

**Metabolic Responses to a Westernized Diet Administered to Rats
Selectively Bred for High and Low Amounts of Voluntary Exercise.**

A Thesis

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By

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled:

Metabolic Responses to a Westernized Diet Administered to Rats Selectively Bred for High and Low
Amounts of Voluntary Exercise.

Presented by Alex Heese,

A candidate for the degree of Masters of Biomedical Sciences, and hereby certify that, in their opinion, it
is worthy of their acceptance.

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ABSTRACT

Introduction: Obesity is a current American and global epidemic caused mainly by the interactions of genetics, improper dieting, and lack of exercise. Not only is it a huge economic burden, but is associated with many other chronic diseases, including risk factors of prediabetes and metabolic syndrome. Exercise and proper dieting are both imperative to improving the present worldwide health catastrophe of obesity and its co-morbidities. Motivation for exercise may have a genetic basis, but it is unknown whether genes selected based on motivation to physical activity co-select with metabolic genes whose function to protect in the absence of voluntary exercise. Therefore, the purpose of my study was to determine if rats with varying levels of inherent motivation to voluntarily run in wheels, when given *no* access to voluntary running wheels, would retain any protective effects against a Westernized diet. **Methods:** A novel rat model was previously created in the Booth lab in which rats were artificially selected with the putative motivation to produce high or low amounts of voluntary running (termed HVR and LVR, respectively) each night for several generations. A wild type group was added as a nonselected control group. Every Wistar rat (all male) was given zero access to voluntary running in a wheel in an attempt to limit the environmental effects of physical activity on the acquired genetics of the varying levels of motivation for voluntary running by the rat lines. The three rat lines were divided into two groups, half of each line receiving a Western diet (sucrose 34% by food weight, fat 21% by food weight) and the other half receiving a normal chow diet (sucrose 2.6% by food weight, fat 6.5% by food weight). Each diet was administered for eight continuous weeks. Weekly body weights were measured and a pre- and post-body fat percentage was obtained. After seven weeks of dieting, a glucose tolerance test was performed. After the full eight weeks of dieting, tissues and blood were extracted immediately after rats were

sacrificed via CO₂. **Results:** Without access to running wheels, the metabolic markers of HVR rats (having a high inherent motivation to voluntarily run), along with the LVR rats (having a low inherent motivation to voluntarily run) tended to be worse metabolically than that of the WT line. In general, the two selective lines (HVR and LVR) appeared to be closer to a prediabetic-like or pre-metabolic syndrome-like state after the full eight weeks of dieting than WT.

Conclusion: These results suggest that the process of selective breeding for high or low motivation for voluntary running likely co-selected genes that can be undesired or even deleterious on their ability to minimize the negative health consequences of a Western diet, at least when they were not allowed to voluntarily exercise in a wheel. The translation of these results to humans suggests that individuals with a high motivation/drive for voluntary physical activity see diminished or no health benefits in regards to their metabolic state if they do not actually exert this drive for daily exercise by partaking in voluntary physical activity.

INTRODUCTION

Obesity Epidemic

Obesity is a current global epidemic and has specifically become a monumental health care burden within the United States, with nearly 35% of the population obese and 6.4% of the population morbidly obese (Lakka & Bouchard, 2005; Ogden, Carroll, Kit, & Flegal, 2014; Sturm & Hattori, 2013). For medical purposes, obesity is defined as an excess of body fat, however for clinical purposes, obesity and overweight are most commonly determined by body mass index (BMI) (Krebs et al., 2007). BMI is the ratio of weight (kg) divided by height (m^2) with perimeters for overweight at a BMI of 25-29.9 kg/m^2 and obesity at a BMI of $>30 kg/m^2$. Other measurements in humans are also used to try and provide a better measurement for whole body adiposity, such as waist circumference, waist-hip ratio, dual X-ray absorptiometry (DEXA), computerized axial tomography (CAT), and magnetic resonance imaging (MRI). However, many of these other measurements are not widely used clinically due to cost efficiency among other reasons (Bouret, Levin, & Ozanne, 2015). Regardless of the manner in which adiposity is measured, a major concern with obesity is that it is directly associated to an increased risk for premature death from the onset of various comorbidities, such as type 2 diabetes, cardiovascular disease, cancer, and other metabolic and neurodegenerative diseases (Lakka & Bouchard, 2005; Warburton, Nicol, & Bredin, 2006).

Poor diet and physical inactivity lead to obesity

In 2008, the estimated economic cost of obesity in the United States was nearly \$150 billion or 10% of all medical spending (Finkelstein, Trogdon, Cohen, & Dietz, 2009). A more recent study found that the obesity epidemic is estimated to cost the US in excess of \$215 billion

annually in 2008 dollars, including direct medical costs and indirect costs such as productivity costs, transportation costs, and human capital accumulation costs (Hammond & Levine, 2010). The prevalence of obesity is growing as a financial burden on the economy. Comparing an obese group to a healthy group, one study found that the obese individuals had, on average, a 36% higher average annual health care cost with a 105% higher prescription cost and 39% higher primary-care cost compared to the healthy individuals (Thompson, Brown, Nichols, Elmer, & Oster, 2001). This expensive onset of excess weight has been attributed to an imbalance between energy intake and physical activity (Lakka & Bouchard, 2005). Low levels of energy expenditure and high levels of energy intake lead to a surplus of unused energy that is stored as body lipids, predominantly in adipose tissue (Speakman, Hambly, Mitchell, & Krol, 2008). Animal models have been created where obesity can be induced through diet to study energy regulation and obesity. It is known that there are predisposing genes to obesity that interact with the environment to influence the response to treatments of the disease, such as exercise (Choquet & Meyre, 2011; Speakman et al., 2008). Diets high in fat and sugar complemented by a sedentary lifestyle are promoters for increased weight gain and fat mass. Diet complemented with exercise has been shown to have significant and clinically meaningful initial weight loss, and, though difficult for most, is possible to maintain significant amounts of weight loss over time (Wing & Phelan, 2005). Exercise has a positive correlation with mean lifespan, decreased risk of chronic diseases, and a promotion of a healthier life. This linear relationship of exercise to health status is one where increased exercise correlates with better health status (Goodrick, 1980; Warburton et al., 2006). In other words, increasing exercise prevents gain in body fat, which significantly lowers the chance for chronic diseases and leads to longer healthier lives.

Motivation for exercise has genetic links

So why do people not exercise when there are clear health risks associated with a lack of exercise? Many factors have been shown to influence physical activity levels, such as income, socioeconomic status, education, gender, age, and ethnicity (Trost, Owen, Bauman, Sallis, & Brown, 2002). Another possible answer could be that there is a lack of motivation or drive to want to exercise. Motivation is defined as a force or influence that causes someone to do something (Merriam-Webster, 2015). These forces act as incentives, motivating behavior as well as increasing the frequency of actions that produce them (Robinson, Yager, Cogan, & Saunders, 2014). Physical exercise has been shown to have a genetic or inherent background that provides individuals with various levels of motivation for exercise (Basso & Morrell, 2015; Garland et al., 2011; Herring, Sailors, & Bray, 2014; Roberts et al., 2014). When something is inherent, it is an essential constituent or characteristic of that individual that is therefore present on a genetic level. Heritability of physical activity has been shown to vary in the literature from 9% in a Mexican-American family study (Mitchell et al., 2003) to well over 50% in European twin studies (Eriksson, Rasmussen, & Tynelius, 2006; Maia, Thomis, & Beunen, 2002). Variance in level of genetic influence can differ between race and manner of which the study is measured. Nonetheless, some people may be born lacking the desire to exercise, leading to a sedentary lifestyle which promotes obesity and a shorter lifespan. Others may have genes that give a large drive for activity which results in a better health status and a better quality of life. Thus, what would happen to an individual that has the genes for high motivation, but is not allowed to use them? Are these inherent genes for high or low motivation to be physically active also co-selecting genes that allow a better or worse protection against metabolic disorders produced from a sedentary/unhealthy lifestyle?

