mL)*	66.2 ± 29.6		114.3 ± 57.0		0.001
Baseline DAG (pg/mL)*	81.6 ± 24.3		137.6 ± 57.1		0.002
Baseline Hunger	34.4± 22.7		41.1 ± 22.3		0.31
Baseline Appetite	55.6 ± 17.3		63.3 ± 13.4		0.09
Exercise Sessions	Mod	High	Mod	High	
Duration (min)†	50:52± 0.4	33:54± 0.4	53:14± 0.1	33:00± 0.2	
Energy Expenditure (kcal)	299.9 ± 46.1	296.5 ± 48.4††	251.4± 48.2	243.0± 45.4††	
Average HR (bpm)†	115.9 ± 12.65	157.7 ± 24.08	123.3 ± 22.6	170.6.± 18.7	

*differences between males and females , †, differences between moderate and high intensity within the same group , †† difference groups within the same intensity, p≤ <0.05

Table 2.2. Demographics of study sample between Lean and Obese

	Lean		Obese		P-value
N (F)	8 (5)		8 (3)		-
Age (years)	37.75 ± 12.9		39.75 ± 10.1		0.7
BMI (kg/m2)*	22.6 ± 0.9		28.3 ± 6.2		0.03
BF%*	28.1 ± 6.5		35.2 ± 5.9		0.03
AVF (cm^3)	383.4 ± 131.6		379.6 ± 132.7		0.90
VO_{2peak} (mL/kg/min) *	33.2 ± 5.8		25.1 ± 6.6		0.02
TG (pg/mL)	183.8 ± 60.0		191.7 ± 110.8		0.8
AG (pg/mL)	100.7 ± 58.1		80.1 ± 42.4		0.2
DAG (pg/mL)	113.8 ± 40.4		113.5 ± 43.2		1.0
Baseline Hunger	34.4± 23.2		42.1 ± 17.1		0.22
Baseline Appetite	56.3 ± 21.4		63.3 ± 13.5		0.12
Exercise Sessions	Mod	High	Mod	High	
Duration (min)†	54:02± 0.2	32.41± 0.2	50:04 ± 0.4	34:11± 0.4	
Energy Expenditure (kcal)	284.25± 48.8	280.6 ± 51.5	267.0± 56.95	263.6± 56.6	
Average HR (bpm)†	123.9 ± 20.3	173.0 ±8.3	115.4 ± 15.7	155.9 ± 26.8	

*differences between OG and LG , †, differences between moderate and high intensity within the same group , †† difference groups within the same intensity, p≤ <0.05

Table 2.3. Correlations between AUC data.

	TG	AG	DAG	APP	HUN	LAC	VO_{2peak}
TG	**-**	**0.92***	**0.95***	**0.66***	0.23	**-0.42***	-0.14
AG	**0.92***	**-**	**0.76***	**0.67***	**0.53***	**-0.29^**	-0.06
DAG	**0.95***	**0.76***	**-**	**0.52***	0.19	**-0.47***	0.02
APP	**0.66***	**0.67***	**0.52***	**-**	**0.69***	-0.20	-0.10
HUN	0.23	**0.53***	0.19	**0.69***	**-**	-0.25	-0.03
LAC	**-0.42***	**-0.29^**	**-0.47***	-0.2	-0.25	**-**	0.01
VO_{2peak}	-0.14	-0.06	0.02	-0.10	-0.03	0.01	**-**
BF%	**0.44***	**0.26^**	**0.36^**	0.27	0.16		
AVF	**-0.49***	**0.26^**	**-0.39^**	0.16	0.18		

* denotes significance ($p<0.05$), ^, denotes trending significance

Table 2.4. Correlations between timepoint data.

	TG	AG	DAG	APP	HUN	LAC
TG	**-**	**0.91***	**0.94***	**0.44***	**0.21***	**-0.33***
AG	**0.91***	**-**	**0.72***	**0.49***	**0.36***	**-0.24***
DAG	**0.94***	**0.72***	**-**	**0.36***	**0.18***	**-0.30***
APP	**0.44***	**0.49***	**0.36***	**-**	**0.82***	-0.21
HUN	**0.21***	**0.53***	**0.19***	**0.82***	**-**	-0.20
LAC	**-0.33***	**-0.24**	**-0.30***	-0.20	-0.21	**-**
BF%@	**0.66***	0.31	0.56	0.02	0.11	
AVF	-0.70	-0.08	-0.57	0.04	0.03	

@ denotes a correlation between baseline data, as body composition was not measured over time. * denotes significance ($p<0.05$), ^ denotes trending significance

Figure 2.1. Outline of Testing Visit Measurements

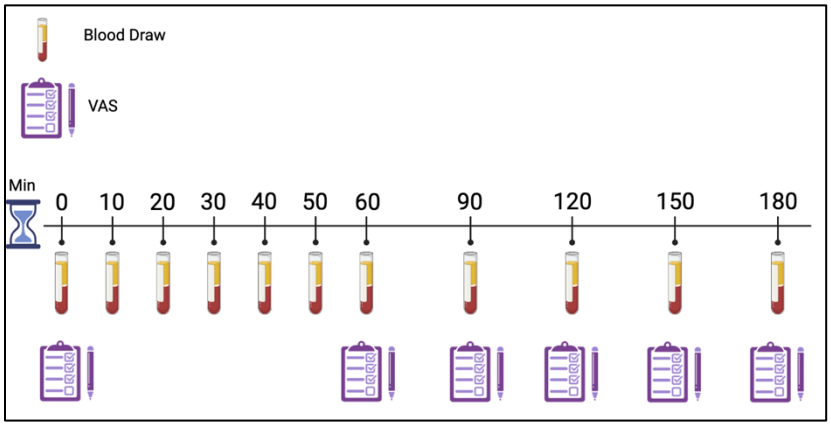
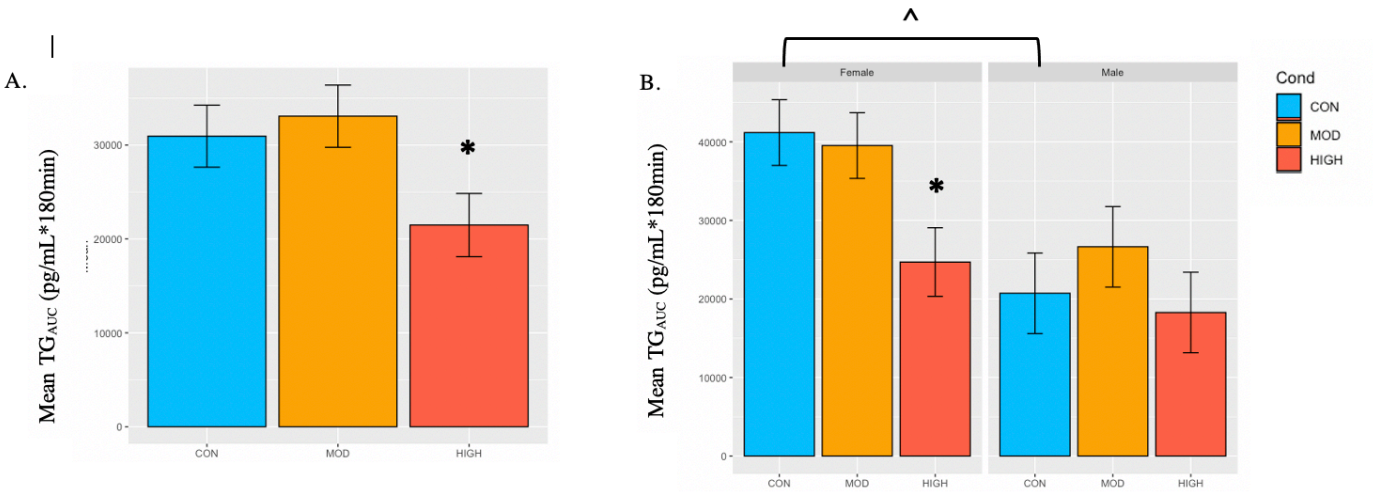
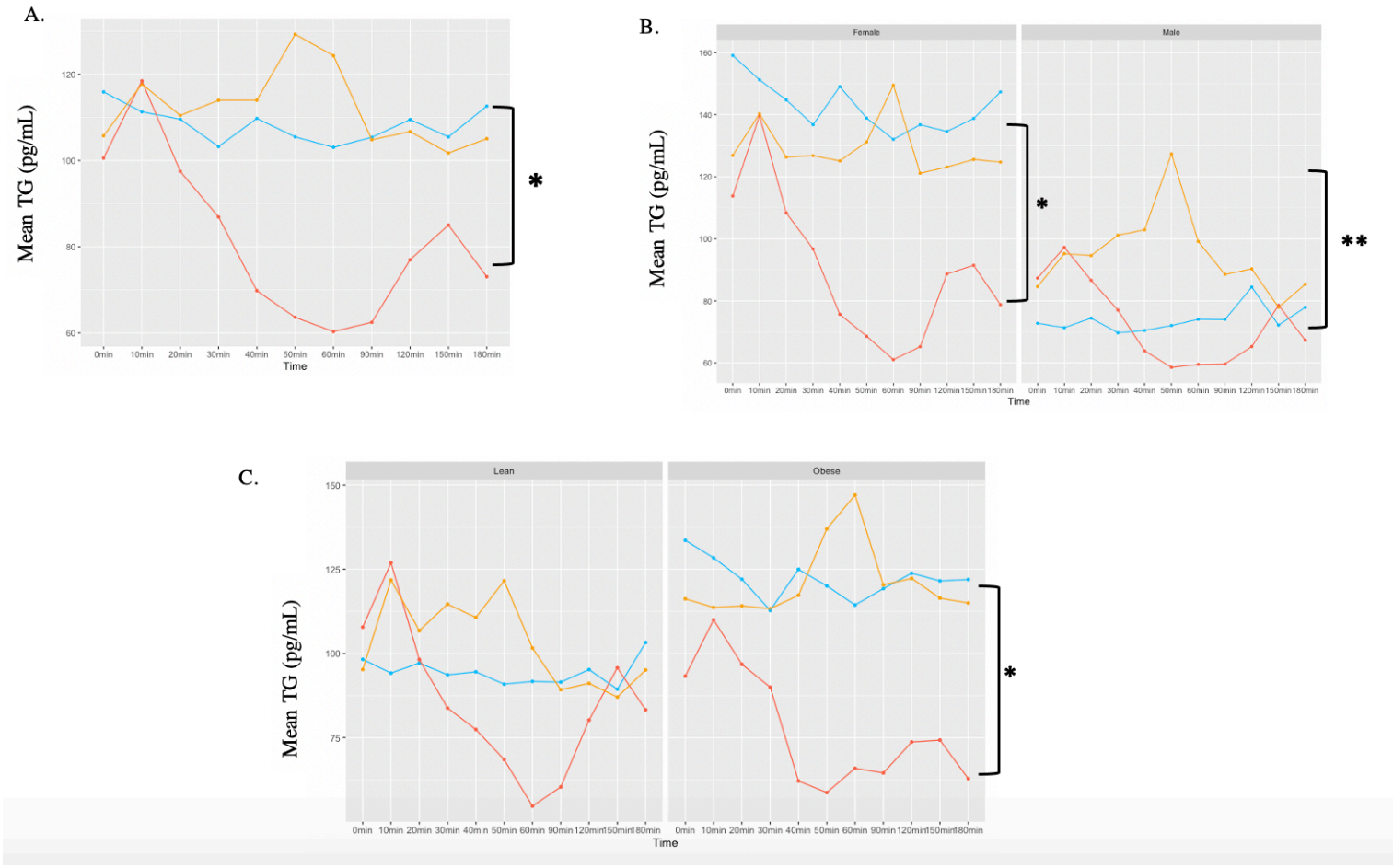


Figure 2.2. Effect of Exercise Intensity on TG_{AUC} by Condition (A) and Condition and Sex (B).



