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THE IMPLICATIONS OF DIFFERENT TYPES OF DIET AND EXERCISE ON  
HUMAN HEALTH

A Thesis  
Presented to  
The Faculty of the School of Kinesiology, Recreation and Sport  
Western Kentucky University  
Bowling Green, Kentucky

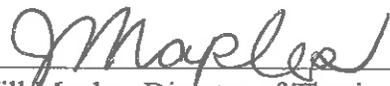
In Partial Fullfillment  
Of the Requirements for the Degree  
Master of Science

By  
BethAnne C Clayton

August 2016

THE IMPLICATIONS OF DIFFERENT TYPES OF DIET AND EXERCISE ON  
HUMAN HEALTH

Date Recommended 6-16-2016

  
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Date

I dedicate this work to my family who set me up for success in every way and who has loved me so sacrificially. My greatest education has come from you. Thank you for teaching me to choose what I cannot lose and to lose what I cannot keep.

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# THE IMPLICATIONS OF DIFFERENT TYPES OF DIET AND EXERCISE ON HUMAN HEALTH

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August, 2016

60 pages

Directed by: Jill Maples, Rachel Tinius, and Lee Winchester

School of Kinesiology, Recreation and Sport

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There is need for enhanced prevention and treatment methods to combat sedentary lifestyle, obesity, and chronic disease by investigating the impact of specific exercise modalities and dietary factors on human health. The purposes of this study were:

- 1) to assess self-selected and perceived exercise intensity during High-Intensity Functional Training (HIFT) between males and females and to determine variables that predict self-selected exercise intensity (%VO<sub>2</sub>max) and/or perceived intensity (RPE) and
- 2) to investigate the impact of obesity on skeletal muscle metabolism in response to lipid oversupply by analyzing the responses of genes linked with fatty acid oxidation and inflammation in lean and obese subjects. Males and females were recruited to complete a 15min HIFT circuit wearing a metabolic analyzer, reporting RPE during and after the exercise bout. Obese and lean females were recruited to provide skeletal muscle cell biopsies for harvesting cell cultures from which to measure change in gene expression after exposure to a high lipid treatment. The first study results demonstrate that females exercised at a significantly higher self-selected exercise intensity while also reporting a lower RPE ( $p < 0.05$ ). The second study revealed differential gene expression response and pathway activation related to lipid metabolism and inflammation between the lean and obese. In conclusion, gender plays a significant role in the intensity self-selected and the RPE reported during HIFT, suggesting HIFT may be an optimal home-based

modality for female clients. Additionally, the skeletal muscle metabolic and inflammatory gene expression of the lean and obese respond differently to a high fat exposure and may provide further evidence of mechanisms linking obesity to metabolic disease.

## Chapter 1: Introduction and Literature Review

### **Introduction**

The prevalence of metabolic diseases, such as type II diabetes, heart disease, and certain kinds of cancer, as well as the prevalence of obesity, continue to pose as a major concern world-wide. Undoubtedly, insufficient physical activity [1] and a high intake of dietary fat [2, 3], are contributors to the high rates of disease and obesity. Still, further investigation of the impact of diet and exercise on various groups of individuals is needed to enhance tailored prevention and treatment methods in the fight against sedentary lifestyle, obesity, and disease.

**Primary Objective:** *To assess actual and perceived exertion during high-intensity functional training*

Healthy adults between the ages of 18 to 65 years old are recommended to participate in at least 150 minutes of moderate intensity aerobic activity each week, or at least 75 minutes of vigorous aerobic activity per week, to maintain good health [4]. Unfortunately, around the world, one-third of adults and four-fifths of adolescents do not meet these physical activity guidelines [1]. High-intensity and body-weight exercise modalities are currently popular fitness trends [5] that can be done in a small amount of space with little to no equipment. High-intensity functional training (HIFT) is an example of this modality. HIFT exercises such as the push-up, sit-up, and body-weight squat, are not only used in traditional strength and conditioning settings but also mimic activities of daily living and their physiological benefits can transfer to the physical demands of daily life [6]. A recent investigation showed short high-intensity bouts may be equally as effective for enhancing fitness measures as continuous exercise [7]. Additionally, a

recent study found many participants intended to continue HIFT on their own after participating in an investigation of inactive overweight and obese adults in a HIFT intervention [6]. Although this modality may be a strong candidate for promoting exercise adherence and improved health, further investigation is needed.

Because of concerns related to the tolerability of high-intensity exercise modalities that would possibly negatively impact adherence rates, two factors have been investigated due to their role in influencing rates of high-intensity physical activity participation, including (1) whether the intensity is prescribed or self-selected [8-10] (2) and the perceptual response (RPE) to a particular bout of exercise [11-13]. In order for an exercise modality to ensure adherence and effectiveness, both factors should be considered.

#### *Self-Selected Exercise Intensity*

Higher intensity is typically associated with reduced pleasure or increased displeasure during aerobic activity[14]. However, a greater level of enjoyment has been associated with high-intensity exercise when the intensity is self-selected by participants rather than imposed [8-10]. The optimization model in the field of bioenergetics helps to predict what intensity individuals will self-select. The model suggests during physical activity most people will gravitate toward a specific and identifiable intensity that is innately selected on the grounds of efficiency [15]. Specifically, the model states the spontaneous selection of the speed and pattern of human gait is determined by the minimization of metabolic cost [15, 16]. Although Lind, Joens-Matre, and Ekkekakis found middle-aged sedentary women self-selected an intensity that was considered

“physiologically effective” (55-65% of maximum heart rate) during a 20 minute treadmill walking bout [12], typically, efficiency of locomotion would not be advantageous for an exerciser attempting to improve fitness and expend calories to maintain or lose body fat. When adequate energy consumption was questionable in human history, efficiency of human movement would have been an evolutionary advantage. However, the goal of exercise prescription is to help ensure fitness goals, including improved body composition.

In order for a home-based or unsupervised exercise program to be effective at eliciting beneficial physiological adaptations (e.g. improved body composition and cardiovascular fitness), the individual must self-select an intensity that is sufficient. While self-selected intensity programs have been found more enjoyable among a variety of groups [8-10, 17], there is much intra- and inter-individual variability in preference for exercise intensity [18].

Previous research suggests there may be variation between the exercise intensity men and women self-select while completing the same exercise. During 20 minutes of aerobic exercise at a self-selected intensity, males were found to work at a significantly higher  $VO_2$  while females achieved significantly higher heart-rates [19], displaying gender discrepancies in self-selected intensity. It is not known what intensity individuals participating in an acute bout of HIFT will self-select or if the selected intensity will be optimal for fitness goals in home-based settings. Due to the discrepancy between male and female self-selected intensity found in past research, it is necessary to investigate the intensity men and women will choose to work during an acute bout of HIFT to assess any trends that may prevent the effectiveness or cause injury due to inappropriate work rate.

### *Perceptual Response - Rating of Perceived Exertion*

Rating of perceived exertion (RPE) is a widely used validated scale to assess exercise intensity [20] as RPE has a positive correlation with aerobic and resistance exercise intensity [21-23]. Higher RPE's are typically reported while performing at higher intensities [21, 24]. Interestingly, one study showed that HIFT, when compared to anaerobic sprint-training, elicited lower RPE values, independent of exercise intensity and duration [11]. Gist, Freese, and Cureton compared peak cardiorespiratory, metabolic, and perceptual responses to acute bouts of sprint interval cycling (repeated Wingate tests) and an intermittent HIFT protocol that consisted of completing as many burpees as possible in 30 seconds with four minute rests in between the four sets. Mean values for percent  $\text{VO}_2\text{peak}$  and percent  $\text{HR}_{\text{peak}}$  were not significantly different between the two modalities, however, immediate post exercise RPE's, though not significant, were lower in those who participated in the burpee protocol. Both protocols produced RPE's described as "Hard" or "Very hard" [11].

It seems possible HIFT will result in lower RPE's despite the high-intensity design and may therefore result in an increased chance of adherence as exercise that is *perceived* as less intense is consistently associated with a higher level of enjoyment [25] and higher enjoyment is found to predict better exercise adherence [26, 27]. However, there is still need of investigation into the perceptual response and level of enjoyment associated with HIFT among men and women. O'Connor et al., observed that young women reported a lower perceived exertion than young males during fatiguing elbow flexion contractions [28]. This suggests gender may be a factor that influences variability in RPE among participants completing the same exercise bout.

While investigating specific exercise modalities and their impact on health is necessary for combating sedentary lifestyle which can lead to obesity and disease, this is only part of the equation that comprises a formula for enhanced tailored prevention and treatment methods. Therefore, the second objective of this thesis was to investigate the impact of a high fat diet on obese metabolism.

**Secondary Objective:** *To investigate the impact of obesity on skeletal muscle metabolism in response to lipid oversupply*

A high intake of dietary fat is one environmental factor contributing to high rates of obesity [2, 3]. In a report by the USDA, between 1977 and 2008, the amount of food being consumed that was prepared away from home increased from 18 to 32% in the U.S. and this food has been found to contain more calories, more saturated fat, and less essential minerals than food prepared in the home [29]. The nutritional patterns termed, the “Western diet” include high fat and high cholesterol foods largely in the form of “fast food” [30]. Metabolically healthy, lean individuals who are exposed to increased dietary fat intake will experience a slow increase in fat oxidation to match nutrient availability [31] as well will experience increase in expression of genes involved with fatty acid oxidation, such as peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ), PPAR $\gamma$ , and coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) in the skeletal muscle [32, 33]. These adaptations signify metabolic flexibility [34]. However, there is much evidence the ability to metabolically and molecularly adapt to a high fat diet is impaired in obese individuals [32, 35, 36]. The impaired metabolic flexibility of the obese, especially in those who follow the “Western diet”, puts them at increased risk of developing insulin resistance and type II diabetes [30, 32, 36, 37].

While there is much evidence skeletal muscle lipid metabolism is reduced with obesity and that the expression of genes linked with lipid oxidation have been found dampened in the obese in response to a lipid stimulus [32, 36, 38], few studies have directly compared the molecular response to a lipid stimulus in skeletal muscle of the lean and obese. Studying changes in gene expression of skeletal muscle will enhance the understanding of the pathophysiology of obesity.

### **Overall Purpose and Objectives**

The overall purpose of this thesis project was to investigate the impact of diet and exercise on various groups of individuals, which will aid in the development of more effective prevention and treatment methods in the fight against sedentary lifestyle, obesity, and chronic disease. The primary objectives of this study were to (a) to investigate gender differences in self-selected intensity and RPE in response to an acute HIFT circuit, (b) as well as assess whether factors such as baseline enjoyment and self-efficacy, age, gender, BMI, and  $VO_2\text{max}$ , can predict self-selected intensity and RPE. The secondary objective of this study was to investigate the impact of obesity on skeletal muscle metabolism in response to lipid oversupply by analyzing the responses of genes linked with fatty acid oxidation and inflammation in lean and obese subjects.