Motivation for exercise has been shown in rat models

In order to study the role of exercise volumes and disease prevention, a rat model in which artificial selection for high and low nightly voluntary running distances was developed (Roberts et al., 2013). Briefly, two-way artificial selective breeding was used to create high voluntary runners (HVR) and low voluntary runners (LVR). The founder population consisted of 80 male and 79 female outbred Wistar rats (Charles River Raleigh, Raleigh, NC). At 28 days of age, these rats were introduced to running wheels and their nightly running distance was monitored for 6 days. At this point, the 26 highest (13 male and 13 females) and the 26 lowest (13 male and 13 female) were randomly selected from the original and bred into 13 HVR families and 13 LVR families. At each subsequent generation, progeny were weaned at 21 days of age and introduced to running wheels at 28 days of age. After 6-days of voluntary running, the highest male and female HVR runners were kept for the next generation of HVR breeding and the lowest LVR male and female runners were kept for the next generation of LVR breeding. At generation 8, there was a large divergent in distance running, time running, and speed of running between the two lines (Roberts et al., 2013). The two lines have developed into a model of exercise motivation with the HVR line having a high motivation for voluntary running and the LVR line having a low motivation.

Objectives for current study

Aim 1: Attempted to induce a prediabetic-like and metabolic syndrome-like profile through diet manipulation methods using two novel lines of Wistar rats selectively bred for high- and low- amounts of inherent motivation for voluntary wheel running as well as a wild-type, nonselected control rat. Both prediabetic-like and metabolic syndrome-like phenotypes are associated with one another based on their metabolic symptoms, but tend to be unclear on precise definitions. Prediabetes, the precursor to type 2 diabetes, is an asymptomatic condition

that is characterized by glycemic variables that are higher than normal but lower than the diabetes thresholds (Tabak, Herder, Rathmann, Brunner, & Kivimaki, 2012). Metabolic syndrome is a complex syndrome characterized by insulin resistance and hyperinsulinemia and often associated with other metabolic traits such as such as dyslipidemia, obesity (especially abdominal), hypertension and glucose intolerance (Carroll & Dudfield, 2004; Timar, Sestier, & Levy, 2000). Both prediabetes and metabolic syndrome are influenced by the presence of obesity and increase the risk of mortality, type 2 diabetes, cardiovascular disease as well as other life shortening chronic diseases (Carroll & Dudfield, 2004; Eckel, Grundy, & Zimmet, 2005). As previously noted, energy expenditure, whether through exercise, dieting, or lifestyle change, is essential for weight loss and reduced obesity (Curioni & Lourenco, 2005). In other words, more energy needs to be used than consumed in order to lose weight. It has been shown that exercise and dieting can also help to reduce insulin resistance along with the other risk factors of metabolic syndrome and even delay the onset of type 2 diabetes (Carroll & Dudfield, 2004). Thus, it is essential to understand what the effect of having genetically predisposed levels of motivation for exercise, such as the HVR line having high levels, is on the ability to metabolically handle a Western diet when the rats are not permitted voluntary running. The big picture is individualized medicine. Individuals with different levels of motivation for physical activity would therefore need individualized plans of exercise prescription when it comes to disease prevention and therapy. The President and NIH Director are now marketing such plans as “precision medicine” (Collins & Varmus, 2015).

Aim 2: After inducing a prediabetic-like and metabolic syndrome-like profile, the rats were investigated for the protective and/or deleterious effects that could be associated with different levels of inherent motivation for exercise. In order to induce the prediabetic-like

symptoms, rats will be administered either a standard chow diet control or a high sugar/high fat “Western” diet for a period of eight consecutive weeks. The Western diet (TD.88137; Harlan Laboratories) contained the following profile: 4.5kcal/g, 15.2% protein (expressed as %kcal), 42.7% carbohydrate (expressed as %kcal), and 42.0% fat (expressed as % kcal). The normal chow diet (LabDiet® Certified CR 14% protein rodent diet) contained the following profile: 3.5kcal/g, 16.1% protein (expressed as %kcal), 69.3% carbohydrate (expressed as %kcal), and 14.6% fat (expressed as % kcal). Additionally, the Western diet contained a high amount of sucrose (34% by food weight) whereas the normal chow diet contained a low amount of sucrose (2.6% by food weight) (Mobley et al., 2014). The experiment will introduce the unique Booth lab rat model of high voluntary nightly running (HVR) and low voluntary nightly running (LVR) rats along with a nonselected control WT rat line (Roberts et al., 2013). On top of specific diets and levels of motivation for exercise, the animals will not have access to running wheels. In other words, we aim to produce rats with prediabetic-like symptoms (via a Western diet) while keeping them sedentary (without wheels for voluntary running). With no access to voluntary running wheels, we are attempting to limit physical activity so that their metabolic outcomes will be more closely based on their genetic predisposition to handle high amounts of sugar and fat.

Hypotheses:

- It is known that Western diets will significantly worsen metabolic markers and pathways in rats (An et al., 2013). After eight weeks of dieting, I hypothesize to see drastic differences in metabolic markers between the two diet groups, regardless of rat line.
- All three rat lines on the Western diet are hypothesized to have higher body weights after the eight weeks compared to the rats on a normal chow diet.

- The HVR rat line contains genes that, on average, gives it the motivation to voluntarily run nearly 10-fold more each night than its LVR rat line counterpart (Roberts et al., 2013). The hierarchy of nightly running distances would therefore be HVR > WT > LVR. Higher levels of activity is associated with positive health benefits, especially in regards to metabolic status (Skop et al., 2015). Based upon this, I hypothesized that the HVR male rat line administered a Western diet will have genes co-selected with the high motivation for exercise that will allow it a better, more effective mechanism of handling the negative metabolic effects caused by a chronic high fat/high sugar diet than both the WT and LVR rat lines. In other words, the HVR line will be the most resistant to poor diet and have less severe metabolic markers, followed by the WT and then the LVR rat line. Figure 1 (page 15) gives a pictorial view of the predicted outcome.

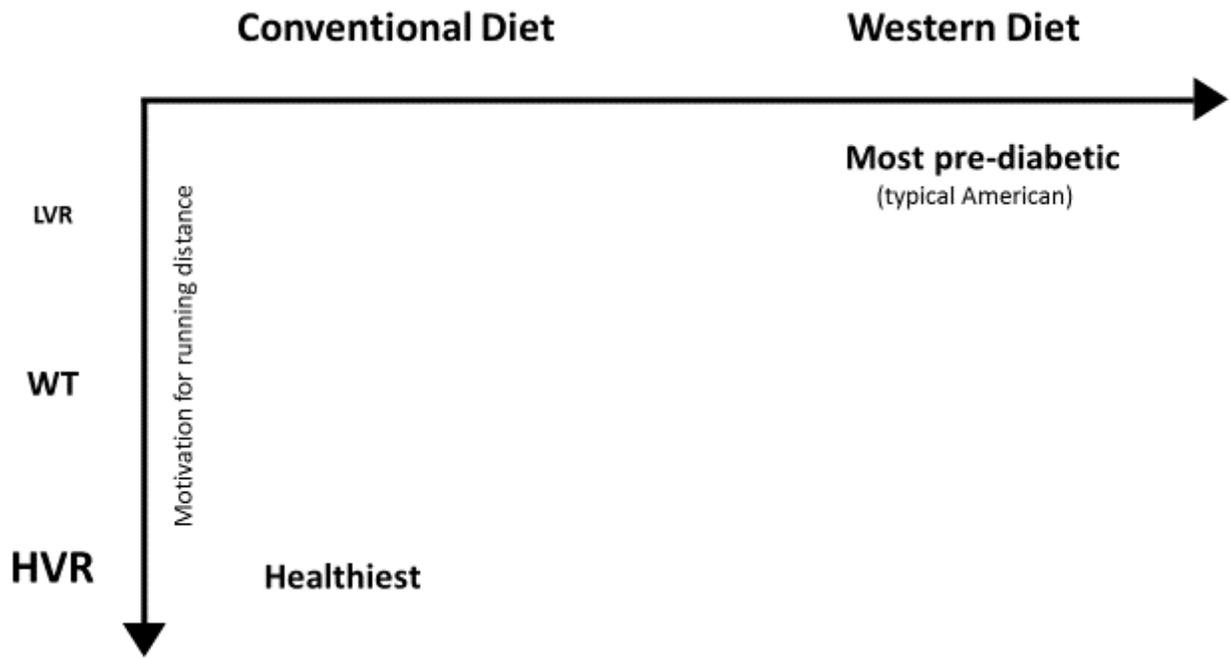


Figure 1: Schematic on hypotheses. The conventional diet (normal chow) will be healthier than the Western diet. The motivation for exercise increases such that $LVR < WT < HVR$. The LVR line will be the least healthy (most prediabetic-like) followed by the WT and then the HVR. The HVR on a conventional diet will be the healthiest of all groups.