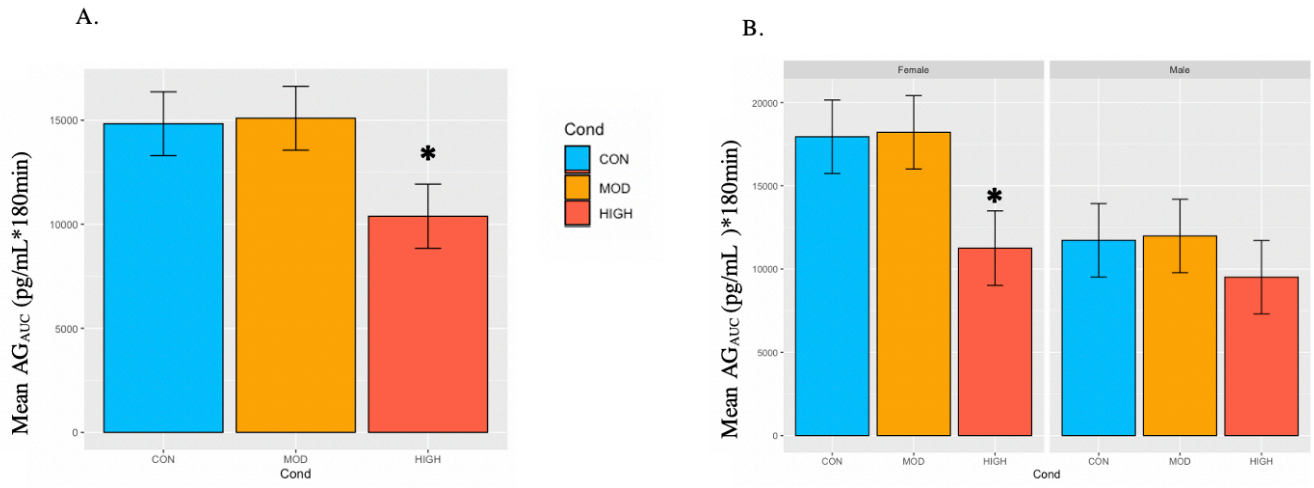
Data are mean ± SE. *Denotes significant effect of condition compared to CON ($p<$ 0.05), ^ denotes trending significance (p=0.06)

Figure 2.3. Effect of Exercise Intensity on $TG_{TIMEPOINT}$ by Condition (A) ,Condition and Sex (B), and Condition and BF (C).



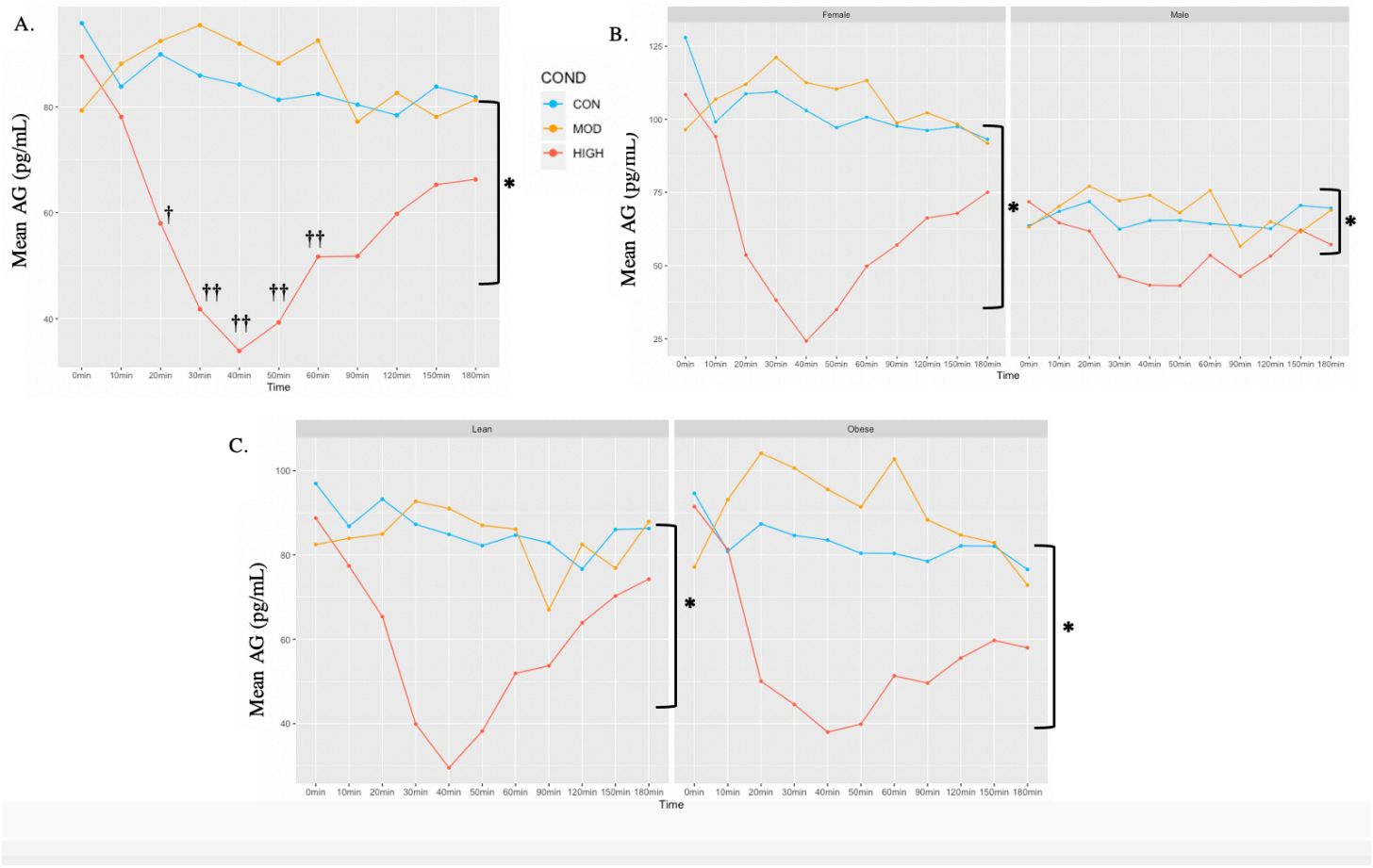
Data are mean ± SE. * Denotes significant effect of HIGH compared to MOD and CON, ** denotes significant effect of MOD compared to CON and HIGH ($p < 0.05$)

Figure 2.4. Effect of Exercise Intensity on AG_{AUC} by Condition (A) and Condition and



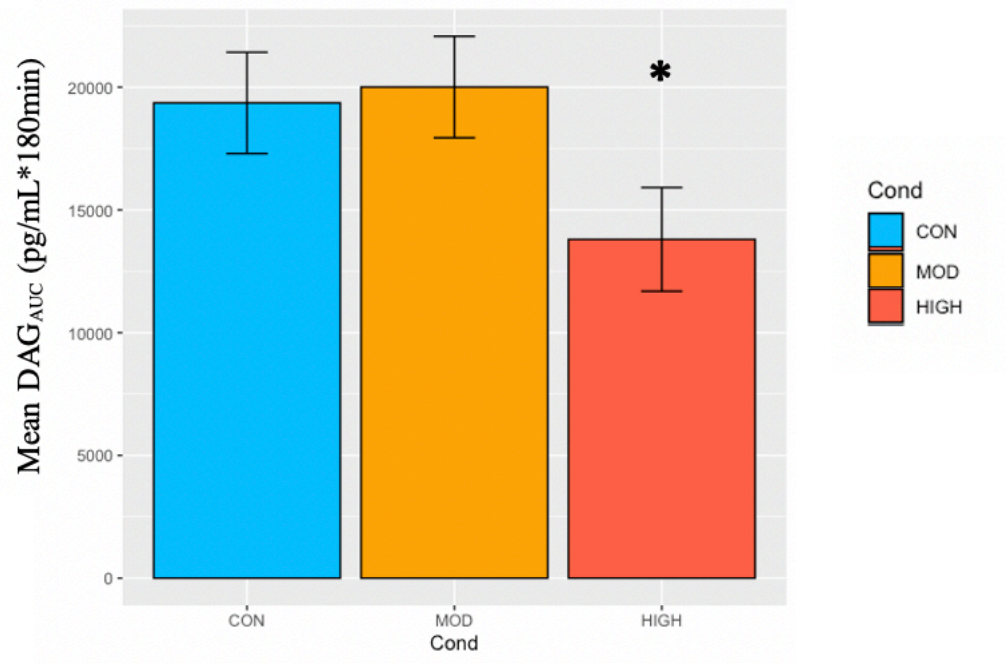
Sex (B). Data are mean ± SE. *Denotes significant effect of condition compared to CON ($p < 0.05$)

Figure 2.5. Effect of Exercise Intensity on $AG_{TIMEPOINT}$ by , Condition and Time (A), Condition and Sex (B), and Condition and BF (C).



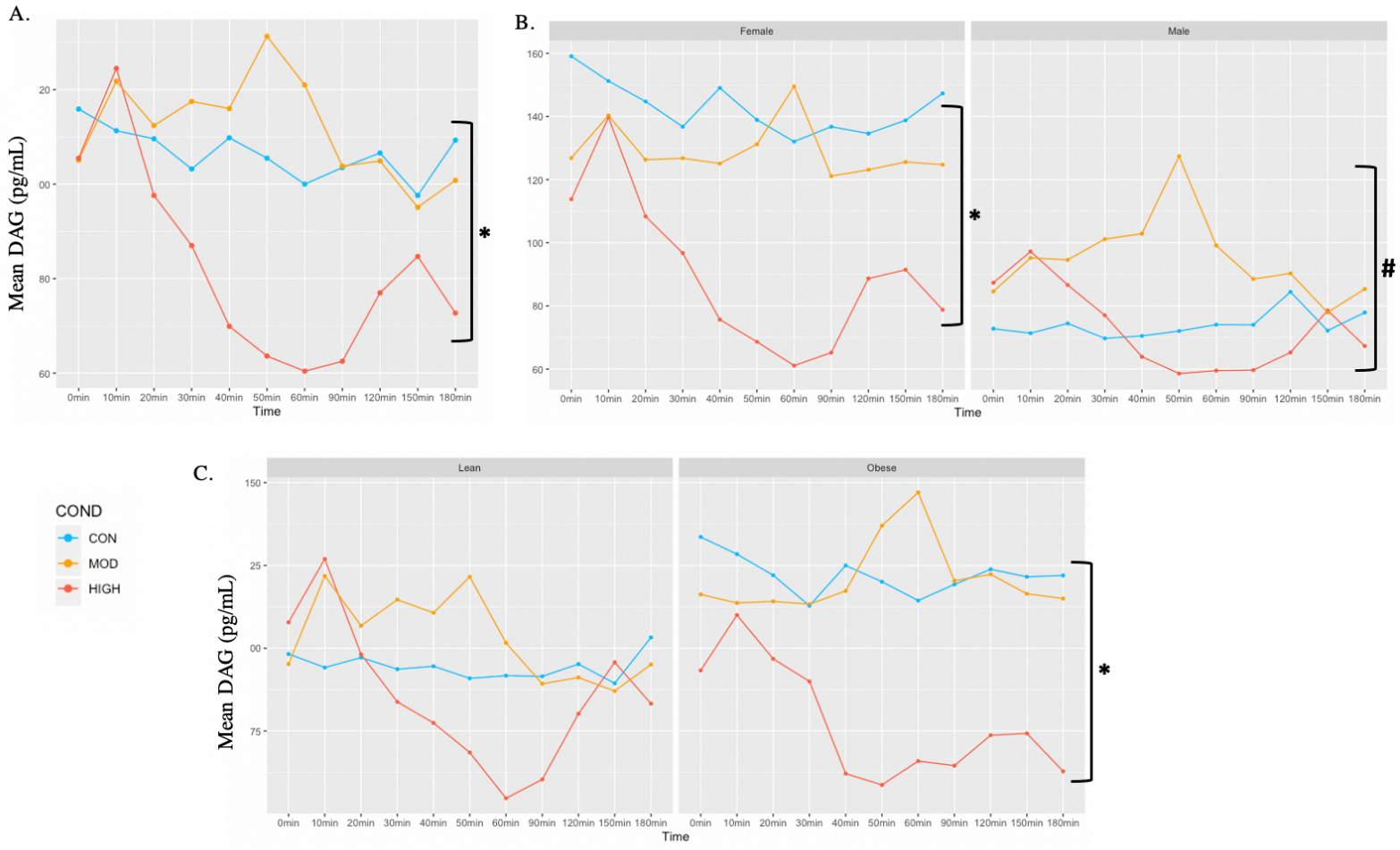
Data are mean ± SE. *Denotes significance ($p < 0.05$). † Denotes significant time effect compared to baseline