## Chapter 2: Methodology

This chapter describes the methods used to meet the Primary and Secondary Objectives of this thesis project. First the methods used to assess the Primary Object (to assess actual and perceived exertion during high-intensity function training) are described. Next the methods used to assess the Secondary Objective (to investigate the impact of obesity on skeletal muscle metabolism in response to lipid oversupply) are described.

**Methodology Used to Meet the Primary Objective:** *To assess actual and perceived exertion during high-intensity functional training*

### **Subjects**

Fifty seven participants were recruited from the campus of Western Kentucky University via e-mail and flyer advertisement. Forty seven of the fifty seven participants completed both study sessions. Participants were eligible if they had no contraindications to exercise and were classified as “low risk” according to ACSM guidelines based on their initial health screening questionnaire [39]. The study was approved by the Western Kentucky University Institutional Review Board.

### **Procedures**

The study consisted of two sessions separated by 24 hours or more. All participants were required to complete an informed consent form before participating in any study procedures. The informed consent form and all testing procedures were explained to each participant by the test administrator and all participants were informed

that they could drop out of the study at any time for any reason and would not be penalized or questioned.

### *Session 1*

During Session 1, eligibility was confirmed through health screening questionnaires. Each participant completed a Physical Activity Readiness Questionnaire to assess their ability to perform the experimental exercise bout. Participants completed a health history questionnaire to fulfill ACSM risk-factor assessment. Additionally questionnaires were administered to assess baseline exercise enjoyment and self-efficacy.

Baseline measures taken included: resting heart rate and blood pressure, height, weight, skin fold measurements to predict percent body fat, and a maximal aerobic capacity test. The participants' body weight (kg) and height (cm) was determined using a Detect-Medic Scale and attached stadiometer (Detecto Scales Inc., New York). Subjects were asked to remove their shoes and were wearing a t-shirt and shorts. Body Mass Index (BMI) was calculated from the height and weight measurements using the equation:  $BMI = \text{Weight (kg)} / \text{Height (m)}^2$ . The participants' body composition was measured using calibrated Lange skinfold calipers. Maximum aerobic capacity ( $VO_{2max}$ ) testing was conducted on a standard treadmill using the Bruce ramp protocol in the Exercise Physiology lab. Gases were measured by indirect calorimetry using Parvo Medics TrueOne 2400 metabolic cart (East Sandy, Utah). Participants were instructed to follow the protocol until exhaustion, which was defined as the point at which the subject could no longer continue running at the set pace. At this point, the treadmill was stopped immediately. A walking cool-down was optional.

Following baseline testing procedures, each participant was informed of the exercise bout that they would be required to complete during their next session. They were informed about wearing the portable metabolic analyzer during the course of the bout. The test administrator would then explain the exercise bout and go over each of the movements. The participant was informed to not consume any caffeine, alcohol, or drugs and to avoid heavy exercise within the 24 hours before Session 2.

### *Session 2*

At least 24 hours after Session 1, participants reported to the lab for the high-intensity functional exercise bout. Upon arrival to the lab, the participant was fitted with a Polar heart rate monitor and was instructed to complete a 5 minute walking treadmill warm-up at a self-selected pace. The participant was then fitted with the portable metabolic analyzer, the K4 Cosmed (Concord, California), to measure oxygen uptake and heart rate throughout the bout using telemetric capabilities. Participants were instructed to complete as many rounds of the body-weight exercises as possible during the 15 minute time allotment. The Omni-Walk/Run (0-10) RPE scale was used to assess perceived exertion at minute 7:30, minute 15 (immediate post-exercise), and 15 minutes post exercise (Session RPE). Upon completion of the 15 minute bout, the metabolic analyzer was removed from the participant and he or she was immediately asked to complete the exercise enjoyment and exercise self-efficacy questionnaires once again. After 15 minutes had passed from the time of the end of the bout, the researchers acquired the participant's Session RPE by asking the participant how they would rate the bout as a whole, considering the difficulty, on a scale of 0-10.

### *15 minute High Intensity Functional Training Circuit*

During the 15 minute exercise bout, participants were required to complete as many rounds as possible of 12 repetitions each of seven-functional-movements including the air squat, butterfly sit-up, push-up, alternating forward lunge, pull-up, step-up, and high knees (See Table 1). Participants were instructed on proper form for each movement prior to the bout and performed practice repetitions until proper form was understood to ensure safety. During the air squat, participants were asked to keep their core tight and descend into a bottom position while driving their knees out and keeping their heels flat on the floor until thighs were parallel to the floor or lower, and then they ascended to the standing position to begin another repetition.

To complete the butterfly sit-up, participants were asked to lie on their backs on a mat on the floor with their hips abducted and knees flexed until the soles of their feet were together. From the lying down position, subjects were to throw their extended arms forward until they came to an upright, seated position. Their hands touched the floor in front of their feet before returning to the starting position.

During the push-ups, participants were instructed to assume a plank position with arms extended, and then flex the elbows, while keeping the elbows in close to the torso, until the chest touched the floor. For those who could not complete standard push-ups, they completed a modified (inclined) push-up on an 18 inch bench in which they flexed the elbows until touching their chest to the bench while with their toes planted on the floor.

During the alternating forward lunge, participants were asked to start in a standing position and then step forward with one leg until the knee of the rearward leg came close to the floor. The knee of the forward leg was to remain in line with the forward ankle so that the lower leg was perpendicular with the floor. During the pull-up, participants who could not complete the standard pull-up were given the option to modify the movement and choose an Olympic ring row. Whether completing pull-ups or rows, the participants were required to fully extend the arms during the eccentric portion of the movement. The rings were suspended from the ceiling and adjusted to each participant's chest level. The participant then assumed a standing, diagonal position to the floor while holding on to the rings. With both feet planted and legs fully extended, the participant then pulled the rings to the chest, driving the elbows backward.

Alternating step-ups were completed using a 20 inch wooden box with a 12 inch box available to those who needed modification. During each step-up, the participant was asked to step onto the box with one foot and extend the knee until legs were fully extended and both feet were planted on the top of the box. Then the participant would step back down and alternate feet. High knees are a high-impact aerobic movement that requires the participant to bring the knee of one leg to hip level while standing and then alternating legs so that only one foot remains on the ground at a time.

**Table 1.** Experimental High Intensity Functional Training (HIFT) Exercise Protocol

---

Complete as many rounds as possible in 15 minutes

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12 air squats

12 butterfly sit-ups

12 push-ups (modified = 18 in incline push-ups)

12 forward alternating lunges

12 pull-ups (modified = ring row)

12 step ups (20" box)

12 high knees

---

### *Questionnaires*

International Physical Activity Questionnaire: The International Physical Activity Questionnaire (IPAQ) long form was administered to calculate MET-minutes of physical activity across leisure time, domestic, work-related, and transportation-related physical activity in order to assess degree of total physical activity. After total MET-minutes were calculated from each domain, participants could be classified into “low”, “moderate”, or “high” physical activity groups based on guidelines given by the IPAQ scoring guide. Participants also reported their average hours of sedentary time per day.

Satisfaction Scale for Physical Activity: To assess exercise enjoyment, participants completed a Likert-type Satisfaction Scale for Physical Activity (SSPA), which has been traditionally used to indicate level of “enjoyment” associated with two

items, 1) walking 2) moderate to vigorous PA [40]. The SSPA has been validated in adult populations [40, 41]. Two blocks, each representing one of the two items, is composed of three questions. The three-point scale modification was created to apply to a wider range of education levels in a previous study [40]. The walking and moderate to vigorous PA blocks were scored separately.

**Exercise Self-efficacy Questionnaire:** An exercise self-efficacy questionnaire was administered at baseline and immediately post-exercise. The questionnaire was based on a 3-point Likert scale with [1] being “none”, [2] being “a little”, and [3] being “a lot” in regard to how much the participant agreed with a statement regarding exercise self-efficacy. The 7-item scale was taken from a previously validated instrument [42, 43] and modified to apply more so to adult ages and to be on a three-point scale instead of a five-point scale. One item was removed due to redundancy after a wording modification was made.

#### *Rating of Perceived Exertion*

The rating of perceived exertion (RPE) is a widely used validated scale to assess exercise intensity [20]. The session RPE has been deemed an accurate reflection of the overall intensity of bout of exercise ranging from low to high-intensity modalities [44]. The Omni-Walk/Run (0-10) scale was used to assess perceived exertion at minute 7:30, immediately post exercise, and 15 minutes post exercise (Session RPE). The Walk/Run as opposed to the Omni-RES pictorial scale was selected because, although the protocol is classified as circuit-style body-weight resistance training, participants only used their own body weight and the bout design was intended to elicit a strong cardiovascular

response. The participants were instructed to rate their overall exertion on a scale of zero to 10. The Omni RPE scale, as opposed to the Borg (6-20 scale), was selected because it was easier for participants to report as they were able to hold up zero to 10 fingers to indicate their RPE while exercising at high-intensity with their mouths covered by the metabolic analyzer mask.

### **Statistical Analyses**

All analyses were performed using SPSS for Windows software (version 23.0; SPSS, Inc., Chicago, IL, USA). Descriptive statistics (mean  $\pm$  SD) were generated for baseline tests. All data were assessed for normality and equal variance. If data were not normally distributed and/or had unequal variance across comparison groups, non-parametric analyses were conducted. Independent samples t-tests were applied to detect differences in group means between males and females. A One-way repeated measures Analysis of Variance (ANOVA) was used to assess differences between genders in self-selected intensity across three time points during the bout (“beginning” = 0:00-4:59, “middle” = 5:00-9:59, and “end” = 10:00-15:00). Regression analyses were conducted to evaluate if such factors as age, gender, BMI, and  $\text{VO}_2\text{max}$ , as well as baseline enjoyment or self-efficacy, were significant predictors of exercise intensity ( $\%\text{VO}_2\text{max}$ ) or RPE immediately post exercise or 15 minutes post. Statistical significance for all comparisons was set at  $p < 0.05$ .

**Methodology Used to Meet the Secondary Objective:** *To investigate the impact of obesity on skeletal muscle metabolism in response to lipid oversupply*

## **Subjects**

Nine lean and ten obese women were recruited for the study. Participants were Caucasian, relatively young, free from overt disease, nonsmokers, and not taking medications known to alter metabolism. All procedures were approved by the East Carolina University Institutional Review Board.

### *Primary Human Skeletal Muscle Cell Cultures*

Skeletal muscle biopsies were obtained from the vastus lateralis under local anesthesia (0.01% lidocaine). Satellite cells were isolated from approximately 50-100mg of fresh muscle and cultured according to methods by Berggren et al. [45]. Cells were then sub-cultured into T-150 flasks and 10cm dishes. Upon reaching approximately 80-90% confluence, differentiation was induced by switching the growth media to low serum differentiation media containing 2% heat-inactivated horse-serum, 0.05mg/mL fetuin, and 5 $\mu$ g/mL gentamicin.