My expected results based upon the literature are given in the next set of bullets.

- When obese and overweight adults exert high amounts of activity, it has been shown to improve their dyslipidemia profile by raising high-density lipoprotein (HDL) cholesterol and lowering triglycerides (Carroll & Dudfield, 2004). Though these rats will not be given access to running wheels, I believe that the inherent effects of increased motivation for exercise will lead to better metabolic outcomes after a period of Western dieting.
- The opposite will go for the LVR rat line. This line contains genes that promote a sedentary, inactive lifestyle. Inactivity has been shown to promote obesity which increases the chances of premature death and many chronic illnesses (Lakka & Bouchard, 2005; Warburton et al., 2006). Though these rats will not be given access to running wheels, I hypothesize that the inherent effects of a low motivation for exercise will lead to negative metabolic markers that resemble a rat with a more severe prediabetic-like or metabolic syndrome-like state compared to the other rat lines.

MATERIALS AND METHODS

Animals and Selective Breeding

All animal procedures were approved by the University of Missouri's Animal Care and Use Committee. LVR, WT, and HVR rats were bred at the University of Missouri. Rats were housed in temperature controlled facilities on a 12hr: 12hr light: dark cycle and allowed *ad libitum* access to food and water. Methods describing the selective breeding process for the creation of HVR and LVR rat lines have been previously published (Roberts et al., 2013). Briefly, we have developed a rat model with two distinct, selectively bred phenotypes in which one rat line voluntarily runs much longer nightly distances [high voluntary runners (HVR)] than the other rat line [low voluntary runners (LVR)].

Experimental Design

The experimental design for this experiment included using only male rats from our generation 12 and 13 HVR and LVR lines. With males, the estrous cycle does not need to be taken into account. However, I realized after the start of the experiment the importance of having a nonselected control group for the study to provide a control for the selective breeding model. The late addition of the wild type (WT) group added 13 rats to the study. There was no access for any experimental group to a running wheel during any parts of this experiment. Each rat was raised according to Booth lab protocol (Roberts et al., 2013). At 33-35 days of age, each rat was single-caged and underwent a week-long feed efficiency test consuming normal chow diet (sucrose 2.6% by food weight, fat 6.5% by food weight). WT rats were unable to undergo the feed efficiency test because they were too old when we added them to the experimental timeline. At 40-43 days of age, each rat had one of two diets provided, continuation of normal chow diet (sucrose 2.6% by food weight, fat 6.5% by food weight.) or switching to Western diet,

WD, (sucrose 34% by food weight, fat 21% by food weight). Specific diets were assigned at random for each rat so that from the 16 HVR, 14 LVR, and 13 WT rats, half of each diet group received a normal diet and the other half received a WD (WT had n=7). Due to the limited amount of animal racks for cage space, rats were double caged when starting their specific 8 weeks of dieting which meant that food intake would be the average of two rats. Seven of the WT rats started their eight week dieting at day 50 of age. Though these seven rats were 7-10 days older than the rest of the WT, HVR, and LVR rats, every rat in the study went through 8 full-weeks of specific dieting before being sacrificed. Figure 2 (page19) offers a visual representation of the timeline for the rats beginning the diet treatment at two different ages.

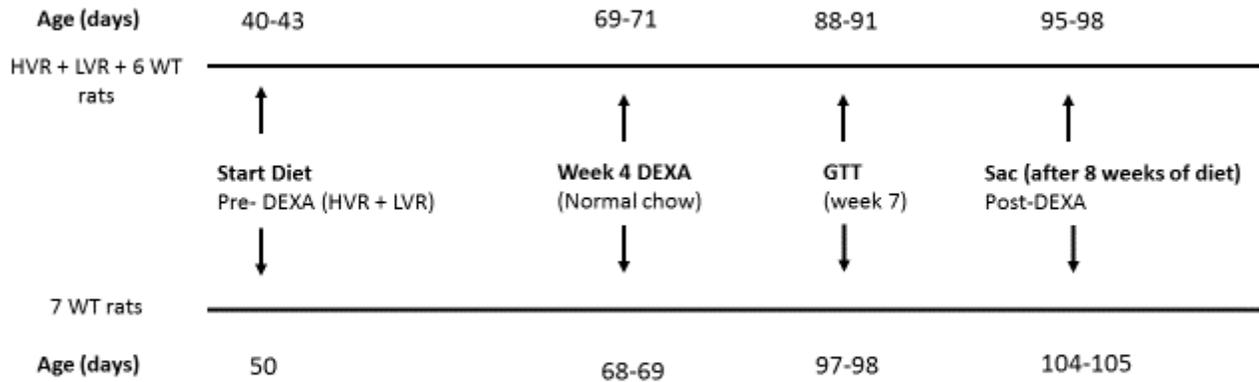


Figure 2: Timeline for rats. The top line indicates all HVR, LVR, and 6 of the WT rats that began their specific diet between 40-43 days of age. The bottom line indicates the 7 WT rats that began their specific diet at 50 days of age.

Food Intake and Body Weight Measurements

During the eight weeks of dieting, food end weights and body weights were recorded weekly. After measuring food weights at the end of each week, a new total amount of food was recorded and added to each cage. Water was refilled as needed to provide *ad lib* supply. I performed all these measurements.

Dual X-ray Absorptiometry (DEXA)

DEXA scans are used to measure body composition. The DEXA machine was calibrated for rats and allowed the measurement of body fat percentage (Hologic, QDR). These scans were performed on all rats from 40-43 days of age before they started their specific diets. Due to seven of the WT rats starting 7-10 days of age later, there was a second DEXA scan performed to all normal chow rats in all three lines 69-71 days of age. This was done so a baseline could be established for comparing the WT, HVR, and LVR groups after a final DEXA was taken at the end of the experiment (95-98 days of age). The final night before sacrifice (94-97 days of age), each rat was fasted. Upon sacrifice, a final DEXA scan was rapidly performed on all animals before tissues were extracted. I performed all these measurements.

Glucose Tolerance Testing

The protocol for the glucose tolerance testing (GTT) was based and modified off of Vieira-Potter et al. (Vieira-Potter et al., 2015), who kindly taught me the procedure. Testing was first done on the HVR and LVR rats before starting the Western diet. This established a baseline glucose tolerance curve for the LVR (n=6) and HVR (n=7) rats. From then on, testing occurred to every rat after seven weeks of dieting. The rats were fasted overnight and given a GTT the following morning. Briefly, a baseline blood sample was collected using 25G x 3/4" winged needles (Terumo, Long Beach, CA) followed by injection of 50% dextrose (Vetone, Grand Island

NE). The amount injected was calculated by multiplying the body weight (g) by four ($g \times 4$). This gave the total amount needed for injection in microliters. From here, blood samples were taken at time points 15, 30, 45, 60, and 120 minutes after the injection of 50% dextrose. For each time point, around 100-200 μ l of blood was taken (Vieira-Potter et al., 2015). Using an Alpha Trak 2 blood glucose monitoring system with a new Alpha Trak 2 blood glucose test strip (Abbott, North Chicago, IL) for each measurement, we were able to obtain blood glucose concentrations for each time point. From here, glucose tolerance curves were created for each rat and then averaged together to create glucose tolerance curves per rat line. After the GTT, the rats were placed back in their respective cages and proceeded on their assigned diet for one more week which was the final week prior to sacrifice. I performed all these measurements.

Tissue Extraction

After the full eight-weeks of dieting, rats were fasted overnight and sacrificed the following morning. Directly following sacrifice, a heart stick was performed to obtain blood for a lipid panel. The brain was sliced and four areas were extracted, which were the nucleus accumbens (NAc), hippocampus (hippo), lateral hypothalamus (LH), and the arcuate nucleus (Arc). These areas of the brain were stored for possible use at a later time. The whole heart was extracted and weighed (data not shown). The left ventricle was then removed from the whole heart for storage. Three types of muscles were extracted from the hind legs of the rats which included the soleus, plantaris, and gastrocnemius muscle. The median lobe of the liver was extracted from each rat in order to measure the triglyceride concentration. Finally, omental adipose tissue around the internal organs was extracted. All extracted tissue was placed in liquid nitrogen immediately after removal from the rat, and stored in a -80°C freezer. I performed all these measurements.