Figure 2.6. Effect of Exercise Intensity on $DAG_{AUC.}$



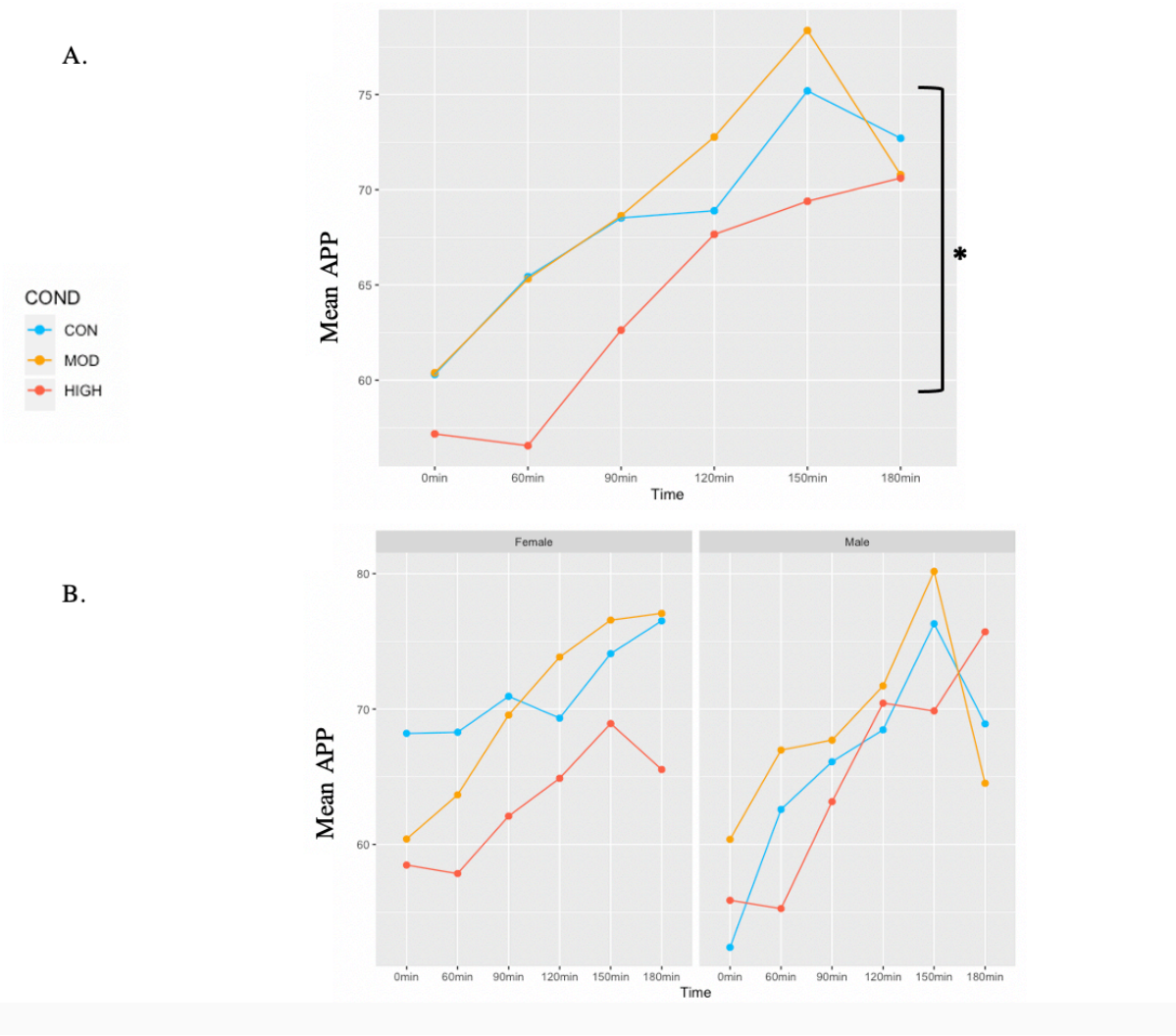
*Denotes significant effect of condition ($p < 0.05$).

Figure 2.7 . Effect of Exercise Intensity on $DAG_{TIMEPOINT}$ by Condition (A) , Condition and Time (B), Condition and Sex (C), and Condition and BF (D).



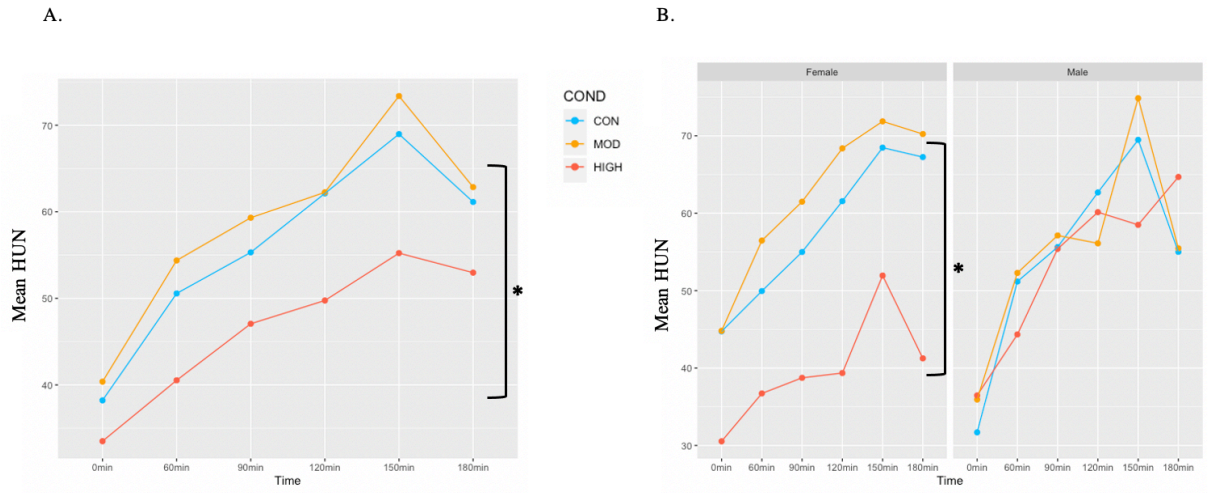
Data are mean ± SE. * Denotes significant effect of HIGH compared to MOD and CON ($p < 0.05$), # denotes a significant effect of MOD compared to HIGH and CON

Figure 2.8. Effect of Exercise Intensity on $APP_{TIMEPOINT}$ by Condition (A) and Condition and Sex (B).



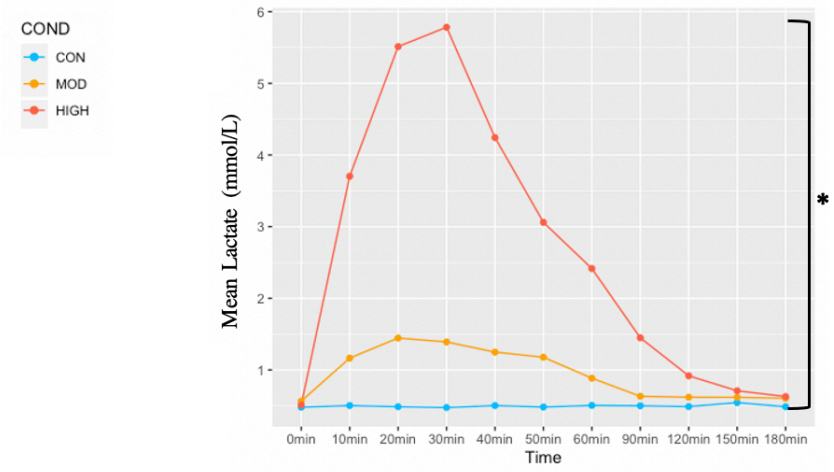
Data are mean ± SE *Denotes significant effect of HIGH compared to MOD and CON ($p < 0.05$).

Figure 2.9. Effect of Exercise Intensity on $HUN_{TIMEPOINT}$ by Condition (A) , Condition and Sex (B).



Data are mean ± SE *Denotes significant effect of HIGH compared to MOD and CON ($p< 0.05$).

Figure 2.10. Effect of Exercise Intensity on $LAC_{TIMEPOINT}$ between Condition.



Data are mean ± SE. * denotes significant effect of HIGH compared to MOD and CON ($p<0.05$)

Figure 2.11. Association between APP_{AUC} and TG_{AUC} (A), AG_{AUC} (B), DAG_{AUC} (C). Blue line represents slope, shaded area represents confidence interval.