On day five of differentiation, myotubes were given fresh differentiation media supplemented with either control or lipid treatment. The control condition included 0.01% bovine serum albumin (BSA) $\pm$ 1mM carnitine. The lipid treatment included 250 $\mu$ M of a 1:1 ratio oleate:palmitate bound to 0.01% BSA $\pm$ 1mM carnitine. The supplementation samples were then incubated for 48 hours. Myotubes were harvested on day seven.

### *mRNA Quantification*

Total RNA was isolated using the RNeasy minikit (Qiagen, Valencia, CA) with on-column DNase digestion using the RNase-Free DNase Set (Qiagen, Valencia, CA) to remove residual DNA. RNA was quantified using the NanoDrop 1000 Spectrophotometer from ThermoScientific (Wilmington, DE, USA). Concentration was determined by measuring absorbance at 260nm and purity was assessed using the 260:280 ratio. Two µg RNA was reverse transcribed into cDNA and PCR was performed in triplicate using the Applied Biosystems ABI 7900HT sequence detection instrument and software with Taqman Universal PCR MasterMix and Taqman gene expression Assays (Applied Biosystems, Foster City, CA) in accordance with manufacturer instructions. Reaction were run with the following thermal cycling conditions: 50°C for 2 minutes; 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. mRNA content was measure in triplicate using the comparative Ct method with a multiplexed endogenous control (18S) and converted to a linear function by using a base 2 antilog transformation.

All chemical reagents/substrates were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated. Dulbecco's phosphate-Buffered Saline (DPBS), fetal bovine serum, heat-inactivated horse serum, gentamicin, 0.05% trypsin EDTA, and Kanks balanced Salt Solution were obtained from Invitrogen (Grand Island, NY, USA). Growth media and differentiation media consisted of low glucose (5mmol/L) Dulbecco's Modified Eagles Medium from Invitrogen. Type I collagen-tissue culture plates were obtained from Becton Dickinson (Franklin Lakes, NJ, USA). PCR reagents were purchased from Applied Biosystems (Foster City, CA, USA).

## **Statistical Analysis**

Statistical analyses were performed using PASW Statistics 19 Software (SPSS Inc., Chicago, IL, USA) on raw or log-transformed data. Comparisons between muscle cell cultures from lean and obese donors were performed with repeated measures ANOVA with emphasis on a “weight status” (lean, obese) X “treatment” (control, lipid-treated) interaction indicating the lean and severely obese individuals responded differently to lipid oversupply. All data met assumptions of sphericity and homogeneity of variance. Data are presented as mean  $\pm$  SEM.

## **Functional Analysis**

GeneGo MetaCore (<http://www.genego.com/>,Thompson-Reuters, MetaCore 6.19) was used to analyze the pathways of significant ( $p \leq 0.01$ ) genes from the “weight status” X “treatment” analysis. Enrichment analyses were used to show how different ontology terms (pathways, processes, disease biomarkers) are relatively represented by the expression data by mapping (matching identifiers). Enrichment is calculated as a probability of the observed overlap between genes/proteins from the experiment and the selected ontology term [46].

## Chapter 3: Actual and Perceived Exertion during High-Intensity Functional Training in Males and Females

### **Abstract**

The purposes of this study were: 1) to assess self-selected and perceived exercise intensity during High-Intensity Functional Training (HIFT) between males and females and 2) to determine variables that predict self-selected exercise intensity (%VO<sub>2</sub>max) and/or perceived intensity (RPE). Twenty-four males and twenty-nine females completed baseline testing and a 15min HIFT circuit wearing a metabolic analyzer. RPE was assessed half-way through (7:30), immediately post (15:00), and 15 minutes post (Session RPE) exercise. The results of this study demonstrate that females exercised at a significantly higher self-selected exercise intensity indicated by %VO<sub>2</sub>max over the course of the whole bout. However, women also reported a significantly lower immediate-post exercise RPE ( $p < 0.05$ ). The results of regression modeling revealed gender, BMI, age, VO<sub>2</sub>max, baseline exercise enjoyment and self-efficacy did not significantly predict self-selected intensity indicated by %VO<sub>2</sub>max at any time point (beginning, middle, or end). The model did not significantly predict RPE. In conclusion, females may self-select a higher intensity during HIFT while not perceiving the work as intensely as male counterparts. Health and fitness professionals should consider the discrepancy between genders in self-regulating intensity when prescribing HIFT.

## **Introduction**

Body-weight resistance training and high-intensity interval training are two top fitness trends according to a 2015 survey [5]. A high-intensity circuit-style workout, that may utilize only one's body weight, termed high-intensity functional training (HIFT) [6], is an ideal exercise modality in terms of economy and functionality. Several body-weight exercises mimic movements used in activities of daily living (e.g. squats, step-ups, etc.); providing functional fitness benefits. HIFT requires little to no equipment and can be done in a variety of environments (e.g. outside, at home) and in relatively small spaces. There are few studies investigating HIFT, but one study reported that a similar modality led to cardiovascular responses very similar to a sprint interval training protocol consisting of repeated Wingate tests in college-aged men and women [11]. It is a priority of health and fitness professionals to prescribe exercise that is effective while also facilitating adherence. High-intensity functional training is a modality that may meet these criteria, however, it is a relatively new modality that warrants further investigation.

Some important factors still to be investigated regarding HIFT are the self-selected intensity and perceived exertion ratings achieved by men and women during a HIFT bout. These factors influence the effectiveness and adherence rates of individuals to most any exercise modality [10, 12, 16, 47] but have yet to be evaluated for HIFT. Regarding effectiveness, intensity is a key component influencing the extent to which exercise training will lead to physiological adaptations (e.g. improved aerobic capacity, muscular strength). While there are no specific guidelines prescribing duration and intensity to achieve optimal physiological benefits from HIFT, it is understood that as

exercise intensity increases, duration can concomitantly decrease while providing positive muscular adaptations [48].

Despite high intensities, when individuals are permitted to self-select their work rate, they report greater enjoyment of exercise [8-10]. However, it is not known what exercise intensity men and women will self-select during a HIFT circuit. This is important because working at an intensity that is too high or too low can have undesirable outcomes (e.g. overtraining; lack of beneficial physiologic adaptations). A bioenergetics theory suggests that individuals will self-select an intensity level that maximizes efficiency of energy utilization during walking and running exercise [12, 16]. Still, investigation of the intensity men and women will self-select is needed to ensure the effectiveness of this modality is maintained when performed without direct professional supervision, such during a home-based exercise program.

Regarding adherence, an individual's rating of perceived exertion (RPE) to a bout of exercise is an important consideration, as lower perceived exertion is associated with higher exercise enjoyment [16] and exercise enjoyment contributes to increased exercise adherence [17]. RPE has a positive correlation with aerobic and resistance exercise intensity [21-23]. Interestingly, one study showed that HIFT, when compared to anaerobic sprint-training, elicited lower RPE values, independent of exercise intensity and duration. It is possible that, despite exercising at a high intensity, ratings of perceived exertion during HIFT may be relatively lower, resulting in increased exercise enjoyment, and subsequent increased exercise adherence. Additionally, exercise enjoyment and exercise self-efficacy are known predictors of physical activity (PA) behavior [9, 27, 49]. To our knowledge, no study has reported the level of exercise enjoyment or self-efficacy

among men and women in response to an acute bout of HIFT. HIFT may be the ideal exercise modality to combat perceived barriers to regular PA (and therefore be associated with an increase in exercise self-efficacy) [50], while also resulting in lower RPE and increased enjoyment (therefore contributing to increased effectiveness and adherence).

Previous research suggests there may be variation between the exercise intensity men and women self-selected as well as the RPE they will report while completing the same exercise. During 20 minutes of aerobic exercise at a self-selected intensity, males were found to work at a significantly higher  $VO_2$  while females achieved significantly higher heart-rates [19], displaying gender discrepancies in self-selected intensity. O'Connor et al., observed that young women reported a lower perceived exertion than young males during fatiguing elbow flexion contractions [28]. For this reason, examining the impact of gender on self-selected intensity and RPE during HIFT is warranted before professionals can safely and effectively prescribe this modality to patient populations. Therefore, the primary objectives of this study were to 1) investigate self-selected exercise intensity and RPE during a HIFT circuit and 2) to assess the influence of gender on measures of self-selected exercise intensity and RPE. The secondary objective of this study was to investigate factors (e.g. gender, exercise enjoyment, exercise self-efficacy) that predict self-selected exercise intensity and RPE. It is hypothesized that self-selected exercise intensity and RPE during HIFT will be significantly different between males and females.

## **Methods**

### *Experimental Approach to the Problem*

Male and female participants were recruited to participate in the experimental exercise bout to assess gender differences in self-selected intensity and perceived exertion, measured by RPE, during a HIFT circuit. Gender was the independent variable and self-selected exercise intensity and RPE were the dependent variables. The percentage of maximal aerobic capacity (%VO<sub>2</sub>max) was the dependent variable used as a marker of self-selected exercise intensity. Session 1 consisted of baseline testing and questionnaires. Session 2 consisted of the HIFT circuit; scheduled at least 24 hours after Session 1.

### **Subjects**

Forty-seven participants were recruited from the campus of Western Kentucky University. The study was approved by the University Institutional Review Board. Following a comprehensive explanation of procedures all participants signed written informed consent. To be eligible, participants had to be free of injury and be classified as “low risk” according to ACSM risk stratification or receive physician approval for participation if above “low risk”.

### **Procedures**

#### *Session 1*

During Session 1, eligibility was confirmed through health screening questionnaires. Each participant completed a Physical Activity Readiness Questionnaire

(PARQ) to assess their ability to perform the experimental exercise bout. Participants completed a health history questionnaire to complete ACSM risk-factor assessment. Two participants were considered above “low” risk and physicians consent was obtained prior to their completion of Session 2. Physical activity level was assessed using the International Physical Activity Questionnaire (IPAQ). Additionally questionnaires were administered to assess baseline exercise enjoyment and self-efficacy. Baseline measures were taken, including resting heart rate and blood pressure, height, weight, and VO<sub>2</sub>max test. The max test using the Bruce treadmill protocol indicated an individual’s maximal aerobic capacity (VO<sub>2</sub>max).

### *Session 2*

At least 24 hours after Session 1, participants reported to the lab for the HIFT session. Upon arrival to the lab, the participant was fitted with a Polar heart rate monitor and was instructed to complete a 5 minute walking treadmill warm-up at a self-selected pace. Participants were fitted with the K4 COSMED (Concord, California) portable metabolic analyzer to measure oxygen consumption and carbon dioxide production throughout the bout using telemetric capabilities. Participants were instructed to complete as many rounds of the body-weight exercises as possible during the 15 minute time allotment. The Omni-Walk/Run (0-10) scale was used to assess RPE at minute 7:30, minute 15, and 15 minutes post exercise (Session RPE). Upon completion of the 15 minute bout, the COSMED was removed from the participant and he/she was immediately asked to complete the exercise enjoyment and exercise self-efficacy questionnaires once again. Fifteen minutes post-exercise, the participant’s Session RPE was assessed by asking the participant to report an overall RPE for the exercise bout.