Blood Lipid Measurements

All rats were fasted for approximately 14 hours before sacrifice. Directly after sacrifice, a heart stick was performed using a 22G1 needle with a 3 mL syringe to extract ~1 mL of blood. The blood was placed in 200 ul of chilled 0.1M EDTA and kept on ice until it was centrifuged at 7000 rcf for 10 minutes at 4°C and the top layer of plasma was carefully extracted via a pipette. The plasma was then placed in a new test tube and stored in a -80°C freezer until all samples were collected. I performed all procedures to this point. This plasma was sent to Charles E. Wiedmeyer Laboratory (Comparative Clinical Pathology Services, Columbia, MO) for a blood lipid panel which measured high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, total cholesterol, triglycerides, and nonesterified fatty acids (NEFA) concentration in the blood.

Plasma Isolation for Insulin Measurement

During GTT, blood was collected in a test tube at time points described previously (basal, 15, 30, 45, 60, and 120 minutes post dextrose injection). The blood was then centrifuged at 7000 rcf for 10 minutes at 4°C and the top layer of plasma was carefully extracted via a pipette. The plasma was then placed in a new test tube and stored in a -80°C freezer until all samples were collected. Each time point of plasma was sent to Charles E. Wiedmeyer Laboratory (Comparative Clinical Pathology Services, Columbia, MO) where an ELISA was performed to measure the insulin concentration per sample.

Hepatic Triglyceride Measurements

Hepatic Triglyceride concentration was measured using the median lobe of the liver from each rat via a triglyceride (TAG) assay. First, a 30-mg liver sample was homogenized with a mixture of 2:1 chloroform: methanol using the Tissue lyzer for 2X2 min at 20 Hz. The test tubes

were put in a cold room on a rotating wheel overnight. The following day, 1 ml of 4mM MgCl₂ was mixed in each tube, followed by centrifuging for 1 hour at 1000xg at a cold temperature. Eventually layers form, allowing the extraction of the organic phase (bottom layer). This organic solvent phase is left to evaporate in a hood overnight. In the morning, only dried lipids remained. Butanol: Triton-x114 mix (3:2 vol: vol) was mixed in the test tubes dissolving the dried lipids. Finally, using a 96-well plate loaded with 300 ul of mixed glycerol and TAG reagent, 3 ul of standards and samples were added into each well. The plate was then incubated for 5 minutes at 37°C (or 15 min at room temperature) followed by a reading at 540 nm. Greg Ruegsegger performed all these measurements.

Corticosterone Concentration

Corticosterone was measured from the blood collected during GTT as a representation of stress levels of the rats, as increased corticosterone will increase blood glucose. Any stress will raise blood corticosterone levels. An ELISA was performed on two plasma time point samples collected during each rat's GTT (Arbor Assays, Ann Arbor, MI). Specifically, the basal time point and 30 minutes post-injection of dextrose were the two time points tested. I performed all these measurements.

Insulin sensitivity index

Quantitative insulin sensitivity check index (QUICKI) uses the fasting blood glucose and plasma insulin concentrations to provide an accurate index of insulin sensitivity (Chen, Sullivan, & Quon, 2005; Chen, Sullivan, Yue, Katz, & Quon, 2003; Hanley et al., 2003; Katz et al., 2000; Mather et al., 2001). QUICKI is one of the most thoroughly studied and validated surrogate index for insulin sensitivity (Muniyappa, Lee, Chen, & Quon, 2008). Thus, $QUICKI = 1 / [\log$

(fasting insulin, uU/ml) + log (fasting glucose, mg/dl)]. Here, the smaller the value is the less insulin sensitive or more insulin resistant the subject is. I performed all these calculations.

Data Analysis

Data analysis was done using the software SAS v9 (SAS Institute Inc., Cary, NC, USA) by Richard Madsen, Ph.D., Professor Emeritus at University of Missouri. In particular, the Mixed procedure was used with an appropriate correlation structure when repeated measures were involved. Examination of the data showed heterogeneous variances and this was accounted for in the analysis. Residuals were examined for normality. Models using baseline values as a covariate were considered as appropriate. Initial models included interaction terms. If interactions were significant, then differences for a given factor were examined while holding other factors constant. Differences were compared using least squares means with adjustments being made for multiple comparisons. Results for glucose, insulin, and lipids were analyzed separately and the results will be given below. All significant differences had a $p < 0.05$ when indicated as significantly different in the text, unless noted otherwise as $p < 0.01$. Analysis of other markers was performed using the software SigmaPlot where I performed two-way and one-way ANOVAs on the data. Table 1 (page 25) simplifies the six different variables present in the study with the two main effects of diet and rat-line.

Two-way ANOVA				
		Line		
		HVR	LVR	Wild Type
Diet	Normal			
	Western			

Table 1: Two-way ANOVA. This table represents what was analyzed using the two-way ANOVAs. The two main effects are diet and line. There are two different groups within the diet factor, normal diet and Western diet. There are three different groups within the line factor, HVR, LVR, and Wild type. Together, there are 6 different comparisons between the two factors.

RESULTS

Body Weight

Body weight was calculated weekly during the course of the study (Table 2; page 45). Analysis of the body weights was performed to show values of what each rat started at before the 8 weeks of specific dieting. At 8 weeks, WT animals weighed significantly more than both LVR and HVR animals (WT: 259.6; LVR: 213.8; HVR: 207.4g; $F = 18.09$; effect of line: $P < 0.001$). There was no significant difference between the HVR and LVR line at this point in time. Starting age of some WT rats was older by 7-10 days. Importantly, however, six of the WT rats were the same age as all other HVR and LVR rats and these WT rats showed the similar difference in a larger starting body weight as WT 7-10 days older had. Since the WT rats weighed significantly more with and without the group of rats a week older, the older rats were included in all other measurements and calculations in order to have a larger n size for the WT group. Another analysis of body weight was performed after seven weeks of specific dieting. Week seven body weights were used in analysis instead of week 8 because week 8 measurements were affected by two nights of fasting (before GTT and before sacrifice). The average rat fed a Western diet was significantly heavier after 7 weeks than the rat on a normal chow diet ($F = 13.049$; effect of diet: $p < 0.001$). Both HVR and LVR lines on a Western diet had significantly higher body weights than the same line on a normal chow diet ($p = 0.039$ and 0.009 , respectively). The WT line on a Western diet was not significantly heavier in body weight than the WT on normal chow. Refer to table 3 (page 46) for a visual rank order of body weight.

Wild Types gained the least amount of body fat

Body fat percentage was calculated at various time points during the experiment (Table 2; page 45). At 40-42 days of age, the HVR and LVR rat lines had no significant difference in body fat percentage ($p=.226$). At 69-71 days of age, the three lines eating a normal diet had the same body fat percentage. After the eight weeks of specific diet treatment a final DEXA was performed to get an end body fat percentage for each rat. Intriguingly, no significant main effect for line was observed. However, the end body fat percentage showed that all three lines had a significantly higher body fat percentage when fed a Western diet than when fed a normal chow diet ($F = 74.846$; effect for diet: $P < 0.001$). The WT gained the least percentage of body fat on an 8-week Western diet with a final average of 28.4% body fat ($n=7$) and a 9.27% increase in body fat percentage from the age-matched normal chow diet WT rats. The HVR line on a Western diet had an average final body fat percent of 33.2% body fat ($n=8$) and a 16.2% increase in body fat percentage from the age-matched normal chow diet HVR rats. The Western diet LVR rats had the highest average final body fat percent with 35.2% body fat ($n=6$) and a 15.7% increase in body fat percentage from the age-matched normal chow diet LVR rats. Refer to table 3 (page 46) for a visual rank order list of body fat percent.