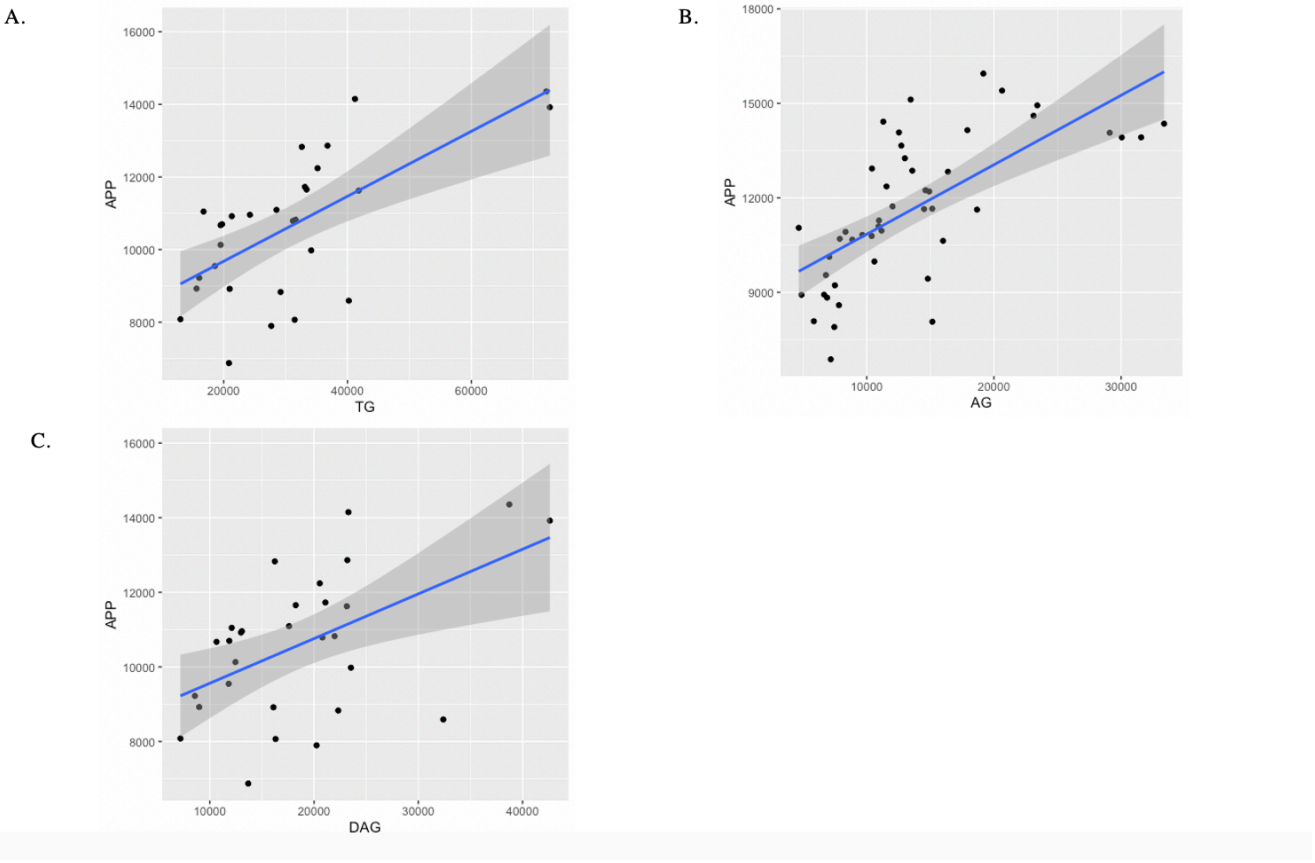
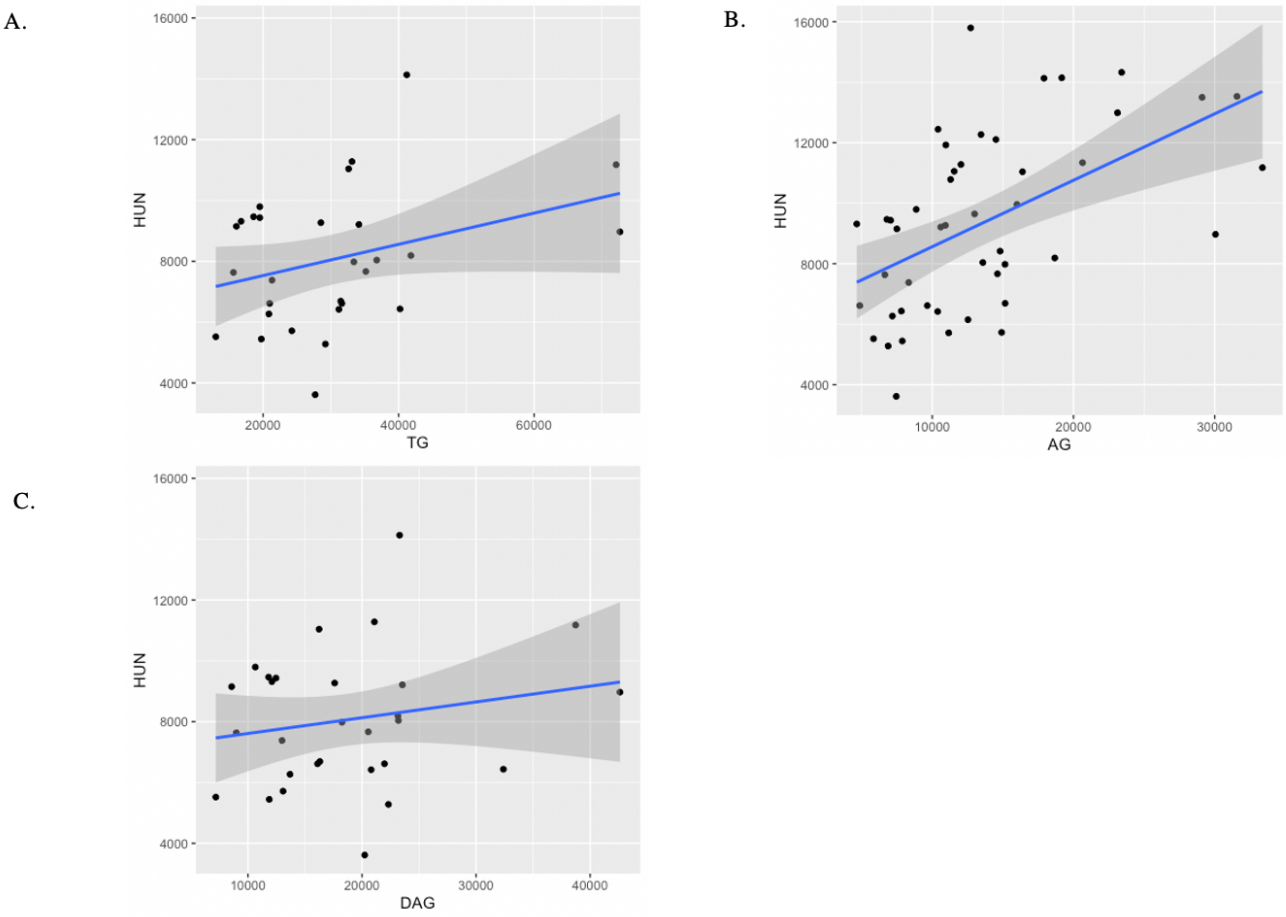
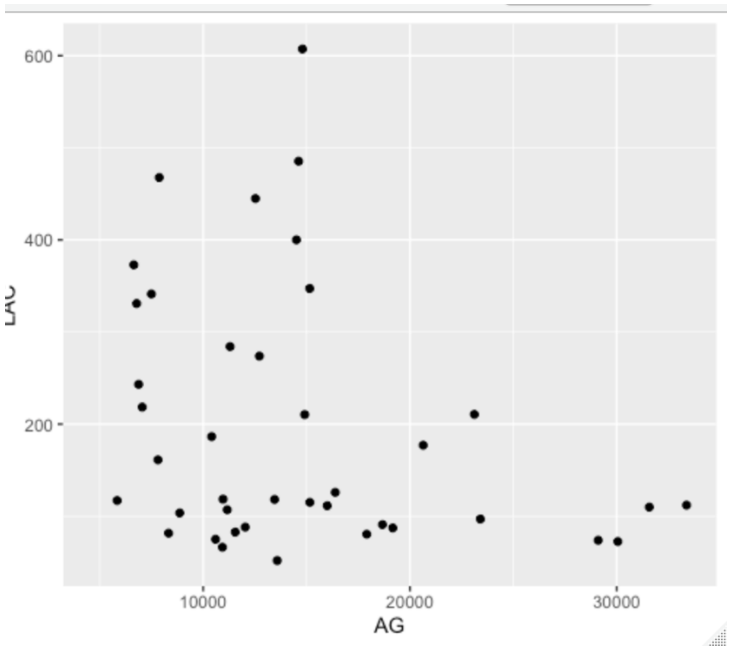


Figure 2.12. Association between HUN_{AUC} and TG_{AUC} (A), AG_{AUC} (B), and DAG_{AUC} (C).



Blue line represents slope, shaded area represents confidence interval.

Figure 2.13. . Association between LAC_{AUC} and AG_{AUC} .



SECTION II:

MANUSCRIPT III

The Effect of Exercise Intensity on Ghrelin Levels and Flow Mediated Dilation in Lean, Normoglycemic Individuals and Individuals with Obesity and Prediabetes

Abstract:

Background: Both AG and DAG have been shown to have a beneficial effect on the vasculature in human and animal models, evidence suggests AG and DAG maintain endothelial function by optimizing the balance of NO and ET-1. Acute exercise has been shown to alter endothelial function as well as ghrelin; however sex, obesity status, and differences in exercise dose have led to inconsistent results, and whether ghrelin release is a contributing factor to exercise-induced endothelial function is unclear. *Purpose:* To investigate the effects of exercise intensity, and associated ghrelin release on endothelial function in two separate groups: males and females, and lean and obese individuals *Methods:* Peak oxygen consumption (VO_{2peak}) and lactate threshold (LT) were determined via an incremental test on a cycle ergometer. Subjects had their body composition assessed via dual energy x-ray absorptiometry (DEXA) to measure total body fat percentage (BF%) and an abdominal CT scan to measure abdominal visceral fat (AVF). The testing period consisted of three randomized visits: Control (CON, no exercise), Moderate intensity exercise (MOD, power output at LT), and high intensity exercise (HIGH, power output associated with 75% of the difference between LT and peak). The caloric expenditure was kept consistent within each subject for the exercise conditions. AG, TG, DAG, and lactate were measured at the following timepoints: 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 minutes. Brachial flow mediated dilation (FMD) was measured at baseline, 30, 60, 90, and 120 minutes post-exercise. Subjects were split using previously determined cutoffs where females with a BF% >37.1% and males with a BF% > 25.8% were placed in the obese group (OG). Area under the curve and data at each individual timepoint were examined for TG, AG, DAG and FMD. *Results:* Eleven males (age: 40.7 ± 12.2y; BMI: 27.0 ± 6.1;

VO_{2peak}: 30.0 ± 9.6 mL/kg/min) and nine females (age: 35.1 ± 10.4 y; BMI: 25.2 ± 5.97 kg/m2; VO_{2peak}: 27.7 ± 5.79 mL/kg/min) completed all measures for the study. For the peak FMD , there was a significant main effect for Condition ($p<0.0001$). HIGH and MOD had a greater %FMD than CON (both; $p<0.0001$). For the model examining FMD over time, there was a significant main effect for condition and time (both ;$p<0.0001$).There was also a significant interaction between condition and time ($p=0.002$), and condition and sex ($p=0.03$). HIGH had a higher %FMD than CON, and MOD had a higher %FMD compared to CON (both; $p<0.0001$). No ghrelin isoform was significantly related to FMD (all; $p>0.05$). *Discussion:* Isocaloric acute exercise of moderate and high intensity both improved FMD to a similar extent. Although all ghrelin isoforms were suppressed following high intensity exercise, changes in FMD were not associated with changes in ghrelin levels regardless of obesity status.

Introduction

Kojima and Kangawa discovered ghrelin as an endogenous ligand to growth hormone secretagogue receptor 1a (GHSR1a) in 1999[1]. First studied to stimulate growth hormone (GH) release from the anterior pituitary, research on ghrelin expanded to examine its effects on outcomes related the endocrine, cardiovascular, digestive, and immune systems[2]. Ghrelin circulates in two forms, acylated (AG) and deacylated (DAG), and the majority circulates as DAG (~78% of total ghrelin (TG)). The less abundant AG (~22% of TG) is the form that binds to GHSR1a, and is catalyzed by ghrelin O-acyltransferase (GOAT)[2,3]. Conversely, DAG binds to a receptor that has yet to be identified. Differentiating between the two forms is important due to data showing AG and DAG can act independently, synergistically, or antagonistically within the body[2].

Both AG and DAG have been shown to favorably affect vascular function. In healthy individuals, there is a balance between vasoconstrictor and vasodilator factors to maintain homeostasis within the endothelium[4]. In disease states such as obesity and insulin resistance, this balance becomes disrupted due decreased nitric oxide (NO) availability, coupled with increased vasoconstrictor production in the form of endothelin-1 (ET-1)[4]. Evidence suggests AG and DAG maintain endothelial function by optimizing the balance of NO and ET-1. DAG has been shown to increase NO production in porcine endothelial cells[5]. In humans, an infusion of AG restored the balance of NO and ET-1 in individuals with metabolic syndrome[6]. The PI3k-Akt pathway has been implicated in ghrelin's effect on the vasculature, a pathway that coincides with the insulin signaling pathway[5,7]. This suggests that altering ghrelin release may restore vascular function in insulin resistant

states. As endothelial dysfunction precedes the development of atherosclerosis, identifying precise treatment strategies that target the endothelium is critical[8].

Exercise improves vascular function and decreases cardiovascular disease risk in both healthy individuals and in disease states[8–11]. The exercise response may be mediated, in part, through the effects of ghrelin as exercise also affects both AG and DAG levels[12–16]. Acute exercise has been shown to alter endothelial function as well as ghrelin; however sex, obesity status, and differences in exercise dose have led to inconsistent results[17–19]. Concerning exercise dose, results are conflicting; where high intensity had either no effect[20], enhanced[18], or impaired[17] endothelial function post-exercise while moderate intensity exercise had no effect[18]. One study found that although high intensity exercise enhanced endothelial function in their lean cohort, this effect was blunted in obese individuals[18]. With regard to ghrelin levels, limited data indicates the response to exercise may differ by isoform in obese individuals, having a greater suppression of DAG and attenuated changes to AG compared to lean individuals [16,21].

Identifying the exercise dose to optimize endothelial function in clinical populations such as obesity is critical to prevent the development of overt cardiovascular disease. Furthermore, elucidating the contributing factors (e.g., ghrelin release) that explain the effects of acute exercise can contribute toward the development of a precision exercise prescription. A recent meta-analysis published by our group determined that exercise suppresses ghrelin levels, and that exercise intensity moderates that relationship, however most studies included in the analysis utilized a moderate intensity exercise bout[22]. Although

we examined all forms of ghrelin, the meta-analysis lacked power to show effects of individual ghrelin forms as the majority of included studies just measuring AG.

The purpose of the present study was to investigate the effects of exercise intensity, and associated ghrelin release on endothelial function in two separate groups: males and females, and lean and obese individuals. We hypothesized that high intensity exercise will lead to the greatest alterations in ghrelin levels (i.e., decrease in AG, increase in DAG) and improvement to vascular function in lean individuals. As data show individuals with obesity have an impaired or absent FMD response to exercise[18], we also hypothesized that obese individuals will have an intensity dependent blunted FMD as well as ghrelin response to exercise.