### *Self-selected Exercise Intensity*

The percentage of maximal aerobic capacity (%VO<sub>2</sub>max) was the variable used to represent self-selected exercise intensity. For analysis, the 15 minute bout was divided into three five-minute segments in order to investigate the participants' self-selected exercise intensity at the beginning (minutes 0:00-4:59), middle (minutes 5:00-9:59), and end (minutes 10:00-15:00) of the HIFT circuit. Therefore, %VO<sub>2</sub>max for each participant was estimated by dividing the average VO<sub>2</sub> assessed during the three time segments (beginning, middle, end) divided by their VO<sub>2</sub>max to calculate the participant's "Beginning %VO<sub>2</sub>max," "Middle %VO<sub>2</sub>max," and "End %VO<sub>2</sub>max".

### *Rating of Perceived Exertion*

The rating of perceived exertion (RPE) is a widely used validated scale to assess exercise intensity [20]. The session RPE has been deemed an accurate reflection of the overall intensity of bout of exercise ranging from low to high-intensity modalities [44]. The Omni-Walk/Run (0-10) scale was used to assess perceived exertion at minute 7:30, minute 15(immediate post-exercise), and 15 minutes post exercise (Session RPE). The Walk/Run as opposed to the Omni-RES pictorial scale was selected due to the aerobic nature of the bout's design. The participants were instructed to rate their overall exertion on a scale of zero to 10.

### *Questionnaires*

Satisfaction Scale for Physical Activity: To assess exercise enjoyment, participants completed a Likert-type Satisfaction Scale for Physical Activity (SSPA), which has been traditionally used to indicate level of "enjoyment" associated with two

items, 1) walking 2) moderate to vigorous PA [40]. The SSPA has been validated in adult populations [40, 41]. Two blocks, each representing one of the two items, is composed of three questions. The three-point scale modification was created to apply to a wider range of education levels in a previous study [40]. The walking and moderate to vigorous PA blocks were scored separately.

**Exercise Self-efficacy Questionnaire:** An exercise self-efficacy questionnaire was administered at baseline and immediately post-exercise. The questionnaire was based on a 3-point Likert scale with [1] being “none”, [2] being “a little”, and [3] being “a lot” in regard to how much the participant agreed with a statement regarding exercise self-efficacy. The 7-item scale was taken from a previously validated instrument [42, 43] and modified to apply more so to adult ages and to be on a three-point scale instead of a five-point scale. One item was removed due to redundancy after a wording modification was made.

### **Statistical Analyses**

All analyses were performed using SPSS for Windows software (version 23.0; SPSS, Inc., Chicago, IL, USA). Descriptive statistics (mean  $\pm$  SD) were generated to show group characteristics. All data were assessed for normality and equal variance.

Independent samples t-tests were applied to detect differences in group means between males and females. A One-way repeated measures Analysis of Variance (ANOVA) was used to detect differences in self-selected intensity between males and females across three five-minute time segments during the bout. Self-selected exercise intensity was determined by analyzing %VO<sub>2</sub>max. Regression analyses were conducted to evaluate if

such factors as age, gender, BMI, and VO<sub>2</sub>max, as well as baseline enjoyment or self-efficacy, were significant predictors of self-selected intensity across all three time segments (beginning, middle, and end) during the bout, or RPE immediately post exercise or 15 minutes post (Session RPE). All data were assessed for linearity, independence of residuals, homoscedasticity, and normality of residuals. Statistical significance for all comparisons was set at  $p < 0.05$ .

## Results

### *Participant Characteristics*

Participant characteristics are presented in Table 1. At baseline, there was no significant age difference between males or females, however males had a significantly higher BMI ( $t(45)=2.216$ ,  $p = 0.03$ ) and VO<sub>2</sub>max than females ( $t(51)= 6.322$ ,  $p = 0.00$ ).

**Table 1.** Participant Characteristics (mean  $\pm$  SD).

	Men (n = 22)	Women (n = 25)	Combined (n = 47)
Age (y)	27.0 $\pm$ 11.2	24.3 $\pm$ 09.5	30.0 $\pm$ 1.1
Height (cm)	176.0 $\pm$ 07.4**	165.7 $\pm$ 05.0	168.6 $\pm$ 7.4
Weight(kg)	76.1 $\pm$ 09.4**	65.5 $\pm$ 12.1	68.7 $\pm$ 12.3
BMI(kg·m <sup>-2</sup> )	24.0 $\pm$ 05.6*	22.1 $\pm$ 02.6	24.0 $\pm$ 4.1
VO <sub>2</sub> max (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	52.4 $\pm$ 05.6**	43.6 $\pm$ 03.9	48.3 $\pm$ 9.8

BMI = Body Mass Index; VO<sub>2</sub>max = maximal oxygen consumption

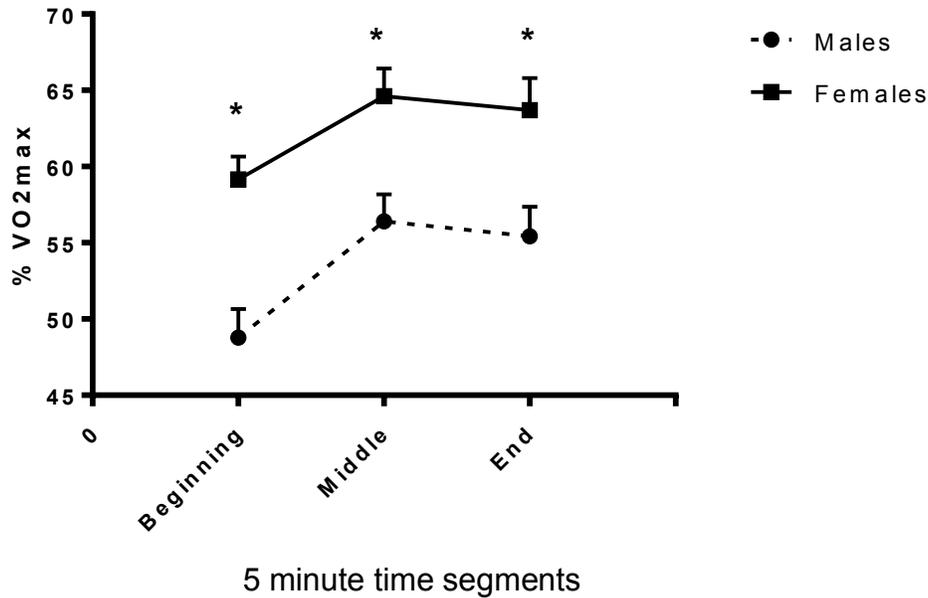
\* Significant difference ( $p < 0.05$ ) between males and females.

\*\* Significant difference ( $p < 0.001$ ) between males and females.

### *Self-Selected Intensity*

There was a significant difference in self-selected exercise intensity between males and females as indicated by a significant between subjects effect in %VO<sub>2</sub>max ( $F(1,44) = 12.46, p = 0.00$ ). There was also a significant main effect for time during the HIFT (beginning, middle, and end) ( $F(1,44) = 35.19, p = 0.00$ ). Paired samples t-tests revealed, as a whole, participants worked out at a significantly ( $p < 0.00$ ) greater %VO<sub>2</sub>max during the middle and end of the HIFT session compared to the beginning. The beginning %VO<sub>2</sub>max (54.2 +/- 9.6) was approximately 6% lower compared to the middle %VO<sub>2</sub>max (60.6 +/- 9.6) and the end %VO<sub>2</sub>max (59.6 +/- 10.6). There were no significant differences in %VO<sub>2</sub>max between the middle and end time segments. Independent samples t-tests showed females consistently worked at a significantly higher %VO<sub>2</sub>max compared to males at the beginning (females-59.0±7.5%; males-52.2±6.3%), (females-63.3±9.3%; males-60.0±7.0%), and end (females-62.9±10.0%; males-59.7±5.9%) of the HIFT bout. Females worked at a %VO<sub>2</sub>max that was approximately 9% higher than the %VO<sub>2</sub>max the males worked throughout the HIFT circuit. Figure 1 shows this comparison.

**Figure 1.** %VO<sub>2</sub>max as a marker of self-selected exercise intensity comparing males and females during three five-minute time segments



As a whole, participants worked at a significantly higher %VO<sub>2</sub>max from “Beginning” to “Middle”, and between “Beginning” and “End”, with no significant difference in %VO<sub>2</sub>max between “Middle” and “End”.

“Beginning” = minutes 0:00-4:59, “Middle” = minutes 5:00-9:59, and “End” minutes 10:00-15:00).

Data are presented as means ± SEM.

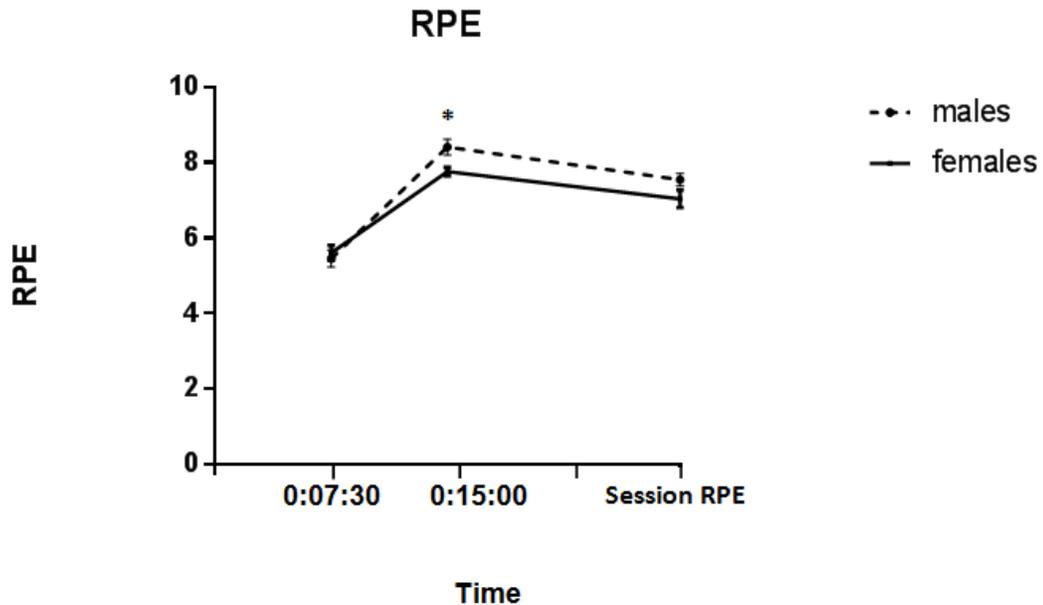
\*Significant difference between males and females ( $p < 0.00$ ).