Total energy intake

Total energy intake is approximated by taking the amount of food consumed by a rat (kcal) and dividing it by the body weight of that rat (grams) (Figure 3; page 36). The analysis was done keeping the baseline as a covariate. The initial analysis showed a couple of outliers, so the analysis was rerun by Dr. Madsen with those outliers excluded. However, Dr. Madsen recommended that differences only be considered to be significant when they appear to be significant with and without outliers. This part of the analysis uses a significance level of 0.01 due to the above. When analyzing the data, the time points for week 7 of specific dieting were

excluded due to GTT and a night of fasting affecting energy expenditure. There was a common trend of decreasing energy intake with all lines and diets as the rats' age. First, a main effect of line was analyzed. At week 2, 3, and 5 the Western-diet HVR line has a higher total energy intake than the Western-diet WT line, $p = 0.0029$, $p < .0001$, and $p = 0.0064$, respectively. Similarly on the normal chow diet, the week 2 and 3 HVR line had a higher total energy intake than the normal chow diet WT line, $p=0.0023$ and 0.0004 respectively. Both week 3 normal chow and Western diet LVR rats had significantly more energy intake than the normal chow and Western diet WT rats at week three, $p = 0.0006$ and 0.0041 respectively. Next, a main effect of diet was analyzed. The Western HVR line had a significantly higher energy intake at weeks 3 and 4 of specific dieting compared to the HVR line on a normal chow diet, $p = 0.0019$ and 0.0008 . The Western LVR line had a significantly higher energy intake at weeks 4 and 5 of specific dieting compared to the LVR line on a normal chow diet, $p = 0.0036$ and 0.0048 respectively. The Western WT line had a significantly higher energy intake at weeks 3 and 4 of specific dieting compared to the WT line on a normal chow diet, $p = 0.0093$ and 0.0002 respectively. Refer to table 3 (page 46) for a visual rank order list of total energy intake.

Blood Lipid Panel:

The lipid panel measured the concentration of five different lipid markers in the blood: cholesterol, TAG, NEFA, LDL-cholesterol, and HDL-cholesterol (Figure 4, page 37). My diet hypothesis was that rats on a Western diet would have elevated lipid markers in all five panels compared to the normal chow diet rats. For line effects, I hypothesized that the HVR line, regardless of diet, would show healthier levels of lipid markers followed by the WT line, and finally the LVR line. In terms of healthier, if considered by rank-ordering highest to lowest values for cholesterol, triglycerides, NEFA, and LDL would be: $LVR > WT > HVR$. The results

for HDL would be reversed (because the highest HDL is the healthiest) so that HVR > WT > LVR.

As an overview for lipid data presentations in the next paragraphs, interaction differences between line and diet will be discussed. Analyses showed that three of the lipids – cholesterol, TAG, and NEFA – have significant interaction terms, so that the interpretation of their results for one factor must be done for each level of the other factors. For line effect (with 3 levels), Dr. Madsen used the adjusted $p < 0.05$ for significance. For the remaining two lipid analyses – HDL-cholesterol and LDL-cholesterol – analyses did not show significant interactions, so Dr. Madsen looked at main effect differences. Each of the five lipids will be described next with the three with interactions described first. Refer to table 4 (page 47) for a visual rank order list of each lipid marker.

Analysis of blood total cholesterol concentrations showed interaction. Here, the HVR and LVR lines on normal chow had significantly more circulating cholesterol in comparison to the WT line on normal chow diet (Figure 4A; page 37). Looking at the three genetically diverse lines of Wistar rat lines on a Western diet showed that they all had significantly different concentrations of circulating total cholesterol (specifically, LVR > HVR > WT). Between diets, the WT line showed no significant diet differences. However, both HVR and LVR lines had significantly higher cholesterol concentrations on Western diet than on normal chow diet ($p = 0.0034$ and 0.0035 , respectively). Thus, my hypothesis of LVR > WT > HVR was not held.

Analysis of blood TAG concentrations showed that HVR and WT lines on normal chow had significantly less circulating TAG than the LVR line on the same diet (Figure 4B; page 37). The three genetic Wistar rat groups on a Western diet all had significantly different concentrations of circulating TAG from one another (specifically, LVR > HVR > WT). Between

diets, the WT line showed no significant diet differences. However, both HVR and LVR lines had significantly higher TAG concentrations on Western diet than on normal chow diet ($p = <.001$ and 0.002 , respectively). Thus, my hypothesis that the LVR line would have the most TAG was held but I did not predict the WT line to have the lowest values.

Analysis of blood NEFA concentrations showed that the LVR line on a normal chow diet had significantly more circulating NEFA than the WT and HVR line on the same diet (Figure 4C; page 37). The WT line on a Western diet had significantly less circulating NEFA than both LVR and HVR lines on the same diet. This biochemical marker showed a significant diet difference in all three lines whereby the HVR, LVR, and WT lines on a Western diet had significantly more circulating NEFA than their respective groups on a normal chow diet ($p = <.001$, 0.0054 , and 0.0381 , respectively). Taken together, my hypothesis that the LVR line would have the most NEFA was held in the normal chow diet, but I did not predict the WT line to have the lowest values for the Western diet.

Analysis of blood LDL-cholesterol levels showed no interactions, so only main effects were performed. Rats on a Western Diet had a greater concentration of LDL-cholesterol than the rats on a normal chow diet ($p = 0.0089$) (Figure 4D; page 37). Also, the HVR line had the greatest concentrations of LDL-cholesterol than both LVR and WT lines. Thus, my hypothesis of $LVR > WT > HVR$ was not held.

Analysis of blood HDL-cholesterol levels showed no interactions. No significant differences existed between diets of the three lines. Looking within the diets' main effect, the HVR, LVR, and WT lines all had significantly different concentrations of HDL-cholesterol from one another (specifically, $LVR > HVR > WT$) (Figure 4E; page 37). Thus, my hypothesis of $HVR > WT > LVR$ was not held.

Liver triacylglyceride concentration showed a diet difference but no line difference

Triglyceride concentration was measured from the median lobe of the liver from each rat (Figure 5, page 38). Analysis was performed and a main effect of diet was observed but there was no significant difference between the three lines. The rats fed a Western diet had significantly more triglyceride concentration in the liver than rats fed a normal chow diet ($p < .001$). The LVR line showed a trend toward greater hepatic triglyceride concentrations than both HVR and WT lines ($p = 0.0967$ and 0.0351 , respectively). Refer to table 3 (page 46) for a visual rank order list of triglyceride concentrations.

Glucose Tolerance Test (Blood Glucose Levels)

Glucose Tolerance Tests (GTT) results were analyzed, and the area under each curve (AUC) was calculated in order to provide a good summary of the body's glucose tolerance (Figure 6, 7; page 39, 40). Analysis of the AUC showed that the interaction term was not significant, so the model was re-run looking only at main effects (Figure 6; page 39). Main effects for diet and line were shown to be significant. The Western diet had a higher mean by about 13230 units (95% confidence interval estimate (9692, 16777)). For line effect, HVR was significantly higher than LVR and WT. Refer to table 3 (Page 46) for a visual rank order of the AUC for the GTT.

Significant interaction terms were observed when analyzing the time course of glucose tolerance tests, so Dr. Madsen looked for significant Diet effects for fixed Line and Time. Likewise, Dr. Madsen looked for significant Line effects with the other factors fixed. Since there are numerous pairwise comparisons, a 0.01 level of significance was used rather than the usual 0.05. There was no significant difference between lines or diets at the basal, pre-dextrose level (time point 0). For rats on normal chow, the HVR line had significantly higher glucose

levels than LVR at time points 15 and 30 minutes post dextrose injection and tended to be higher than the LVR and WT line for all other time points (Figure 7A; page 40). Similarly, HVR rats on a Western diet had significantly higher glucose levels for time points 15, 30 and 45 minutes post dextrose injection than the LVR line (Figure 7B; page 40). The HVR line was also significantly higher than the WT line at time points 45 and 60 minutes post dextrose injection and tended to be higher on all other time points than both LVR and WT lines. There were significant differences between the two diets when time and line were held constant. Throughout each line, the Western diet tended to have higher glucose levels at each time point than the same line on normal chow. Specifically, the HVR and LVR line on a Western diet showed significantly higher glucose levels at the 30, 45, 60, and 120 minutes post dextrose injection than the HVR and LVR line on a normal chow diet. The WT line on a Western diet showed significantly higher glucose levels at 15, 30, 45, and 60 minutes post dextrose injection than the WT line on a normal chow diet. Taken together, it is clear that the Western diet caused hyperglycemia in all three rat groups compared to the rats on a normal chow diet.