Methods

Participants

Individuals between the ages of 18-55 years old were recruited for this study. They were included if they were untrained, non-smoking, and weight stable (<3 kg over 3 months) and had a BMI between 18.5-25 and 30-35 kg/m2. Criteria for exclusion included: history of T2DM, pregnancy/fertility treatments, disorders of the endocrine and gastrointestinal system, and/or any medications/treatments that effected the ability to safely exercise or measure hormones. Individuals arrived at the Clinical Research Unit (CRU) between 7 and 9 am and after an overnight fast for all visits. Subjects were asked to refrain from exercise and alcohol for 24 hours, and tobacco products for 12 hours prior to each CRU admission. The study was conducted in accordance with the Declaration of Helsinki, and the protocol

was approved by the University of Virginia Institutional Review Board (IRB-HSR # 200241), and all subjects provided written informed consent.

Screening Period

BMI was determined with obesity defined as a BMI between 30-40kg/m^2. Peak oxygen consumption (VO_{2peak}) and lactate threshold (LT) were determined via an incremental test on a cycle ergometer (Lode Model 960900). Subjects began at an initial power output of 50 W and power output was increased by 25 W every 3 minutes until volitional exhaustion. Indirect calorimetry using standard open circuit spirometry (Vyntus, Viasys, Yorba Linda, CA) was used to measure oxygen consumption and carbon dioxide production (as well as provide min-by-min kcal in order to equate caloric expenditure between the two exercise bouts). Blood was sampled at the end of each stage and assayed for lactate (YSI 2900D, Yellow Springs, OH, US). The lactate threshold was determined as the power output just prior to the curvilinear increase in blood lactate and the VO_2 at this power output was chosen as VO_2 LT. The highest 1 min VO_2 attained was chosen as VO_2 peak. During the screening period, subjects' percent body fat was measured via dual energy x-ray absorptiometry (DEXA) and abdominal visceral fat (AVF) was measured by CT Scan.

Testing Period

The testing period consisted of three randomized visits: Control (CON, no exercise), Moderate intensity exercise (MOD, power output at LT), and high intensity exercise (HIGH, power output associated with 75% of the difference between LT and peak). Females were tested during the early follicular phase of the menstrual cycle, and there was

a minimum of 72 hours between exercise sessions. In the 24 hours before each visit, participants were asked to standardize their diet. They filled out a diet log before the first experimental visit and were instructed to follow that log each day prior for the remaining two visits.

Exercise session caloric expenditure was kept consistent within each individual. At each visit, subjects were observed for three hours (Figure 3.1)**.** An indwelling venous catheter was inserted and blood was sampled at baseline, every 10 mins for the first hour, and then every 30 mins for the remaining two hours (Figure 1) to measure TG, AG, DAG, and lactate. Brachial flow mediated dilation (FMD) was measured at timepoints illustrated in Figure 1**.** Participants were instructed to lie supine with right forearm extended. The location of the probe was marked during the first testing visit at baseline and a measurement from the antecubital fold to the probe was recorded to allow for consistent probe placement for repeat measurements. A manual blood pressure cuff was placed distally from the antecubital fold and was inflated 50 mmHg above each participant's resting blood pressure for each 5-min forearm occlusion. A high-resolution ultrasound (Philips EPIC-Q7) and a 7.5MHz linear array transducer were used to obtain images at baseline, during five minutes of forearm occlusion, and on r-wave trigger for 2 minutes following cuff release to determine peak diameter . All images were obtained by a single investigator (KCA) and measured via Brachial analyzer program. The percent change in brachial artery diameter was calculated as follows:((peak post–hyperemia diastolic diameter–baseline diastolic diameter)/baseline d

iastolic diameter)*100. Images were analyzed by a single investigator blinded to condition. Interclass correlation coefficient for our lab group is 0.91.

Biochemical Analyses

Blood lactate collected during exercise was immediately analyzed (YSI Instruments 2900). Blood to measure TG, AG, and DAG was collected in EDTA vacutainers containing protease inhibitor AEBSF and was centrifuged for 10 minutes at 3,000 rpm at 4ºC. Hydrochloric acid 1N was added to the plasma aliquots immediately after centrifugation. Plasma ghrelin was stored at -80ºC for later analysis. Ghrelin was analyzed using Bertin Pharma ELISA kits by University of Virginia Center for Research in Reproduction, Ligand Assay and Analysis Core. All timepoints were run in duplicate.

Statistics

Based on previous literature[16], assuming a power of 80% for an ANOVA with significance of $\alpha = 0.05$, an adequate sample size of n=8 per group was determined *a priori* to assess group differences between acute exercise and ghrelin levels. A sample of 21 per condition was determined *a priori* as an adequate sample size to determine differences in FMD by experimental condition. Power calculations were made with G*Power version 3.1. Data was analyzed via R (Version 4.0.2). To examine percent body fat (BF% ; Obese vs Lean) subjects were split using previously determined cutoffs from Macek et al., where females with a BF% >37.1% and males with a BF% > 25.8% were 2-4 higher times more likely to develop a cardiovascular risk factor[23] (Obese Group, OG).

Baseline comparisons were evaluated using independent sample t-tests, and normality was assessed using Q-Q plots and the Shapiro-Wilk tests . Area under the curve (AUC) was calculated for TG, AG, DAG from each testing visit via the trapezoidal method. FMD at each timepoint was analyzed utilizing the relative change from baseline of each condition. We examined area under the curve (AUC) and timepoint data for both TG, AG, DAG, and FMD. Several linear mixed models were used examine the ghrelin response to exercise. For timepoint data ($FMD_{TIMEPOINT,}$ $TG_{TIMEPOINT}$, $AG_{TIMEPOINT}$, $DAG_{TIMEPOINT}$): Subject as a random factor and sex, condition, time, and body fat (BF) group ("Lean" or "Obese") were fixed factors. For AUC (TG_{AUC}, $AG_{AUC,}$ DAG_{AUC}) data: Subject was a random factor and sex, condition, and BF group were fixed factors. We also inputted the max %FMD regardless of time in a model with the same specifications as above. Satterwaite's approximation was utilized to determine significance. F tests of nested models were used to determine differences in fixed effects. Estimated marginal means (EMM) were utilized to estimate the means that are adjusted for the factors in each model. Associations were determined using spearman rank correlations if data was not normal and/or relationship between variables of interest was not linear, while Pearson product moment correlations were utilized in normal and linearly related data. We ran correlations using the %FMD (change from baseline) as well as the peak value of %FMD during each condition regardless of time. Significance was accepted as $p < 0.05$. Data are reported as mean ± SD unless otherwise noted.

Results

Eleven males (age: 40.7 ± 12.2y; BMI: 27.0 ± 6.1; VO_{2peak}: 30.0 ± 9.6 mL/kg/min) and nine females (age: 35.1 ± 10.4 y; BMI: 25.2 ± 5.97 kg/m2; VO_{2peak}: 27.7 ± 5.79 mL/kg/min) completed all measures for the study. Due to supply chain issues, AG data is available for 16 subjects, and DAG/TG data is available for 10 subjects. Baseline differences between sex are in Table 3.1. There were significant differences in AG (p=0.001), DAG (p=0.001), and TG (p=0.002), with females having higher levels of all isoforms compared to males. Males had significantly larger baseline brachial diameters than females (p<0.05), and a trend toward larger AVF values (p=0.06). Females had higher %BF (p=0.01).

Baseline differences between BF% group are in Table 3.2. There were significant differences in BF%, AVF, and VO_{2peak} between Lean (LG) and Obese (OG). OG had lower VO_{2peak} (p=0.001), and higher BF% (p=0.01) and AVF (p<0.05). There were no differences observed for baseline TG, AG, or DAG.

Total Ghrelin

The TG_{AUC} model revealed a significant main effect for condition (p=0.005), and a significant interaction effect for condition and sex (p=0.04, Figure 3.2). HIGH was lower TG levels than MOD and CON (p<0.01). The EMM contrasts for the interaction showed that the females had lower TG levels in HIGH compared to MOD and CON (both; p<0.05). There was a trending difference between the CON levels of TG between males and females (p=0.06).

The model for $TG_{TIMEPOINT}$ showed a significant main effect for condition ($p<0.0001$) and time ($p=0.007$), and significant interaction effects for condition and sex, and condition and BF% (both; $P<0.0001$, Figure 3.3). For the main effect of condition, TG levels was 47.3 pg/mL lower in HIGH than CON, and 60.7pg/mL lower in HIGH than MOD (both; $p<0.0001$). Regardless of condition, TG levels were elevated at 10 minutes compared to 90 minutes ($p<0.05$). With the condition and sex interaction, TG levels decreased in HIGH compared to CON and MOD in females (both; $p<0.0001$). For males , TG levels were decreased in CON compared to MOD ($p<0.05$), and increased in MOD compared to HIGH ($p=0.01$). Concerning the condition and BF% comparison, those in OG had significantly lower TG levels in HIGH compared to CON, and HIGH compared to MOD (both; $p<0.0001$). There were no differences in conditions within the Lean group.

Acylated Ghrelin

The model for AG_{AUC} (Figure 3.4) revealed a significant main effect for condition ($p<0.0001$), and a significant interaction for condition and sex ($p<0.05$). EMM contrasts showed that CON and MOD were greater than HIGH (both; $p<0.0001$). Regarding the interaction within females, HIGH was 6, lower than CON and MOD (both; $p<0.0001$). No condition was significantly different within males.

$AG_{TIMEPOINT}$ (Figure 3.5) had a significant main effect for condition and time. Significant interactions included condition and time, condition and sex, and condition and BF% group. For condition, AG in HIGH was less than CON and MOD (both; $p<0.0001$). Overall,

baseline AG was higher than the 40, 50 , and 90 minute timepoints regardless of condition (all; $p<0.01$). The condition and time interaction showed AG in HIGH being significantly lower than CON at 30, 40, 50, and 60 minutes, and lower than MOD at 20, 30, 40, 50, and 60 minutes (all; $p<0.01$). AG levels in females during HIGH was significantly lower than MOD and CON (both, $p<0.0001$). For males, the AG HIGH was also significantly lower than MOD and CON (both; $p=0.01$). The condition by BF% group showed that OG and LG had higher AG levels in the MOD and CON than HIGH (all; $p<0.0001$).

Deacylated Ghrelin

The DAG_{AUC} exhibited a significant main effect for condition ($p<0.0001$, Figure 3.6). EMM contrasts showed that CON ($p<0.01$) and MOD ($p<0.05$) were greater than HIGH.

The $DAG_{TIMEPOINT}$ had a significant main effect for condition and time. Significant interactions include condition and time, condition and sex, and condition and BF% (Figure 3.7). For condition, HIGH was less CON and MOD (both; $p<0.0001$). Overall, DAG levels at 10 minutes were higher than the 60 and 90-minute timepoints ($p<0.05$). For the condition and time interaction, DAG levels in HIGH were significantly lower than MOD at 50 and 60 minutes ($p<0.05$). With the condition and sex interaction, females during HIGH had DAG levels less than in the CON and MOD conditions (both; $p<0.0001$). Males during CON exhibited DAG levels less than MOD ($p=0.05$) and a trending difference compared to MOD and HIGH ($p=0.054$). Lastly, OG significantly lower DAG levels in

HIGH compared to CON and MOD (both; $p<0.0001$). There were no differences within LG by condition.

Flow Mediated Dilation

For the peak FMD , there was a significant main effect for Condition ($p<0.0001$, Figure 3.8). HIGH and MOD had a greater %FMD than CON (both; $p<0.0001$).

For the model examining FMD over time, there was a significant main effect for condition and time (both ;$p<0.0001$).There was also a significant interaction between condition and time ($p=0.002$), and condition and sex ($p=0.03$). HIGH had a higher %FMD than CON, and MOD had a higher %FMD compared to CON (both; $p<0.0001$, Figure 3.9). There was no difference between MOD and HIGH ($p>0.05$). %FMD in MOD and HIGH was higher at every timepoint compared to their respective baseline (all; $p<0.05$). Males and Females had significantly lower %FMD in CON compared to MOD and HIGH (both; $p<0.001$, Figure 9).

Correlations

The maximum %FMD (regardless of time) was not significantly associated with BF% (rho=0.14, $p>0.05$) or VAT (rho=0.21, $p>0.05$). %FMD was not significantly associated with delta TG (rho=-0.08, $p>0.05$), AG (rho=0.07, $p>0.05$), or DAG (rho=0.007 $p>0.05$). There was a trending inverse association between AG_{AUC} and LAC_{AUC} (rho=-0.29, $p=0.07$).