#### *Rating of Perceived Exertion (RPE)*

Males reported a higher RPE (females-8.0±0.7; males-8.0±1.3) immediately post exercise compared to females ( $t(45)=2.725, p = 0.01$ ). RPE half-way through (females-6.0±1.0; males-6.0±0.9) and the Session RPE (females-7.0±1.2; males-8.0±0.8) were not significantly different between males and females. Figure 2 shows the RPEs between males and females during after the exercise bout. A Pearson correlation showed no

significant correlation between RPE and %VO<sub>2</sub>max at minute 7:30 or immediately post exercise.

**Figure 2.** Ratings of Perceived Exertion (RPE) between males and females



7:30 = half-way through; 15:00 = immediate post-exercise; Session RPE taken 15 minutes post exercise.

Data are presented as mean  $\pm$  SEM.

\*Significant difference between males and females ( $p < 0.05$ ).

### *Exercise Enjoyment*

The SSPA used to measure exercise enjoyment contains two items, 1) enjoyment regarding walking and 2) enjoyment regarding moderate to vigorous physical activity, each containing three questions worth 2 points each. The items were score separately.

The SSPA was administered at baseline and immediately post exercise. Males had a slightly higher enjoyment level compared to females in regard to moderate to vigorous physical activity immediately after the exercise bout ( $t(44)=2.386, p = 0.02$ ), equal

variances not assumed. There were no other significant differences in enjoyment between males and females. See Table 2.

*Exercise Self-Efficacy*

There was no significant difference in baseline or immediately post-exercise self-efficacy scores between the males and females. See Table 3.

**Table 2.** Exercise Enjoyment and Self-efficacy Scores at Baseline and Immediately Post-exercise

	Enjoyment of Moderate-Vigorous Activity Items		Enjoyment of Walking Items		Exercise Self-efficacy	
	Baseline	Post-exercise	Baseline	Post-exercise	Baseline	Post-exercise
Males	6.0±0.7	6.0±0.4*	5.0±0.4	5.0±0.7	12.0±1.7	12.0±1.2
Females	5.0±1.3	5.0±1.2	6.0±0.1	5.0±0.8	11.0±2.4	11.0±2.2

Data are presented as Means ± SD

\* $p = 0.02$

*Predictors of Self-Selected Exercise Intensity*

Models including the independent variables age, gender, BMI, VO<sub>2</sub>max, baseline enjoyment scores for the walking and moderate-to vigorous items, as well as baseline self-efficacy, were not found to significantly predict %VO<sub>2</sub>max during the beginning, middle, or end of the circuit, nor did it successfully predict RPE at minute 7:30, immediately post exercise, or the Session RPE. Regression model results in the form of slope coefficients and confidence intervals for %VO<sub>2</sub>max and RPE are presented in Tables 4 and 5 respectively.

**Table 3.** Linear regression model using select demographic characteristics baseline test results to estimate the degree to which such factors predict self-selected %VO<sub>2</sub>max (N=47)

<b>%VO<sub>2</sub>max Model</b>						
<b>Factors</b>	<b>Beginning</b>		<b>Middle</b>		<b>End</b>	
	<b>B</b>	<b>CI</b>	<b>B</b>	<b>CI</b>	<b>B</b>	<b>CI</b>
<b>Gender</b>	5.38	(-6.44-17.19)	-1.65	(-14.42-11.12)	0.76	(-12.95-14.46)
<b>BMI</b>	-0.12	(-1.07-0.84)	0.10	(-0.91-1.11)	0.29	(-0.79-1.37)
<b>Age</b>	0.12	(-0.24-0.48)	-0.08	(-0.47-0.30)	-0.04	(-0.46-0.39)
<b>VO<sub>2</sub>max</b>	-0.28	(-1.10-0.84)	-4.23	(-1.31-0.45)	-0.16	(-1.10-0.79)
<b>High- intensity enjoyment</b>	-2.94	(-8.37-2.49)	-5.86	(-11.72—0.00)	-3.92	(-10.47-2.63)
<b>Walking enjoyment</b>	-2.00	(-6.93-2.92)	-0.37	(-5.69-4.95)	0.51	(-5.22-6.23)
<b>Self-efficacy</b>	1.62	(-0.94-4.18)	3.18*	(0.12-5.95)	2.58	(-0.43-5.59)

\*  $p < 0.05$

**Table 4.** Linear regression model using select demographic characteristics baseline test results to estimate the degree to which such factors predict RPE(N=47)

<b>RPE Model</b>						
<b>Factors</b>	<b>07:30</b>		<b>15:00</b>		<b>Session RPE</b>	
	<b>B</b>	<b>CI</b>	<b>B</b>	<b>CI</b>	<b>B</b>	<b>CI</b>
<b>Gender</b>	-0.28	(-1.83-1.27)	-0.17	(-1.44-1.11)	-0.59	(-2.12-0.94)
<b>BMI</b>	0.00	(-0.12-0.13)	0.04	(-0.06-0.15)	0.01	(-0.12-0.13)
<b>Age</b>	-0.03	(-0.08-0.02)	0.02	(-0.02-0.06)	0.04	(-0.01-0.09)
<b>VO<sub>2</sub>max</b>	-0.02	(-0.12-0.09)	-0.03	(0.12-0.06)	-0.01	(-0.11-0.10)
<b>High- intensity enjoyment</b>	0.45	(-0.26-1.16)	0.26	(-0.32-0.85)	0.04	(-0.70-0.78)
<b>Walking enjoyment</b>	-0.15	(-0.80-0.49)	-0.67*	(-1.20-0.14)	-0.30	(-1.05-0.45)
<b>Self-efficacy</b>	-0.13	(-0.47-0.20)	0.12	(-0.39-0.16)	-0.14	(-0.48-0.20)

\*  $p < 0.05$

## Discussion

Supportive of the hypothesis, the major findings of this study indicate a significant difference in self-selected exercise intensity and RPE between males and females. Females tended to self-select a higher exercise intensity over the course of a 15 minute bout of HIFT compared to males, as indicated by a significantly higher %VO<sub>2</sub>max (See Fig. 1A) ( $p < 0.05$ ). Interestingly, RPE was higher in males immediately post-exercise despite them working at a relatively lower intensity than females (See Fig. 2).

Kravitz et al., found males worked at a higher %VO<sub>2</sub>max while females worked at a higher heart rate during the same aerobic exercise protocol in which intensity was self-selected by participants [51]. The females in this study worked at a higher intensity than males at the beginning, middle, and end of the bout (See Fig. 1). It is clear gender plays a role in intensity selection made by an individual during high-intensity exercise and this should be considered by health and fitness professionals who wish to employ a HIFT in an exercise program for their clients.

Additionally, males reported a significantly higher immediate post-exercise enjoyment score for moderate to vigorous activity (See Table 3.), despite past findings that suggest higher perceived exertion is less enjoyable [25]. This may be explained by past observations in a review by Ekkekakis and Petruzzello, who found a negative correlation existed between RPE and enjoyment, however, the correlation was positive when intensity was self-selected rather than prescribed [14]. During investigation of a HIFT interval protocol compared to repeated Wingate tests, males and females reported lower RPE in response to the HIFT bout compared to a high-intensity sprint interval bout using a stationary cycle [11]. While the current study did not directly measure adherence,

the results suggest that because the modality allows individuals to self-select their intensity it may result in higher enjoyment even in those who experience higher perceived exertion, and thus increase the likelihood of adherence.

The present study holds some limitations. In future studies, it may be more beneficial to obtain RPE during each minute of a bout alongside blood lactate in order to gain a better assessment of actual and perceptual response to the bout. Using a bipolar scale of affect that measures both pleasure and displeasure minute-by-minute may have provided of a better idea of the participants' feelings toward the exercise modality. Future studies should compare HIFT to other high-intensity modalities in terms of cardiovascular response, RPE, and affect, as well as employ a long-term intervention in order to determine if HIFT is in fact an effective modality that is perceived as less intense and more enjoyable, thus making it the optimal form of training for increasing adherence.

In conclusion, the results of this study indicated that gender was a significant factor in self-selected exercise intensity and RPE and that females may work at higher intensities than males while perceiving the work as less intense. This information is useful for health and fitness professional when considering prescribing HIFT to male and female clients.

### **Practical Applications**

According to the results of this study, females may tend to self-select a higher relative exercise intensity during high-intensity, circuit-style training like HIFT compared to males, and this should be considered when prescribing this exercise modality. While there are relatively few investigations of HIFT and its impact on measures of fitness

compared to other high-intensity modalities, there is evidence HIFT is both comparable to high-intensity sprint interval training in cardiovascular response [11] and perceived as more enjoyable than lower intensity exercise, even among those who are less physical active [6]. As enjoyment is a key motivating factor people give for exercising [52], and enjoyment is associated with a lower perceptual response during exercise [25], HIFT should be considered for exercise prescription when the aim is to retain physiological adaptations while moderating tolerability of exercise, as well as accommodating individuals when there are perceived barriers to exercise, such as lack of access to equipment and bad weather.

## Chapter 4: Differential Response of Metabolic and Inflammatory Gene Expression to a High Fat Diet in Cell Cultures of Lean and Severely Obese Women

### **Abstract**

The inflammatory response is a promising avenue in finding mechanisms linking obesity, diet, and disease. The purpose of this study was to determine whether metabolic and/or inflammatory genes or gene networks were differentially regulated in human skeletal muscle cell cultures (HskMC) of lean and severely obese women as a response to lipid oversupply. We hypothesized there would be differential regulation of metabolic/inflammatory genes between lean and obese HskMC after a lipid treatment. Lean (n=9) and severely obese (n=10) Caucasian women participated in the study. RNA in HskMC from lean and severely obese women were isolated after a 48hr lipid incubation (250 $\mu$ M oleate:palmitate), designed to imitate a high-fat diet. Genome-wide expression data was acquired using Illumina HumanHT-12 v4.0 Expression BeadChip. Network and pathway analysis was done using MetaCore software. Twenty-eight of the significantly differentially regulated genes by the high-fat treatment were associated with inflammatory processes and metabolism. The significantly differentially regulated genes were found in such pathways as Toll-like receptor 2 and 4 signaling, the HMGB/RAGE pathway, and such processes as immune response regulation. In conclusion, the results indicate genes that were differentially regulated between the lean and obese were found to be part of the signaling of several immune response pathways and are mechanistic in inflammation which may suggest a high fat diet may help to facilitate increased expression of inflammatory genes in the obese skeletal muscle, which may help in understanding the pathophysiology linking obesity to metabolic diseases.

## Introduction

The prevalence of severe obesity and associated metabolic diseases, such as type II diabetes, heart disease, and certain kinds of cancer, continue to pose as a major concern world-wide. Additionally, the prevalence of severe obesity ( $BMI \geq 40$ ) continues to rise [53]. These alarming statistics have inspired much investigation of the obese metabolism. It appears the factors driving this epidemic are more complex than “exercising more and eating less”. The resulting body of research has elucidated that both genetics and environmental factors play a role in this epidemic [54]. One such environmental factor may be a high-fat diet (HFD).