Corticosterone concentration

Corticosterone concentration was measured using GTT plasma collected for each rat at two different time points, pre-dextrose injection (basal) and 30 minutes post dextrose injection (Figure 8, page 41). Analysis was performed on the concentration at both time points for each rat and a main effect for time was observed where the 30-minute time point was higher than basal (0-minute) for all treatments (two-way ANOVA, Time Factor: $F [1, 36] = 28.411, p < .001$). There were no significant differences between lines or diets, only between the basal and 30-minute post-dextrose injection of the GTT. Corticosterone levels oppose insulin by increasing blood glucose via inhibiting glucose transport into peripheral tissues, increasing

gluconeogenesis, and increasing glucose output by the liver (Reviews in Environmental Health (1998): Toxicological Defense Mechanics edited by Gary E. R. Hook, George W. Lucier).

Therefore, elevated corticosterone would affect the GTT by increasing blood glucose levels measured. Taken together, lack of difference among lines and diets imply lack of evidence for plasma corticosterone, itself, differentially altering GTT among lines. Refer to table 3 (page 46) for a visual rank order list of corticosterone concentration.

Insulin results had high variability

Insulin was measured from blood plasma obtained during each time point of the GTT (Figure 9, page 42). Analyzing the individual points within the insulin curves, there were great differences in the distribution of the results. Parametric analysis was tried, but residual analysis showed several outliers. Using a log transformation did not help much. There was a suggestion that there may be diet differences, at least at some times. The conclusions were about the same when the analysis was re-run with 4 outliers excluded. Next, Dr. Madsen tried looking at a series of nonparametric (Kruskal-Wallis or Wilcoxon rank sum tests). When Dr. Madsen tested for diet differences for each line and each time, he found two cases with $p < 0.01$ (LVR and WT Western diets both at the time of 120 minutes greater than their respective normal chow diet, which is consistent with what was suggested by the parametric analysis). When he next tested for line differences for each diet and each time, he found no cases with $p < 0.01$. Results were plotted from the six different time points and the AUC was obtained (Figure 10, page 43). Analysis of the AUC showed that there was not enough data for the WT line to be included. The interaction term was not significant between the two main effects, diet and line, for the HVR and LVR lines. However, the diet main effect for the AUC was significant ($p = 0.0135$), which in this case the significance had been set at $p < 0.05$ because of the low number of comparisons (i.e. no repeated

measures). Specifically, the Western diet had a higher mean by about 232 units (95% confidence interval estimate (52, 411)). Refer to table 3 (page 46) for a visual rank order list of the AUC for insulin concentration.

Insulin Sensitivity Indices

The QUICKI was used to measure the insulin sensitivity of the rats after 7 weeks of specific dieting using the glucose and insulin values obtained from the GTT (Figure 11, page 44). The lines on a normal chow diet had a significantly higher insulin sensitivity index, on average, than the lines on a Western diet ($F = 12.333$; effect for diet: $p = 0.001$). There was a significant difference within the LVR line, where the rats on a normal chow diet had a significantly higher insulin sensitivity index than the rats on a Western diet ($p = 0.003$). Refer to table 3 (page 46) for a visual rank order list of QUICKI.

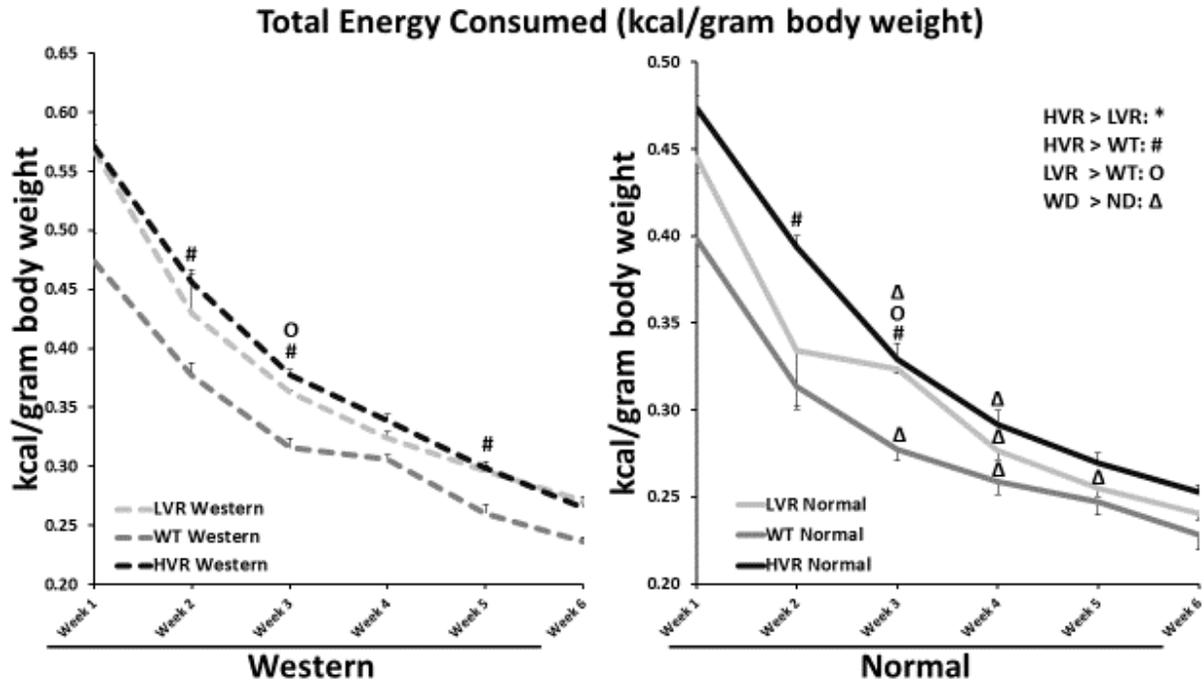


Figure 3: Total Energy Consumed (Kcal/gram body wt.).

The dashed lines on the left graph (A) represent Western chow diets and the solid lines on the right graph (B) indicate Normal chow diet. Note the difference in scale of y-axes. * represents HVR>LVR. # represents HVR>WT. O represents LVR > WT. Δ represents the Western diet > normal chow diet. LVR normal n = 7, WT normal n = 6, HVR normal n = 7, LVR Western n = 7, WT Western n = 6, HVR Western n = 8.

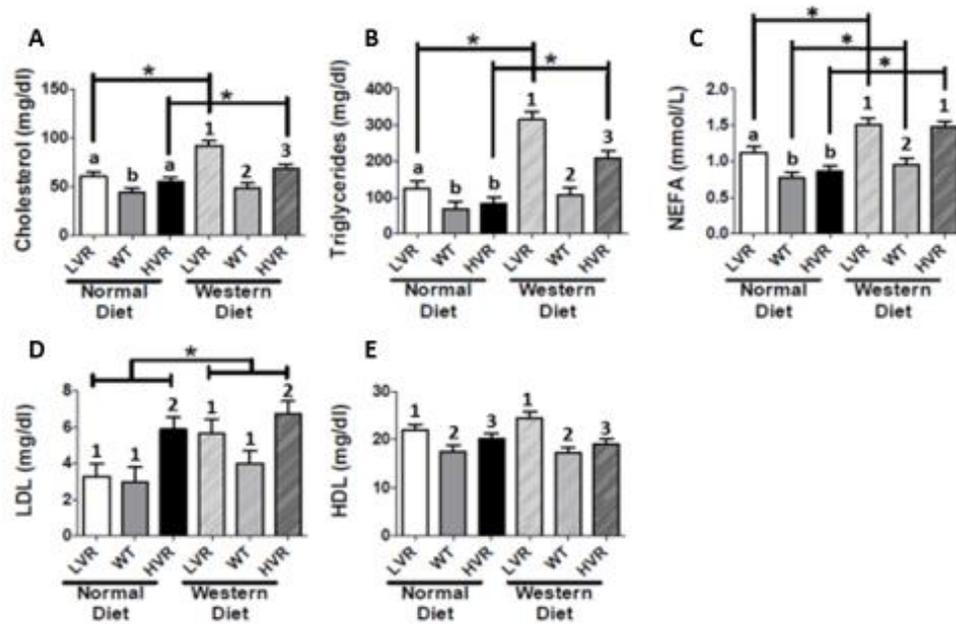


Figure 4: Blood Lipid Panel.