Discussion

Total ghrelin has been suggested to increase vasodilation by increasing NO release and antagonizing ET-1 in human models *in-vitro*[24]. In addition, limited data suggests both AG and DAG have potent vasodilatory properties, suggesting there may be optimal levels of each[25]. Uncomplicated obesity as well as obesity associated co-morbidities (e.g. metabolic syndrome, prediabetes, T2DM) are correlated with endothelial dysfunction and increased risk for cardiovascular disease[26]. The potential for ghrelin to mediate endothelial release of NO make it an attractive potential therapeutic. The mechanism of ghrelin induced NO release appears to be primarily via the PI3K-AKt pathway within the endothelium. This suggests overlap with insulin signaling pathways, and it is possible in insulin resistant states, that altering ghrelin concentrations (e.g. via exercise) could play a significant role in maintaining vascular health and function via different cellular receptors[27,28]. In support of the above, DAG infusion in individuals with metabolic syndrome resulted in a subsequent increase in NO-mediated vasodilation[29]. Similarly, infusion of AG in individuals with metabolic syndrome, improved vasodilation albeit via reduction of excessive vasoconstrictor tone[6]. Importantly, this was not observed in healthy, control subjects in either study. These data suggest ghrelin levels associated with obesity may be implicated in vascular function and provide a potential mechanism by which altering circulating ghrelin may be vaso-protective in these groups at higher risk for cardiovascular disease

The major findings of the present study indicate that: exercise improved flow mediated dilation, independent of exercise intensity; and although all ghrelin isoforms were

suppressed following high intensity exercise, changes in FMD were not associated with changes in ghrelin levels regardless of obesity status. The effect of exercise intensity on post-exercise FMD is equivocal, and differences in sample characteristics (i.e. age, body composition, fitness level, and health conditions) make it difficult to form a consensus[18–20]. Birk et al. found that FMD returned to baseline 1-hour post-exercise regardless of exercise intensity[20]. We report that the FMD for MOD and HIGH remained significantly elevated compared to CON 2 hours after exercise. Hallmark et al. reported that only high intensity exercise improved endothelial function, however their sample contained younger adults than the current study and kept the duration consistent between exercise conditions (as opposed to clamping for kcal in the present study), resulting in higher caloric expenditure in their high intensity condition[18].. Other studies investigating isocaloric bouts of exercise have reported similar results to the present study, with no difference between moderate and high intensity exercise[9,30,31]. One study found a significant difference in their %FMD data between intensities when normalized for shear rate, but not when shear rate was not included in the calculation[31].

Consistent with previous literature our data indicate that FMD was affected by sex[10,32,33]. A study in 4,739 adults found that females have a higher FMD than males the same age until age 70[34].. We found that females had a lower baseline diameter than males, and although females had a higher %FMD in both MOD and HIGH compared to males, it did not reach the level of statistical significance.

We found no effect of BF on our FMD results. This is in contrast previous data from our laboratory, which utilized the same protocol to determine exercise intensity, and showed individuals with obesity display a blunted FMD response at MOD and HIGH intensities[18]. Differences may be due to how obesity was defined; with the current study using BF% cutoffs, and Hallmark and colleagues using BMI and waist circumference[18]. Prior data indicates that individuals with abdominal visceral, but not subcutaneous, obesity have attenuated FMD responses[35], however; we did not find a relationship between visceral adiposity and FMD. This could be explained in part by the fact that although our lean and obese groups had a significant difference between visceral adiposity and total BF%, there was no difference in baseline arterial diameter. In contrast, Hallmark et al. reported individuals with obesity had larger baseline brachial artery diameters than those who were lean[18]. As there is an inverse relationship between vessel size and endothelium-dependent vasodilation, this could be a reason for the discrepancy between findings[36].

In contrast to previous data, we report no relationship between changes in FMD and ghrelin levels. Studies in humans[6,25,27] and animals[27,37] , have identified ghrelin as a vasodilator, likely through NO mediated mechanisms[27]. However, most studies that have illustrated a significant effect on the vasculature have applied a supraphysiological dose of ghrelin, whereas the present study examined physiological response of endogenous levels of ghrelin to exercise of differing intensity.

There are several limitations of this study. We did not match for sex within our obese and lean groups; therefore, we cannot rule out the influence of sex differences on our results

within each BF group. Additionally, our ability to assess DAG and TG may be underpowered, due to our sample size of ten subjects. Lastly, we did not normalize our FMD data to shear stress, however we did compare the raw values with the data allometrically scaled and found no difference in slopes[38].

In conclusion, we report that exercise augments FMD independent of sex and obesity, and ghrelin is transiently suppressed by high intensity exercise., However, the present data do not support our hypothesis that changes in FMD would be related to changes in total and/or acyl or deacyl ghrelin. More work, with adequate sample size, is needed to examine the differential FMD and ghrelin responses to exercise of differing intensity and determine if sex and adiposity impact these responses.

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Table 1. Subject Demographics by Sex

	Male		Female		P-value
N (F)	11		9		-
Age (years)	40.7 ± 12.3		35.1 ± 10.4		0.27
BMI (kg/m2)	27.0 ± 6.1		25.2 ± 5.9		0.52
BF%*	28.6 ± 7.8		35.2 ± 5.9		0.01
VO_{2peak} (mL/kg/min)	30.0 ± 9.6		27.7 ± 5.9		0.51
AVF (cm^2)	271. 6 ± 204.3		166.5 ± 141.4		0.06
Baseline TG (pg/mL)*	130.6 ± 32.3		231.6 ± 97.6		0.001
Baseline AG (pg/mL)*	66.2 ± 29.6		114.3 ± 57.0		0.001
Baseline DAG (pg/mL)*	81.6 ± 24.3		137.6 ± 57.1		0.002
Baseline Brachial Diameter (mm)*	4.06 ± 0.39		3.29 ± 0.58		0.004
Baseline %FMD	6.3 ± 1.2		6.1± 1.3		0.6
Exercise Sessions	Mod	High	Mod	High	
Duration (min)†	47:14± 0.6	31:19 ± 0.4	53:20 ± 0.1	33:45± 0.25	
Energy Expenditure (kcal)	272.8± 72.75	270.4± 71.3	244.55 ± 41.3	247.3± 40.7	
Average HR (bpm)†	118.95 ± 12.2	160.1 ± 23.9	128.6 ± 25.4	170.2 ± 18.5	

*differences between males and females; † denotes a significant difference between MOD and HIGH within the same sex, $p < 0.05$

Table 3.2. Subject Demographics by Obesity Group

	Lean		Obese		P-value
N (F)	9 (5)		11 (4)		-
Age (years)	34.3 ± 13.0		41.4 ± 9.0		0.19
BMI (kg/m2)	24.3 ± 4.1		27.7 ± 6.8		0.18
BF%*	27.4 ± 6.5		35.4 ± 5.6		0.01
AVF (cm^3)*	130.9 ± 82.6		283.1 ± 192.4		0.004
VO_{2peak} (mL/kg/min) *	34.6 ± 6.1		24.2 ± 6.2		0.001
Baseline TG (pg/mL)	183.8 ± 60.0		191.7 ± 110.8		0.8
Baseline AG (pg/mL)	100.7 ± 58.1		80.1 ± 42.4		0.2
Baseline DAG (pg/mL)	113.8 ± 40.4		113.5 ± 43.2		1.0
Baseline Diameter (mm)	3.67 ± 0.63		3.76 ± 0.62		0.75
Baseline %FMD	6.07 ± 1.1		6.29 ± 1.35		0.2
Exercise Sessions	Mod	High	Mod	High	
Duration (min)†	54:02± 0.2	33.04± 0.25	46:40 ± 0.6	31:53± 0.4	
Energy Expenditure (kcal)	277.8± 47.5	280.6 ± 44.7	245.6± 69.6	243.2± 68.6	
Average HR (bpm)†	130.6 ± 23.2	176.7 ±9.9	117.3 ± 13.7	155.4 ± 23.2	

*Denotes significant difference between lean and obese, † denotes a significant difference between MOD and HIGH within the same group, $p < 0.05$

Figure 3.1. Outline of Testing Visit Measurements

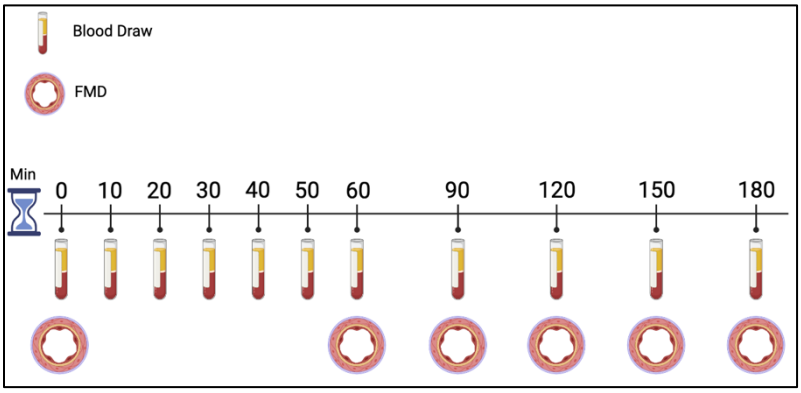
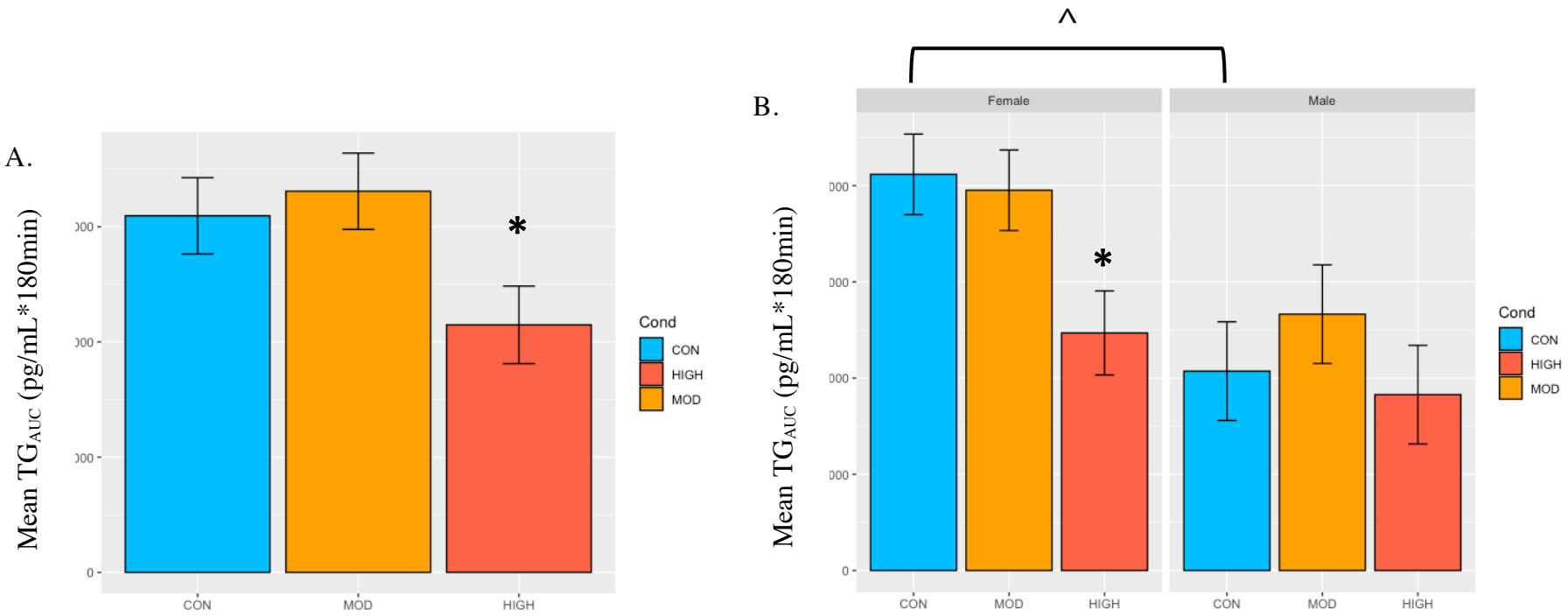
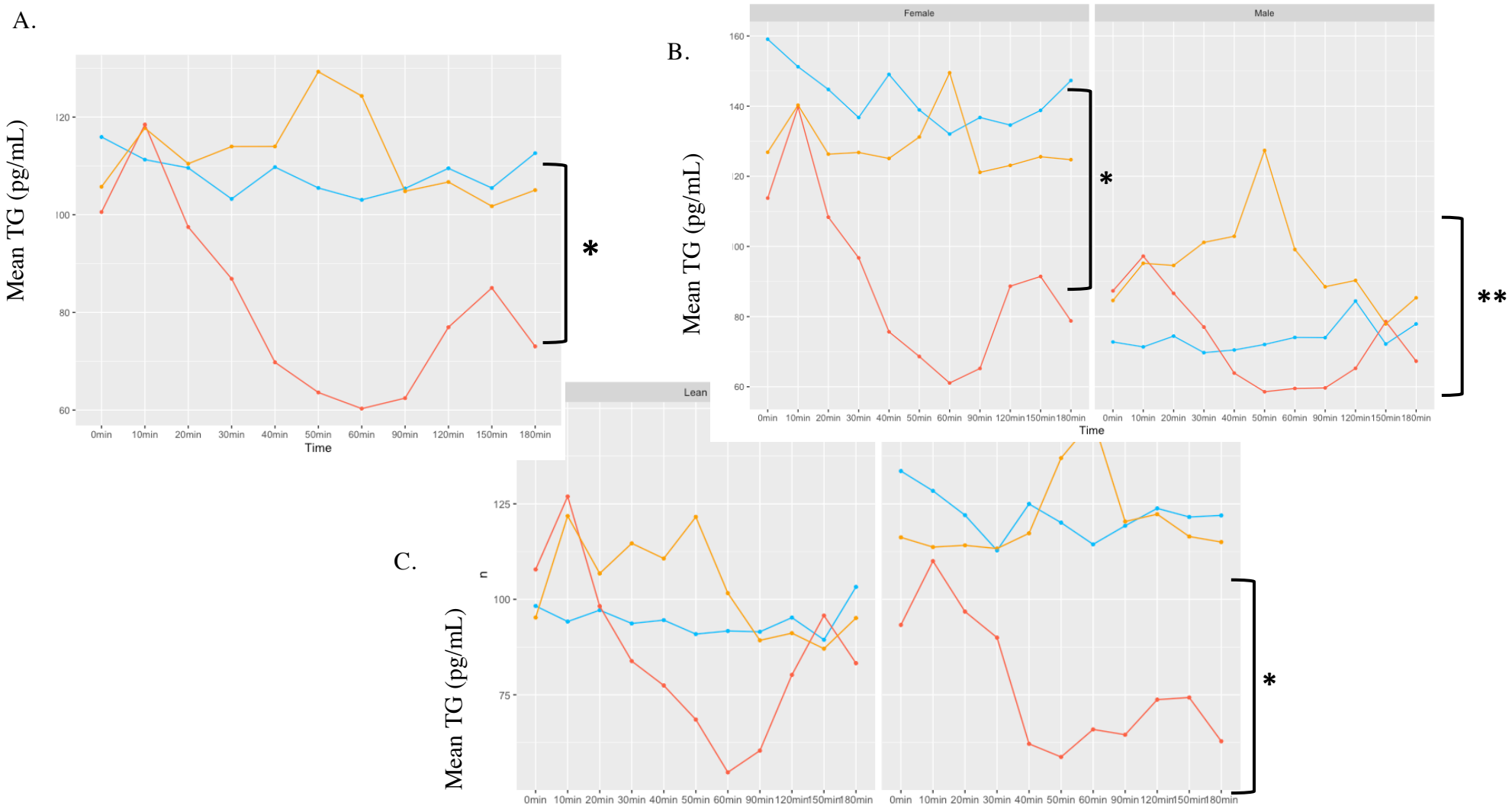