There is substantial evidence that the ability to adapt to high fat intake is impaired in the severely obese [55-57]. This has been termed metabolic inflexibility [58]. Although impaired fat oxidation appears to increase an individual’s fat storing tendency and lead to insulin resistance and ultimately metabolic and cardiovascular disease, the precise biological factors orchestrating the pathophysiology of obesity-related disease states is still not fully understood. There is still need for investigation of changes at the molecular level in metabolically active tissues, such as skeletal muscle, in order to understand the factors at play in obesity-related diseases.

Recent investigations of severely obese individuals suggest epigenetic mechanisms linking skeletal muscle fat oxidation to increased fat deposition [37, 38]. Genes targeted as potential mechanisms have included peroxisome proliferator-activated receptor gamma coactivator 1-alpha ( $PGC-1\alpha$ ), the peroxisome proliferator-activated receptor (PPAR) family, and the nuclear respiratory factor family, due to their role in fat

metabolism [32, 38]. Skeletal muscle oxidative capacity is regulated by an interaction between 5-AMP-activated protein kinase (AMPK), silent mating type information regulation 2 homolog 1 (SIRT1) [59] which are intracellular fuel sensors that respond to changes in nutrient availability [60], and PGC1- $\alpha$  which coactivates transcription factors and nuclear receptors [61, 62]. However, the response of gene networks to increased fat intake in the severely obese is still being uncovered. Boyle et al. compared the molecular adaptations to a HFD between lean and obese humans and found a differential response in genes coordinating oxidative metabolism in the skeletal muscle, including pyruvate dehydrogenase kinase isozyme 4 (PDK4) and PGC-1 $\alpha$  [32].

In addition to genes involved in fat oxidation, expression of inflammatory factors have been targeted as well. Chronic tissue inflammation is an important cause of obesity-induced insulin resistance [63], and is considered a risk factor for metabolic syndrome, type II diabetes and other metabolic dysfunctions [64, 65]. This chronic, low-grade state of inflammation associated with obesity has been termed metainflammation [34] and is characterized by an increase in cytokine proteins and gene expression. While cytokines have been found to regulate system-wide inflammation through endocrine action, less investigation has been made into the molecular changes in skeletal muscle in response to a high-fat diet. Inducers of inflammation in skeletal muscle include pro-inflammatory cytokines and saturated fatty acids which induce increases in intracellular ceramides, triglyceride lipolysis, free fatty acid release, and depressed fatty acid oxidation [66]. Saturated fatty acids have been found to activate inflammatory signaling pathways either directly, through interaction with members of toll-like receptor (TLR) family, or

indirectly, through secretion of cytokines such as tumor-necrosis factor-alpha (TNF- $\alpha$ ), interleukin 1beta (IL-1 $\beta$ ), and IL-6 [67].

While statistical methods are most often applied for identification and quantization of the proteins involved in the obese metabolic processes and related diseases, fewer investigations have taken a “functional approach”. Statistically processed proteomics profiles usually do not allow investigators to distinguish between diseases while functional and pathway analyses that use accumulated knowledge about relationships between proteins allows more interpretations of data [46]. Therefore, the following study was designed to compare the responses of genes linked with fatty acid oxidation and inflammation and to determine what gene networks are associated with mRNA changes in skeletal muscle to lipid oversupply in lean and obese subjects in order to potentially further the identification of mechanisms linking obesity to disease states.

This study focused on genes known to be involved in metabolism and inflammatory signaling in order to help uncover mechanistic links between obesity, high-fat intake, and diseases. It was hypothesized that a differential response would occur between lean and obese gene expression and activated disease pathways.

## **Methods**

### ***Subjects***

Participants were Caucasian, relatively young, free from overt disease, nonsmokers, and not taking medications known to alter metabolism. All procedures were approved by the East Carolina University Institutional Review Board.

## ***Procedures***

### *Primary Human Skeletal Muscle Cell Cultures*

Skeletal muscle biopsies were obtained from the vastus lateralis under local anesthesia (0.01% lidocaine). Satellite cells were isolated from approximately 50-100mg of fresh muscle and cultured according to methods by Berggren et al.[45]. Cells were then sub-cultured into T-150 flasks and 10cm dishes. Upon reaching approximately 80-90% confluence, differentiation was induced by switching the growth media to low serum differentiation media containing 2% heat-inactivated horse-serum, 0.05mg/mL fetuin, and 5 $\mu$ g/mL gentamicin.

On day five of differentiation, myotubes were given fresh differentiation media supplemented with either control or lipid treatment. The control condition included 0.01% bovine serum albumin (BSA) $\pm$ 1mM carnitine. The lipid treatment included 250 $\mu$ M of a 1:1 ratio oleate:palmitate bound to 0.01% BSA $\pm$ 1mM carnitine. The supplementation samples were then incubated for 48 hours. Myotubes were harvested on day seven.

### *mRNA Quantification*

Total RNA was isolated using the RNeasy minikit (Qiagen, Valencia, CA) with on-column DNase digestion using the RNase-Free DNase Set (Qiagen, Valencia, CA) to remove residual DNA. RNA was quantified using the NanoDrop 1000 Spectrophotometer from ThermoScientific (Wilmington, DE, USA). Concentration was determined by measuring absorbance at 260nm and purity was assessed using the 260:280 ratio. Two  $\mu$ g RNA was reverse transcribed into cDNA and PCR was performed

in triplicate using the Applied Biosystems ABI 7900HT sequence detection instrument and software with Taqman Universal PCR MasterMix and Taqman gene expression Assays (Applied Biosystems, Foster City, CA) in accordance with manufacturer instructions. Reactions were run with the following thermal cycling conditions: 50°C for 2 minutes; 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. mRNA content was measured in triplicate using the comparative Ct method with a multiplexed endogenous control (18S) and converted to a linear function by using a base 2 antilog transformation.

All chemical reagents/substrates were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated. Dulbecco's phosphate-Buffered Saline (DPBS), fetal bovine serum, heat-inactivated horse serum, gentamicin, 0.05% trypsin EDTA, and Kanks balanced Salt Solution were obtained from Invitrogen (Grand Island, NY, USA). Growth media and differentiation media consisted of low glucose (5mmol/L) Dulbecco's Modified Eagles Medium from Invitrogen. Type I collagen-tissue culture plates were obtained from Becton Dickinson (Franklin Lakes, NJ, USA). PCR reagents were purchased from Applied Biosystems (Foster City, CA, USA).

### **Statistical Analysis**

Statistical analyses were performed using PASW Statistics 19 Software (SPSS Inc., Chicago, IL, USA) on raw or log-transformed data. Comparisons between muscle cell cultures from lean and obese donors were performed with repeated measures ANOVA with emphasis on a "weight status" (lean, obese) X "treatment" (control, lipid-treated) interaction indicating the lean and severely obese individuals responded

differently to lipid oversupply. All data met assumptions of sphericity and homogeneity of variance. Data are presented as mean  $\pm$  SEM.

### Functional Analysis

GeneGo MetaCore (<http://www.genego.com/>,Thompson-Reuters, MetaCore 6.19) was used to analyze the pathways of significant ( $p \leq 0.01$ ) genes from the “weight status” X “treatment” analysis. Enrichment analyses were used to show how different ontology terms (pathways, processes, disease biomarkers) are relatively represented by the expression data by mapping (matching identifiers). Enrichment is calculated as a probability of the observed overlap between genes/proteins from the experiment and the selected ontology term [46].

### Results

#### *Participant Characteristics*

Participant characteristics are reported in Table 1. The severely obese females had a significantly higher body mass and BMI than the lean. While not significant, the obese females were slightly older than the lean.

**Table 1:** Participant Demographics

Characteristic	Lean (n=9)	Obese (n=10)
Age, yr	23.4 $\pm$ 1.5	30.2 $\pm$ 2.6
Stature, cm	164.7 $\pm$ 1.8	165.5 $\pm$ 2.2
Mass, kg	62.6 $\pm$ 1.3	113.7 $\pm$ 6.3*
BMI, kg/m <sup>2</sup>	22.8 $\pm$ 0.7	41.3 $\pm$ 1.5*

\*Significant difference ( $P \leq 0.05$ ) between lean and obese groups.

### *Gene Expression*

The results of an Analysis of Covariance (ANCOVA) controlling for age revealed significant differential expression of several metabolic and inflammatory genes between the lean and obese. A list of differentially expressed genes between the lean and obese in response to the lipid treatment can be found in Table 2. Several genes that were differentially expressed between the lean and obese in response to the lipid treatment included those involved in the immune response/inflammatory signaling, mitochondrial function, and the regulation of glycolysis. Of the 21 genes differentially regulated in response to lipid oversupply between the lean and severely obese women, 13 genes were increased more in the severely obese compared to the lean. Of these 13 genes, 8 genes were categorized as pro-inflammatory, 3 anti-inflammatory, 1 anti-lipolytic, and 1 tumor suppressor gene. Eight (of the 21) genes increased more in the lean compared to the obese women. Of these 8 genes, 6 were classified as pro-inflammatory and 2 were considered to have an anti-inflammatory effect.

**Table 2:** Gene Expression from HSkMC Between Lean and Obese in Response to Lipid Treatment

<b>Symbol</b>	<b>Category</b>	<b>Gene Name</b>	<b>p-value</b>	<b>Fold change</b>	<b>Description</b>
TRPM3	Pro-inflammatory	Transient receptor potential cation channel subfamily M member 3	0.00272213	1.66673	The encoded protein mediates Ca <sup>2+</sup> entry.
UNC93B1	Pro-inflammatory	Unc-93 B1	0.000921856	1.65079	This encoded protein is involved in innate and adaptive immune response by regulating toll-

					like receptor signaling. It traffics nucleotide sensing toll-like receptors to the endolysosome from the ER.
RETN	Pro-inflammatory	Resistin	0.00995851	1.52201	The mouse homolog of this protein is secreted by adipocytes and may be the hormone potentially linking obesity to type II DM.
MTUS1	Tumor Suppressor	Microtubule associated tumor suppressor 1	0.0030961	1.50666	The encoded protein contains a C-terminal domain able to interact with the angiotensin II (AT2) receptor. One of the transcript variants has been shown to encode a mitochondrial protein that acts as a tumor suppressor and participates in AT2 signaling.
ERG	Encogene; pro-inflammatory	v-ets avian erythroblastos is virus E26 oncogene	0.002243843	1.50666	The encoded transcription factor is a key regulator of cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis.
RG9MT D1	Pro-lipolysis	Also known as TRMT10C	0.00734757	1.42521	The encoded protein may be

		(tRNA methyltransferase 10c, mitochondrial RNase P subunit)			essential for mitochondrial respiration.
NOS2A	Anti-inflammatory	Nitric oxide synthase 2	0.00622015	1.40677	Produces NO which is a messenger molecule with diverse bodily functions; including inflammatory processes.
RPS6KA5/4	Anti-inflammatory	Ribosomal protein S6 kinase, 90kDa, polypeptide 4	0.0045079	1.39879	The encoded protein can histone H3 to regulate certain inflammatory genes.
TOLLIP	Anti-inflammatory	Toll interacting protein	9.74E-05	1.38545	This gene encodes a ubiquitin-binding protein that interacts with several TLR signaling cascade components and is involved in IL-1 receptor trafficking.
APHK1	Pro-inflammatory	Sphingosine kinase 1	0.00696827	1.36182	The encoded protein plays a key role in TNF- $\alpha$ signaling and the NF- $\kappa$ B activation pathway.
PLIN4	Anti-lipolysis	Perilipin 4	0.00733102	1.35522	Coats intracellular lipid storage droplets and essential for mobilization of lipid in adipose tissue.