A-C) The asterisk represents significant differences in concentrations due to diets within a specific rat line. For total cholesterol, the Western LVR > normal LVR ($p = 0.0035$) and the Western HVR > normal HVR ($p = 0.0034$). For Triglycerides, the Western LVR > normal LVR ($p = 0.002$) and the Western HVR > normal HVR ($p < 0.001$). For NEFA, the Western LVR > normal LVR ($p = 0.0054$), the Western WT > normal WT ($p = 0.0381$), and the Western HVR > normal HVR ($p < 0.001$). Different letters and numbers represent differences in concentration between lines within a specific diet. For cholesterol, the Western diet saw LVR > HVR > WT and the normal chow diet saw LVR and HVR > WT. For triglycerides, the Western diet saw LVR > HVR > WT and the normal chow diet saw LVR > WT and HVR. For NEFA, the Western diet saw HVR and LVR > WT and the normal chow diet saw LVR > WT and HVR.

D-E) The asterisk represents a main effect of diet when summing all lines together. For LDL, the Western diet was significantly greater than the normal chow diet. Different numbers indicate a

main effect of line after averaging one line together (both diets). For LDL, the HVR > LVR and WT. For HDL, the LVR line > HVR line > WT line. LVR normal n = 7, WT normal n = 6, HVR normal n = 8, LVR Western n = 6, WT Western n = 7, HVR Western n = 8

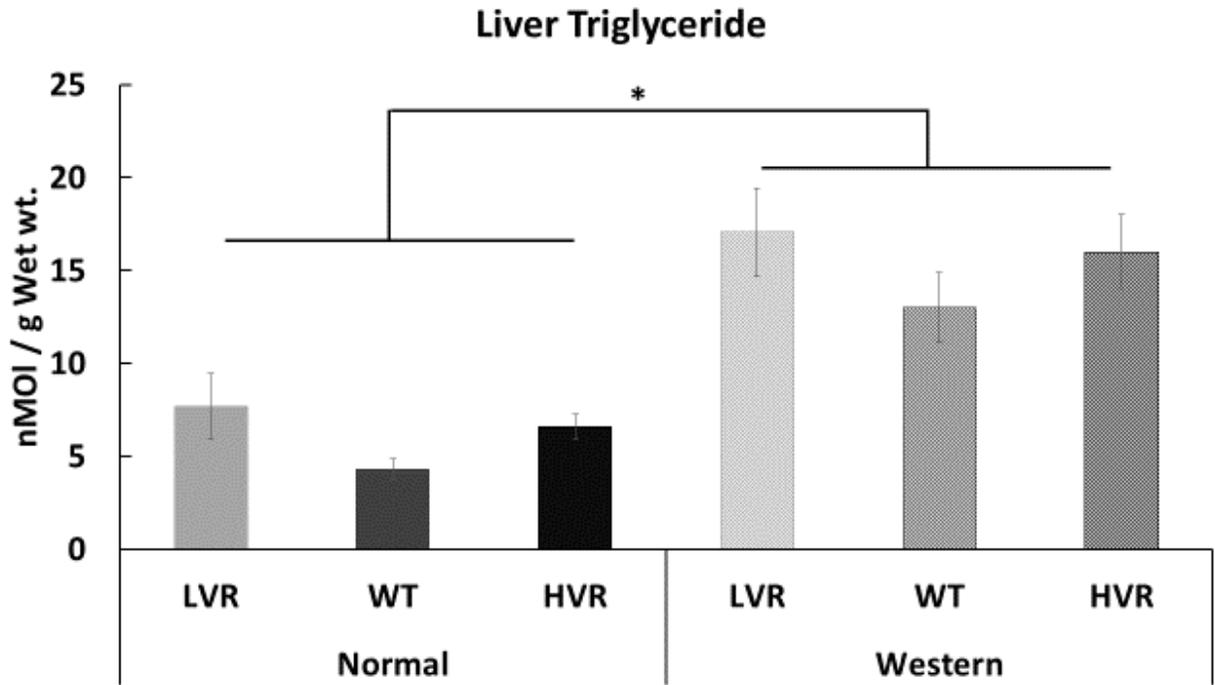


Figure 5: Hepatic Triglycerides.

Bars represent the average of the summed hepatic triglyceride concentrations obtained from the medial lobe of the rat liver. The asterisk represents the Western diet had a higher mean than the normal chow diet ($p < 0.001$). The LVR line showed a trend toward greater hepatic triglyceride concentration than both HVR and WT lines ($p = 0.0967$ and 0.0351 , respectively). LVR normal $n = 7$, WT normal $n = 6$, HVR normal $n = 8$, LVR Western $n = 6$, WT Western $n = 7$, HVR Western $n = 8$.

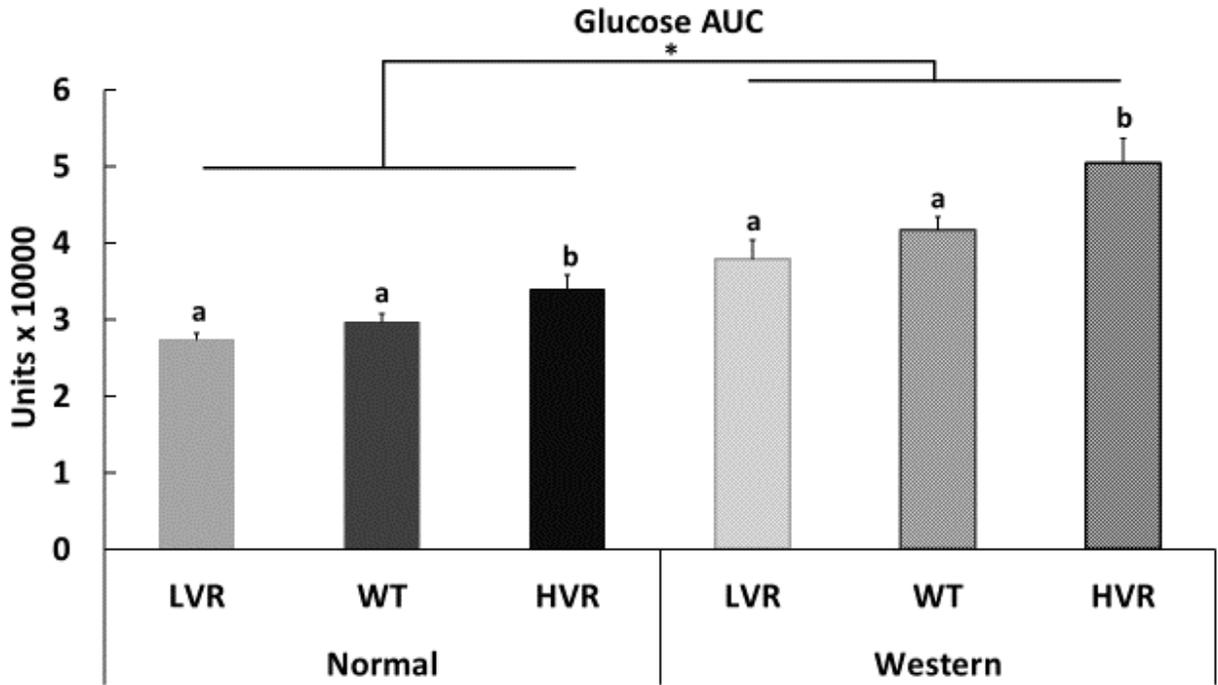


Figure 6: Area under the curve for glucose tolerance tests (GTT).

Bars represent the average of the summed glucose concentrations including all time points measured during GTT. The asterisk represents the Western diet had a higher mean than the normal chow diet ($p < 0.0001$). The small letters represent the line differences, where the $HVR > WT$ ($p < 0.0001$), $HVR > LVR$ ($p = 0.0039$), and there is no significant difference between the LVR and WT lines ($p = 0.1879$). LVR normal $n = 7$, WT normal $n = 6$, HVR normal $n = 8$, LVR Western $n = 7$, WT Western $n = 7$, HVR Western $n = 8$.