Figure 3.2. Effect of Exercise Intensity on TG_{AUC} by Condition (A) and Condition and Sex (B).



Data are mean ± SE. *Denotes significant effect of condition compared to CON ($p < 0.05$), ^ denotes trending significance (p=0.06)

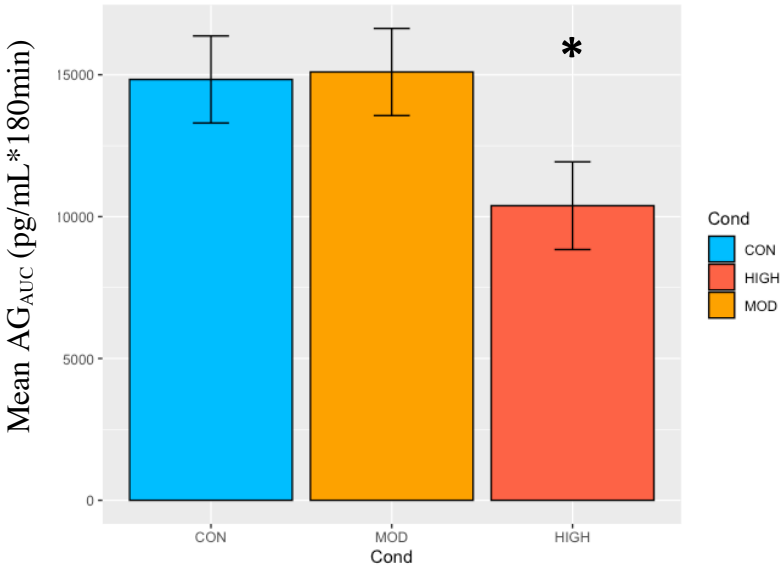
Figure 3.3. Effect of Exercise Intensity on $TG_{TIMEPOINT}$ by Condition (A) ,Condition and Sex (B), and Condition and BF (C).



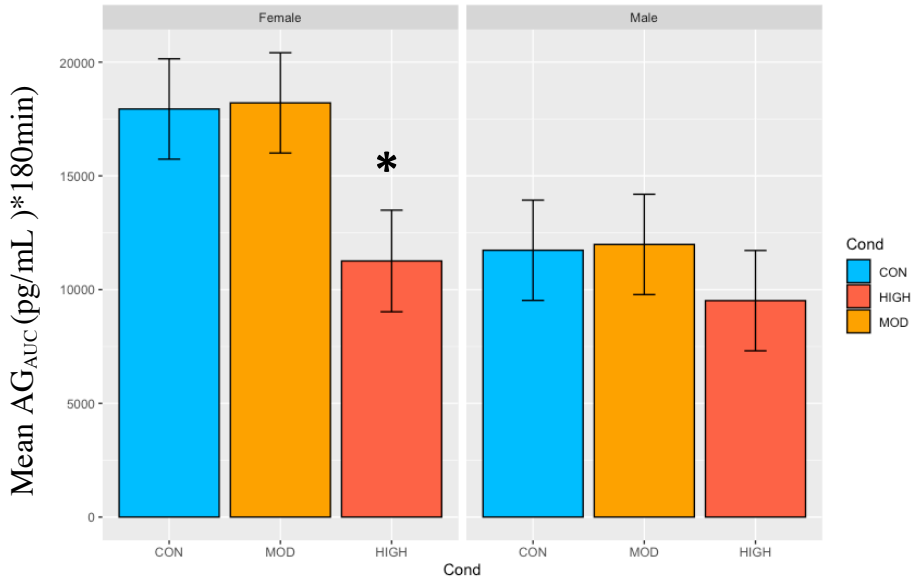
Data are mean ± SE. * Denotes significant effect of HIGH compared to MOD and CON, ** denotes significant effect of MOD compared to CON and HIGH ($p < 0.05$)

Figure 3.4. Effect of Exercise Intensity on AG_{AUC} by Condition (A) and Condition and Sex (B).

A.

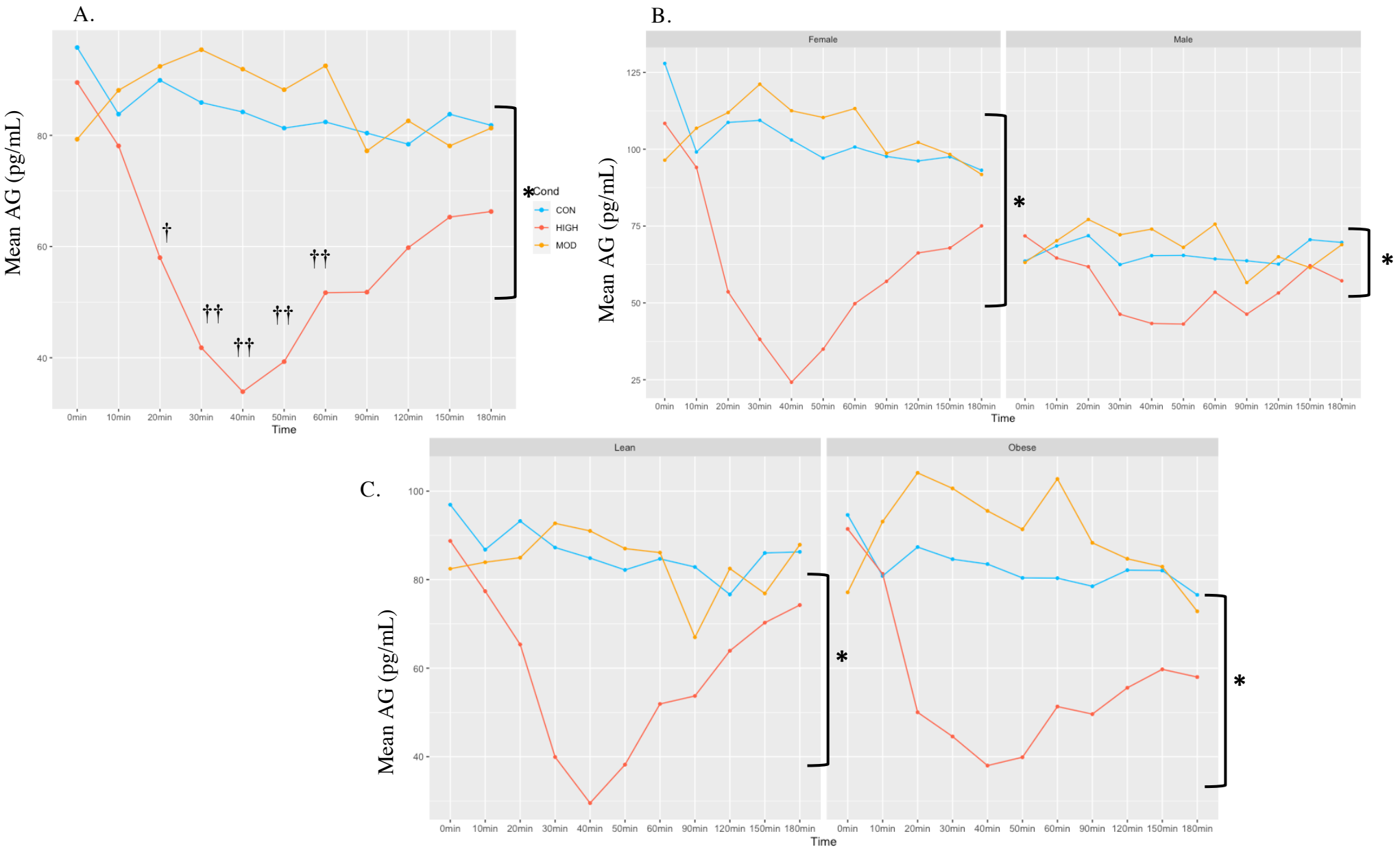


B.