TNFS4	Pro-inflammatory	Tumor necrosis factor superfamily member 4	0.00199646	1.34267	Encodes a cytokine of the TNF family which functions in T cell antigen-presenting cell interactions and mediates adhesion of activated T cells to endothelial cells.
TRAF3	Pro-inflammatory	TNF receptor associated factor 3	0.000763382	1.26757	This protein participates in the signal transduction of CD40, important for the activation of the immune response.
NOX4	Anti-inflammatory	NADPH oxidase 4	0.0104607	-1.30287	The encoded proteins acts as an O <sub>2</sub> sensor and catalyzes the reduction of oxygen to ROS.
TNFRSF10D	Pro-inflammatory	Tumor necrosis factor receptor superfamily member 10d	0.00555366	-1.32744	A member of the TNF-receptor superfamily.
MAPK8	Pro-inflammatory	Mitogen-activated protein kinase 8	0.00986764	-1.35872	The activations of the kinase by TNF- $\alpha$ is found to be required for TNF- $\alpha$ induced apoptosis.
IL3	Anti-inflammatory	Interleukin 3	0.00920206	-1.37196	Plays a central role in cell-mediated immunity.
HGF	Pro-inflammatory	Also known as Interleukin-6(IL-6)	0.0103284	-1.46827	A potent inducer of the acute phase response. Acts as a

					myokine; in response to contraction is secreted to breakdown fat and improve insulin resistance.
IL1RL1	Pro-inflammatory	Interleukin 1 receptor like 1	0.00785627	-1.49476	In mice this receptor can be induced by proinflammatory stimuli, and may be involved in the function of help T cells.
JAK3	Pro-inflammatory	Janus kinase3	0.00299198	-1.61342	Involved in cytokine receptor-mediated intracellular signal transduction.
IL36G	Pro-inflammatory	Interleukin 36, gamma	0.0107873	-1.77934	A member of the interleukin 1 cytokine family.

If the fold change is positive the obese increased in response to lipid treatment more than the lean. If the fold change is negative, the lean experienced increase in expression more than the obese.

#### *Enrichment Analyses*

Pathway/process analyses were performed to identify pathways/processes with differentially expressed genes between lean and obese in response to the lipid treatment. Candidate genes for the enrichment analysis were selected based on p-value less than 0.01. Significant pathways summarized into common pathways, processes and diseases are presented in table 3.

**Table 3:** Enrichment Analysis showing common metabolic and inflammatory pathways and processes with the largest significant expression differences between lean and obese cell cultures following the lipid treatment.

<b>Work Flow</b>	<b>Title</b>	<b>pValue</b>	<b>Ratio</b>
Pathway maps	Immune response HMGB1/RAGE signaling pathway	3.701e-4	5/53
	Immune response TLR2 and TLR4 signaling pathways	5.204e-4	5/57
GO Processes	Regulation of organelle organization	3.060e-10	55/1502
	Immune response-regulating signaling pathway	3.260e-8	31/684
	Innate immune response	2.12e-7	43/1254
Process Networks	Inflammation MIF signaling	4.677e-3	8/140

Ratio indicates number of genes from ANCOVA analysis showing significant differentially regulated genes between lean and obese in response to lipid treatment compared to number of genes comprising the network.

### **Discussion**

The current study investigated skeletal muscle gene expression between lean and obese females after high fat exposure in order to potentially further the identification of mechanisms linking obesity to disease states. In support of the hypothesis, the main findings indicate a differential response of several metabolic and inflammatory genes between the lean and obese and these genes were found in inflammatory disease pathways mapped on the MetaCore database. It is interesting, however, that several pro-inflammatory genes were up-regulated more so in the lean than obese. These results suggest that the relationship between acute inflammatory responses to lipid oversupply and its link to skeletal muscle cell metabolism health is not straightforward.

Gene expression in favor of an enhanced inflammatory response was found to be significantly up-regulated in both the lean and obese, including genes found in the TNF family and Interleukin family. For example, from the MetaCore enrichment analysis, one of the top ten pathways containing differentially regulated genes between the lean and obese in response to the lipid treatment was Immune Response HMGB1/RAGE signaling pathway. HMGB1, through the receptor RAGE, regulates production of pro-inflammatory cytokines and cell motility [68]. In this pathway were five genes differentially influenced by lipid exposure in the lean and obese females: IL-6, iNOS, MAPK8, RPS6KA5, and RPSKA3. IL-6, MAPK8, and RPSKA3 were up-regulated more in the lean than the obese, all of which are involved in inflammatory signaling. iNOS and RPSKA3 were up-regulated more so in the obese and are involved in regulation of inflammatory processes.

## **Conclusion**

The findings of this study indicate a differential response in metabolic and inflammatory genes between the lean and obese cell cultures in response to the lipid treatment. While several pro-inflammatory genes were more up-regulated in the obese in response to the lipid treatment designed to mimic the “Western” high fat diet, there were several pro-inflammatory genes up-regulated more in the lean as well and some genes known to play a role in lipid metabolism were up regulated more so in the obese. These findings suggest a high-fat diet may result in an inflammatory response in both the lean and the obese skeletal muscle.

## Chapter 5: Overall Discussion and Conclusion

The primary findings of this research are supportive of the hypotheses as 1) self-selected exercise intensity and RPE during HIFT was significantly different between males and females and 2) evidence of a differential response between lean and obese gene expression and activated disease pathways was observed in metabolic and inflammatory genes. The results of this investigation on the impact of diet on lean and obese females, and exercise on healthy males and females, may enhance tailored prevention and treatment methods in the fight against sedentary lifestyle, obesity, and disease.

While little investigation has been conducted on high-intensity functional training, there is evidence this modality may elicit a cardiovascular response conducive of health and fitness benefits similar to, but requiring shorter duration than, endurance training [7] while also being perceived as less intense than sprint interval training [11]. Previous studies also found evidence males and females reported variable RPEs after completing the same high-intensity resistance training bout [22, 28]. The present study found similar results as both males and females self-selected high relative intensities (See Chap 3. Figure 1) while females reported lower perceived exertion (RPE) despite working harder than males (See Chap 3. Figure 2).

Another interesting result included that males perceived the HIFT bout as more intense and still reported higher immediate-post exercise enjoyment scores than females (See Chap 3. Table 3). This may be explained by the results of previous studies that

found that an exercise bout may be found enjoyable when the intensity is self-selected rather than imposed, regardless of high-intensities [8-10].

While adherence was not measured in the current study, HIFT may be a great candidate modality for home-based exercise programs for both males and females based on enjoyment. While females worked at a higher intensity, they reported lower RPE. Exercise that is perceived as less intense is consistently associated with a higher level of enjoyment [25] and higher enjoyment is found to predict better exercise adherence [26, 27]. While females reported lower enjoyment scores than males immediately post exercise (See Chap 3. Table 3), their enjoyment level was still relatively high. Additionally, in a study evaluating adherence associated with a short HIFT intervention compared to more moderate-intensity intervention, a greater number of participants in the HIFT group reported intending to continue exercising on their own [6]. However enjoyment was not measured during the study.

In investigated skeletal muscle gene expression between lean and obese females after high fat lipid exposure, the main findings indicate gene expression in favor of an elevated inflammatory response was found to be significantly up-regulated in both the lean and obese, including genes found in the TNF family and Interleukin family (See Chap 4. Table 2). Additionally, such pathways as the immune responses HMGB1/RAGE signaling and TLR2 and TLR4 signaling, were found to be activated by the molecular response in lean and obese tissue to the lipid treatment (See Chap 4. Table 3) which make it unclear whether the “Western” high fat diet alone is a mechanism that may link obesity to metabolic disease.

In conclusion, while self-selected intensity and RPE were significantly different between males and females during HIFT, it may be an optimal home-based modality for both male and female clients based on high enjoyment scores in males and low RPE scores in females. Future research should focus on determining the actual rates of adherence associated with HIFT, as well as enjoyment before, during, and after HIFT bouts. Additionally, the skeletal muscle metabolic and inflammatory gene expression of the lean and obese responded differently to a high fat exposure, but inflammatory pathways were up-regulated in both lean and obese tissue in response to the lipid treatment. The functional analysis revealed several inflammatory pathways containing these genes that may lead to disease, however, a high-fat diet alone appears to activate these pathways in both the lean and obese.