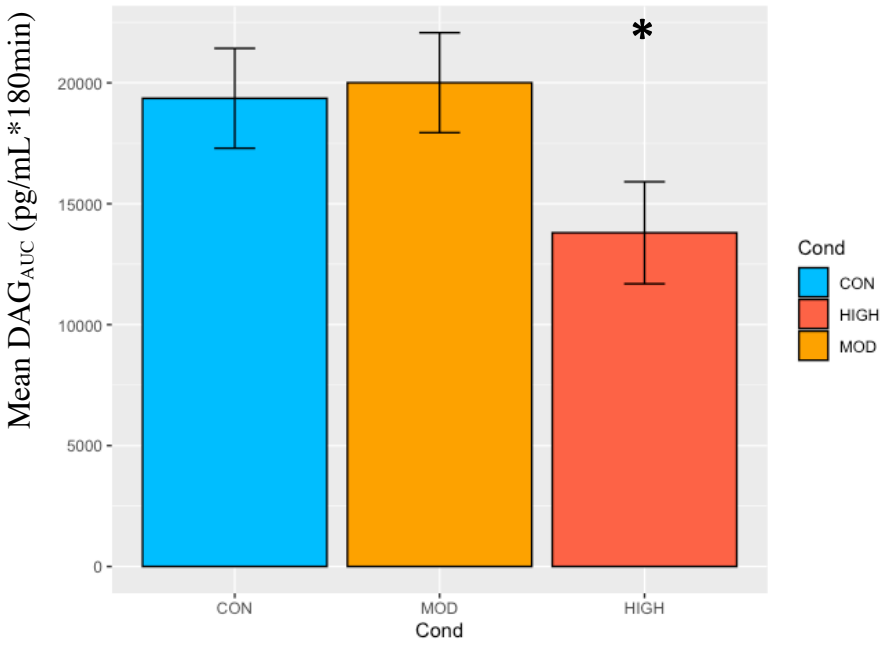
Data are mean ± SE. *Denotes significant effect of condition compared to CON ($p < 0.05$)

Figure 3.5 . Effect of Exercise Intensity on $AG_{TIMEPOINT}$ by , Condition and Time (A), Condition and Sex (B), and Condition and BF (C). Data are Mean ± SE. * Denotes significant HIGH effect compared to CON AND MOD ($p < 0.05$). † Denotes a significant time effect compared to MOD, †† denotes a significant time effect compared to MOD and CON



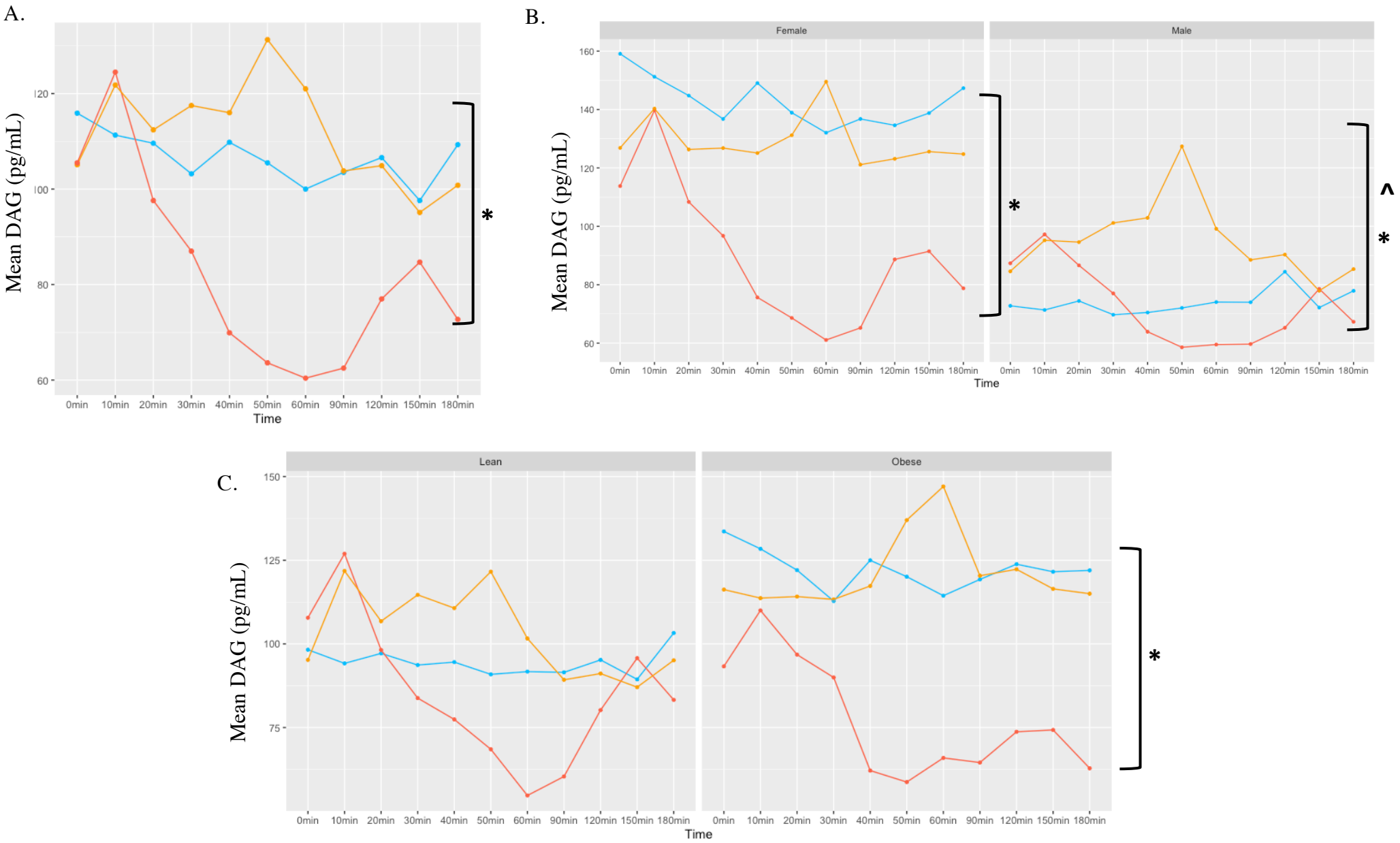
Data are Mean ± SE. * Denotes significant HIGH effect compared to CON AND MOD ($p < 0.05$). † Denotes a significant time effect compared to MOD, †† denotes a significant time effect compared to MOD and CON

Figure 3.6. Effect of Exercise Intensity on $DAG_{AUC.}$ *Denotes significant effect of condition ($p < 0.05$).



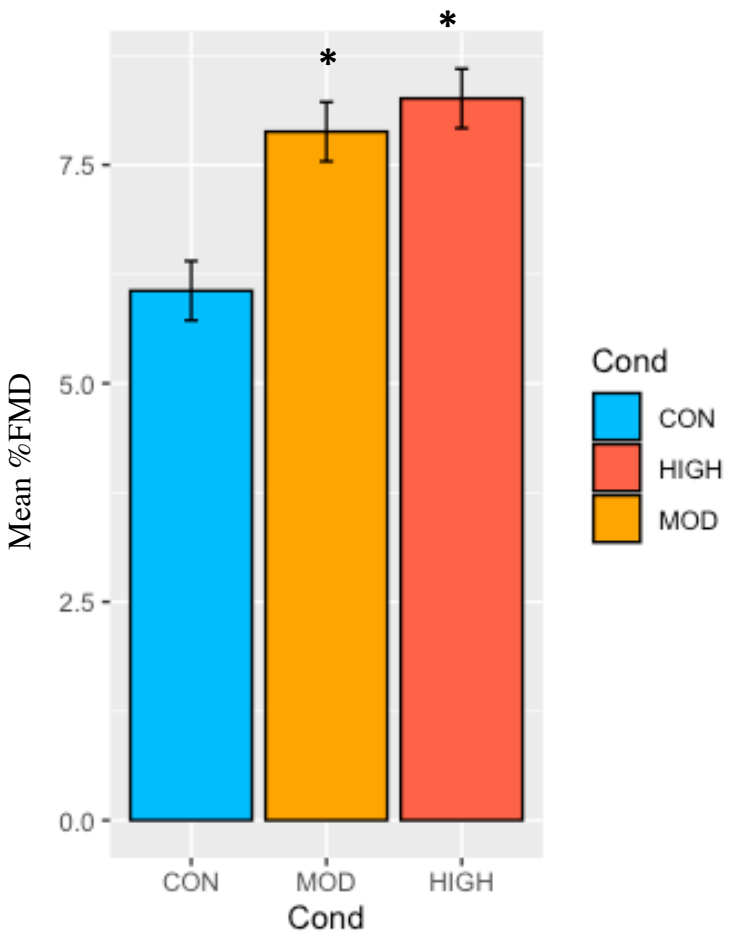
*Denotes significant effect of condition ($p < 0.05$).

Figure 3.7. . Effect of Exercise Intensity on $DAG_{TIMEPOINT}$ by Condition (A) , Condition and Time (B), Condition and Sex (C), and Condition and BF (D). Data are mean ± SE. * Denotes significant effect of HIGH compared to MOD and CON ($p < 0.05$), **, denotes a significant effect of MOD compared to CON , ^ denotes a trending effect of HIGH compared to MOD (p=0.054)



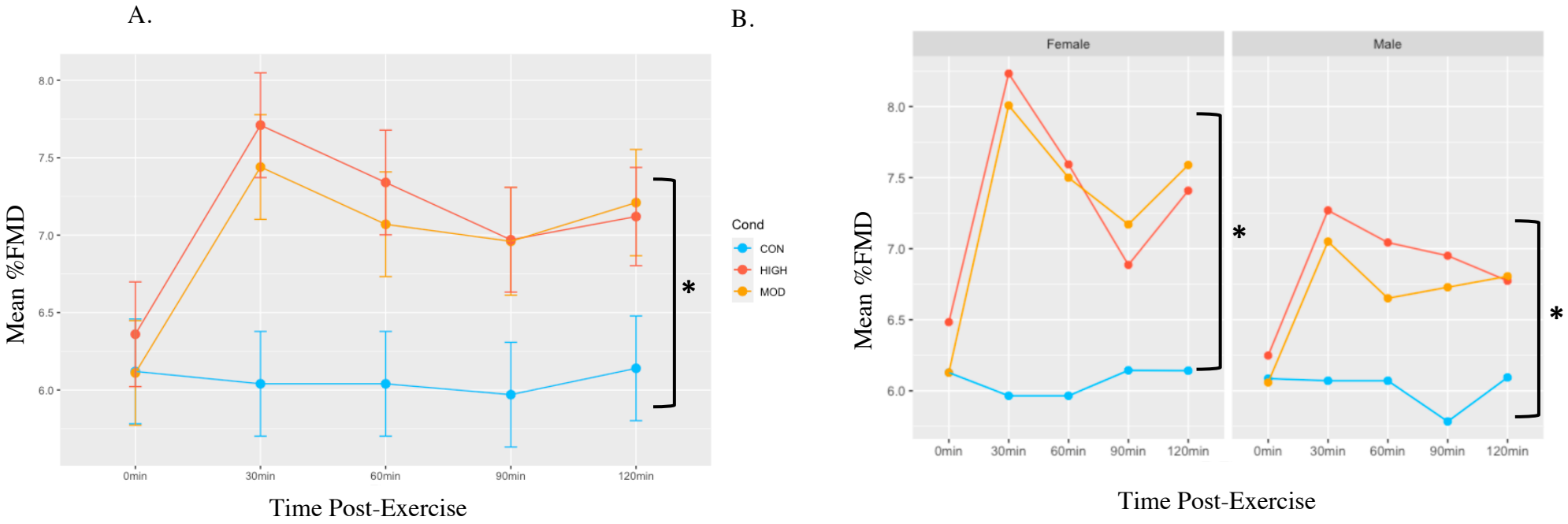
Data are mean ± SE. * Denotes significant effect of HIGH compared to MOD and CON ($p < 0.05$), **, denotes a significant effect of MOD compared to CON , ^ denotes a trending effect of HIGH compared to MOD (p=0.054)

Figure 3.8. Effect of Exercise Intensity on maximum %FMD by Condition



Data are mean ± SE. *Denotes significant effect of condition compared to CON, p <0.05

Figure 3.9. Effect of Exercise Intensity on %FMD by Condition (A) and Sex (B).



Data are mean ± SE. *Denotes significant effect of MOD and HIGH compared to CON, p <0.05

APPENDIX I:

List of Abbreviations

AG: Acylated Ghrelin

AVF: Abdominal Visceral Fat

APP: Appetite

AUC: Area Under the Curve

BF%: Body Fat Percentage

BMI: Body Mass Index

CON: Control Visit

DAG: Deacylated Ghrelin

FMD: Flow Mediated Dilation

HIGH: High Intensity Exercise Visit

HUN: Hunger

LAC: Lactate

LG: Lean Group

MOD: Moderate Intensity Exercise Visit

NO: Nitric Oxide

OG: Obese Group

T2DM: Type II Diabetes

TG: Total Ghrelin

VO_{2peak}: Peak Oxygen Consumption