## BIBLIOGRAPHY

1. Hallal, P.C., et al., *Global physical activity levels: surveillance progress, pitfalls, and prospects*. Lancet, 2012. **380**(9838): p. 247-57.
2. Bray, G.A., S. Paeratakul, and B.M. Popkin, *Dietary fat and obesity: a review of animal, clinical and epidemiological studies*. Physiol Behav, 2004. **83**(4): p. 549-55.
3. Teodoro, J.S., et al., *High-fat and obesogenic diets: current and future strategies to fight obesity and diabetes*. Genes Nutr, 2014. **9**(4): p. 406.
4. Rogers, A.B., *Scientific Report of the 2015 Dietary Guidelines Advisory Committee*, A.B. Rogers, Editor. 2015. p. 1-20.
5. Thompson, W., *Worldwide Survey of Fitness Trends for 2015: What's Driving the Market?* ACSM's Health & Fitness Journal 2014. **18**(6): p. 8-17.
6. Heinrich, K.M., et al., *High-intensity compared to moderate-intensity training for exercise initiation, enjoyment, adherence, and intentions: an intervention study* BMC Public Health 2014. **14**(789): p. 1-6.
7. McRae, G., et al., *Extremely low volume, whole-body aerobic-resistance training improves aerobic fitness and muscular endurance in females*. Appl Physiol Nutr Metab, 2012. **37**(6): p. 1124-31.
8. Ekkekakis, P., G. Parfitt, and S.J. Petruzzello, *The pleasure and displeasure people feel when they exercise at different intensities: decennial update and*

- progress towards a tripartite rationale for exercise intensity prescription. Sports Med, 2011. 41(8): p. 641-71.*
9. Bartlett, J.D., et al., *High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. J Sports Sci, 2011. 29(6): p. 547-53.*
  10. Parfitt, G., E.A. Rose, and W.M. Burgess, *The psychological and physiological responses of sedentary individuals to prescribed and preferred intensity exercise. Br J Health Psychol, 2006. 11(Pt 1): p. 39-53.*
  11. Gist, N.H., E.C. Freese, and K.J. Cureton, *Comparison of responses to two high-intensity intermittent exercise protocols. J Strength Cond Res, 2014. 28(11): p. 3033-40.*
  12. Lind, E., R.R. Joens-Matre, and P. Ekkekakis, *What intensity of physical activity do previously sedentary middle-aged women select? Evidence of a coherent pattern from physiological, perceptual, and affective markers. Preventive Medicine 2004. 40: p. 407-419.*
  13. Ekkekakis, P., E. Hall, and S.J. Petruzzello, *Some Like It Vigorous: Measuring Individual Differences in the Preference for and Tolerance of Exercise Intensity Journal of Sport and Exercise Psychology, 2005. 27(3).*
  14. Ekkekakis, P. and S.J. Petruzzello, *Acute aerobic exercise and affect: current status, problems and prospects regarding dose-response. Sports Med, 1999. 28(5): p. 337-74.*

15. Bertram, J.E. and A. Ruina, *Multiple walking speed-frequency relations are predicted by constrained optimization*. J Theor Biol, 2001. **209**(4): p. 445-53.
16. Alexander, R.M., *Energetics and optimization of human walking and running: the 2000 Raymond Pearl memorial lecture*. Am J Hum Biol, 2002. **14**(5): p. 641-8.
17. Dishman, R.K., *Prescribing exercise intensity for healthy adults using perceived exertion*. Med Sci Sports Exerc, 1994. **26**(9): p. 1087-94.
18. Spelman, C.C., et al., *Self-selected exercise intensity of habitual walkers*. Med Sci Sports Exerc, 1993. **25**(10): p. 1174-9.
19. Kravitz, L., et al., *Exercise mode and gender comparisons of energy expenditure at self-selected intensities* Medicine & Science in Sports & Exercise, 1997. **29**(8): p. 1028-1035.
20. Noble, B.J. and R.J. Robertson, *Perceived Exertion*. 1996: Human Kinetics.
21. Borg, G.A.V., *Physical Performance and perceived exertion.*, in *Studia Psychologica et Paedagogica Serles Altera, Investigationes XI*. . 1962: Gleerup, Lund p. 39-40.
22. Robertson, R.J., et al., *Concurrent validation of the OMNI perceived exertion scale for resistance exercise*. Med Sci Sports Exerc, 2003. **35**(2): p. 333-41.
23. Lagally, K.M. and R.J. Robertson, *Construct validity of the OMNI resistance exercise scale*. The Journal of Strength and Conditioning Research 2006. **20**(2): p. 252-256.

24. Borg, G., *Borg's Perceived Exertion and Pain Scales*. 1 ed. 1998, Champaign, IL. : Human Kinetics
25. Ekkekakis, P., *The study of affective responses to acute exercise: The dual-mode model*. . *New Approaches to Sport and Exercise Psychology*, ed. R. Stelter and K.K. Roessler. 2005, Oxford, United Kingdom: Meyer & Meyer Sport.
26. Wankel, L.M., *The importance of enjoyment to adherence and psychological benefits from physical activity* *International Journal of Sport and Exercise Physiology*, 1969. **24**(2): p. 151-169.
27. Lewis, B.A., et al., *Self-efficacy versus perceived enjoyment as predictors of physical activity behaviour*. *Psychol Health*, 2016. **31**(4): p. 456-69.
28. O'Connor, P.J., M.S. Poudevigne, and J.D. Pasley, *Perceived exertion responses to novel elbow flexor eccentric action in women and men*. *Med Sci Sports Exerc*, 2002. **34**(5): p. 862-8.
29. Lin, B.H. and J. Guthrie, *Nutritional Quality of Food Prepared at Home and Away From Home, 1977-2008*. . U.S. Department of Agriculture, Economic Research Service, 2012(EIB-105).
30. Manzel, A., et al., *Role of "Western diet" in inflammatory autoimmune diseases*. *Curr Allergy Asthma Rep*, 2014. **14**(1): p. 404.
31. Schrauwen, P., et al., *Changes in fat oxidation in response to a high-fat diet*. *Am J Clin Nutr*, 1997. **66**(2): p. 276-82.

32. Boyle, K.E., et al., *A high-fat diet elicits differential responses in genes coordinating oxidative metabolism in skeletal muscle of lean and obese individuals*. J Clin Endocrinol Metab, 2011. **96**(3): p. 775-81.
33. Boyle, K.E., et al., *Mitochondrial lipid oxidation is impaired in cultured myotubes from obese humans*. Int J Obes (Lond), 2012. **36**(8): p. 1025-31.
34. Hotamisligil, G.S., *Inflammation and metabolic disorders*. Nature, 2006. **444**(7121): p. 860-867.
35. Horton, T.J., et al., *Fat and carbohydrate overfeeding in humans: different effects on energy storage*. Am J Clin Nutr, 1995. **62**(1): p. 19-29.
36. Sparks, L.M., et al., *A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle*. Diabetes, 2005. **54**(7): p. 1926-33.
37. Jacobsen, S.C., et al., *Effects of short-term high-fat overfeeding on genome-wide DNA methylation in the skeletal muscle of healthy young men*. Diabetologia, 2012. **55**(12): p. 3341-9.
38. Maples, J.M., et al., *Lipid exposure elicits differential responses in gene expression and DNA methylation in primary human skeletal muscle cells from severely obese women*. Physiol Genomics, 2015. **47**(5): p. 139-46.
39. ACSM, *ACSM's Guidelines for Exercise Testing and Prescription* 9ed. 2013: Lippincott Williams & Wilkins

40. Rech, C.R., et al., *Validation and reliability of the satisfaction scale for physical activity in adults*. Rev Saude Publica, 2011. **45**(2): p. 286-93.
41. Sallis, J.F., et al., *Neighborhood built environment and income: Examining multiple health outcomes*. . Social Science and Medicine, 2009. **69**: p. 1285-1293.
42. Motl, R.W., et al., *Factorial validity and invariance of questionnaires measuring social-cognitive determinants of physical activity among adolescent girls*. Prev Med, 2000. **31**(5): p. 584-94.
43. Hu, L., et al., *Effects of self-efficacy on physical activity enjoyment in college-aged women*. Int J Behav Med, 2007. **14**(2): p. 92-6.
44. Egan, A.D., *Session Rating of Perceived Exertion During High Intensity and Low Intensity Bouts of Resistance Exercise*. Journal of Undergraduate Research, 2003. **6**: p. 1-6.
45. Berggren, J.R., C.J. Tanner, and J.A. Houmard, *Primary cell cultures in the study of human muscle metabolism*. Exerc Sport Sci Rev, 2007. **35**(2): p. 56-61.
46. Bessarabova, M., et al., *Knowledge-based analysis of proteomics data*. BMC Bioinformatics, 2012. **13 Suppl 16**: p. S13.
47. Williams, D.M., et al., *Comparing psychosocial predictors of physical activity adoption and maintenance*. Ann Behav Med, 2008. **36**(2): p. 186-94.

48. Dudley, G.A., W.M. Abraham, and R.L. Terjung, *Influence of exercise intensity and duration on biochemical adaptations in skeletal muscle*. J Appl Physiol Respir Environ Exerc Physiol, 1982. **53**(4): p. 844-50.
49. Crain, A.L., et al., *The long and winding road to physical activity maintenance*. Am J Health Behav, 2010. **34**(6): p. 764-75.
50. Hofstetter, C.R., J.F. Sallis, and M.F. Hovell, *Some health dimensions of self-efficacy: analysis of theoretical specificity*. Soc Sci Med, 1990. **31**(9): p. 1051-6.
51. Kravitz, L., *High Intensity Interval Training in ACSM.org A.C.o.S. Medicine*, Editor. 2015, ACSM ACSM.org p. 1-2.
52. Ryan, R.M., et al., *Intrinsic Motivation and Exercise Adherence*. International Journal of Sport Psychology, 1997. **28**(4): p. 335-354.
53. Sturn, R. and A. Hattori, *Morbid obesity rates continue to rise rapidly in the United States* International Journal of Obesity, 2013. **37**: p. 889-891.
54. Rankinen, T., et al., *The Human Obesity Gene Map*. Obesity, 2006. **14**(4): p. 529-644.
55. Hulver, M.W., et al., *Skeletal muscle lipid metabolism with obesity* Endocrinology and Metabolism, 2003. **284**(4): p. E741-E747.
56. Hulver, M.W., et al., *Elevated stearyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans* Cell Metabolism, 2005. **2**(4): p. 251-261.

57. Berggren, J.R., et al., *Skeletal muscle lipid oxidation and obesity: influence of weight loss and exercise*. *Endocrinology and Metabolism*, 2007. **294**(4): p. E726-E732.
58. Kelley, D.E. and L.J. Mandarino, *Fuel selection in human skeletal muscle in insulin resistance: a reexamination*. *Diabetes*, 2000. **49**(5): p. 677-83.
59. Jager, S., et al., *AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha*. *Proc Natl Acad Sci U S A*, 2007. **104**(29): p. 12017-22.
60. Thomson, D.M. and W.W. Winder, *AMP-activated protein kinase control of fat metabolism in skeletal muscle*. *Acta Physiol (Oxf)*, 2009. **196**(1): p. 147-54.
61. Ruderman, N.B., et al., *AMPK and SIRT1: a long-standing partnership?* *Am J Physiol Endocrinol Metab*, 2010. **298**(4): p. E751-60.
62. Mootha, V.K., et al., *PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes*. *Nat Genet*, 2003. **34**(3): p. 267-73.
63. Lumeng, C.N. and A.R. Saltiel, *Inflammatory links between obesity and metabolic disease*. *J Clin Invest*, 2011. **121**(6): p. 2111-7.
64. Kolb, H. and T. Mandrup-Poulsen, *The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation*. *Diabetologia*, 2010. **53**(1): p. 10-20.

65. Fernandez-Real, J.M. and J.C. Pickup, *Innate immunity, insulin resistance and type 2 diabetes*. Trends in Endocrinology and Metabolism, 2008. **19**(1): p. 10-16.
66. Glass, C.K. and J.M. Olefsky, *Inflammation and lipid signaling in the etiology of insulin resistance*. Cell Metab, 2012. **15**(5): p. 635-45.
67. Haversen, L., et al., *Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages*. Atherosclerosis, 2009. **202**(2): p. 382-93.
68. Sims, G.P., et al., *HMGB1 and RAGE in inflammation and cancer*. Annu Rev Immunol, 2010. **28**: p. 367-88.