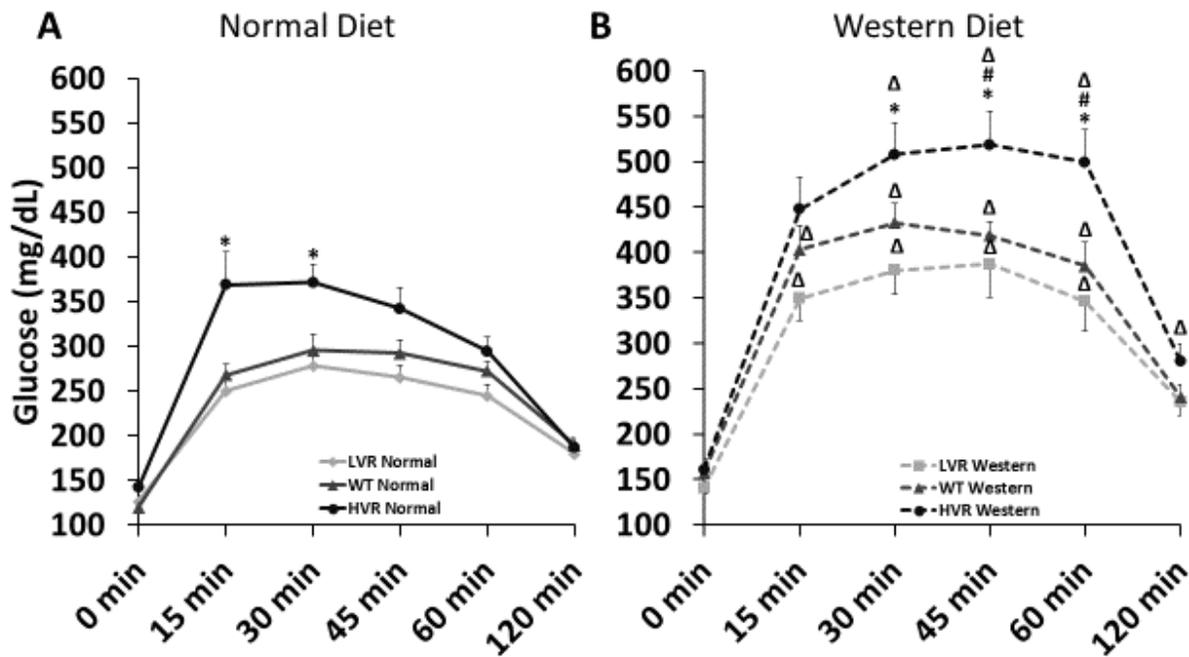


Figure 7: Glucose tolerance test (GTT) curves.

The solid lines on the left graph (A) represent normal chow diets and the dashed lines on the right graph (B) indicate Western chow diet. * represents HVR>LVR. # represents HVR>WT. Δ represents the Western diet > normal chow diet. LVR normal n = 7, WT normal n = 6, HVR normal n = 8, LVR Western n = 7, WT Western n = 7, HVR Western n = 8.

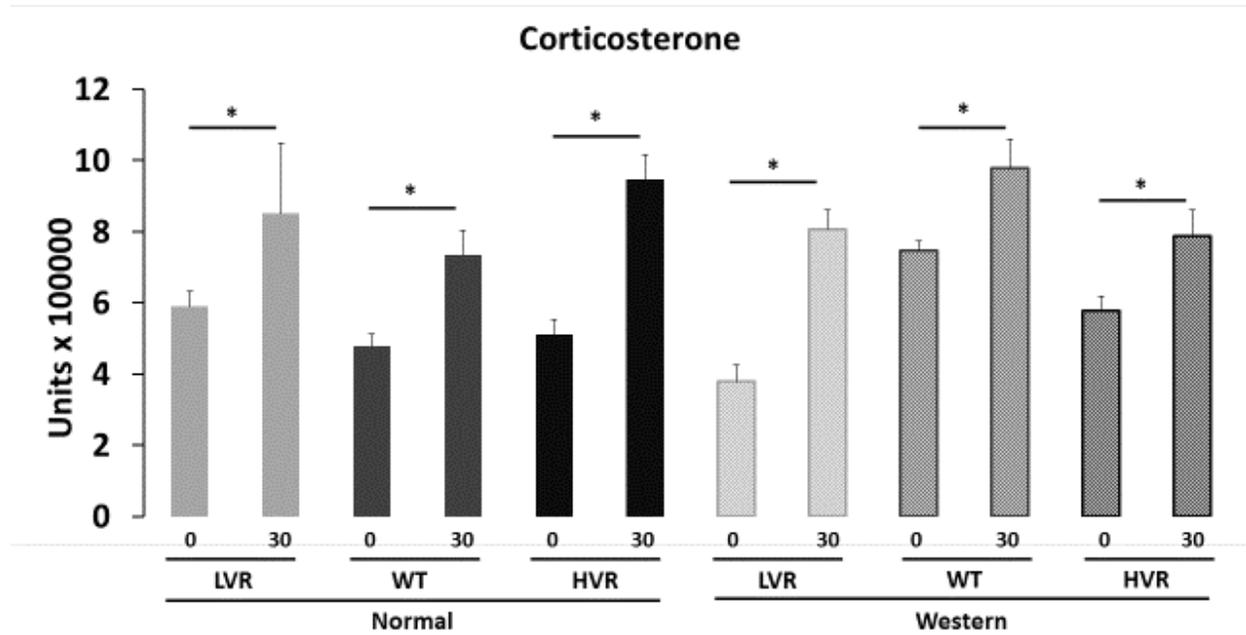


Figure 8: Blood plasma corticosterone concentrations.

Bars represent the corticosterone level measured from the blood plasma of the rats for time points 0 and 30 minutes post dextrose injection of the GTT. For each rat line there was a main effect of time which showed a significant increase in corticosterone concentration from the basal (time 0) to 30 minutes post dextrose injection ($p < 0.001$). LVR normal $n = 7$, WT normal $n = 6$, HVR normal $n = 7$, LVR Western $n = 4$, WT Western $n = 7$, HVR Western $n = 7$.

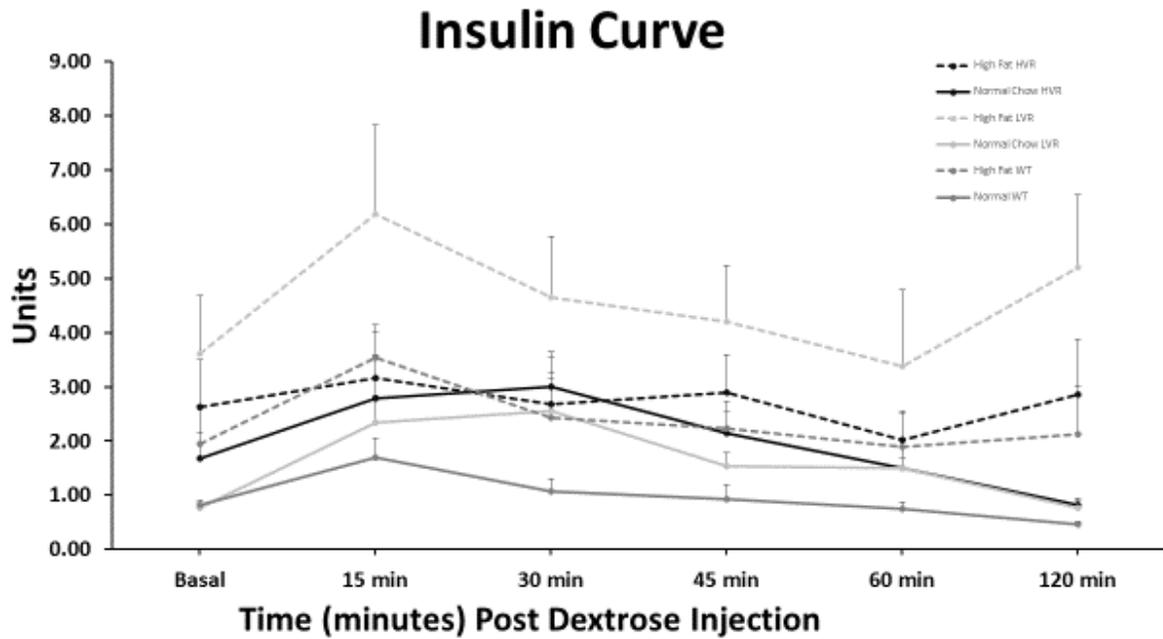


Figure 9: Blood plasma insulin concentrations obtained from GTT.

The solid lines represent the rats on a normal chow diet and the dashed lines represent the rats on a Western diet. LVR normal n = 7, WT normal n = 4, HVR normal n = 7, LVR Western n = 5, WT Western n = 6, HVR Western n = 8.

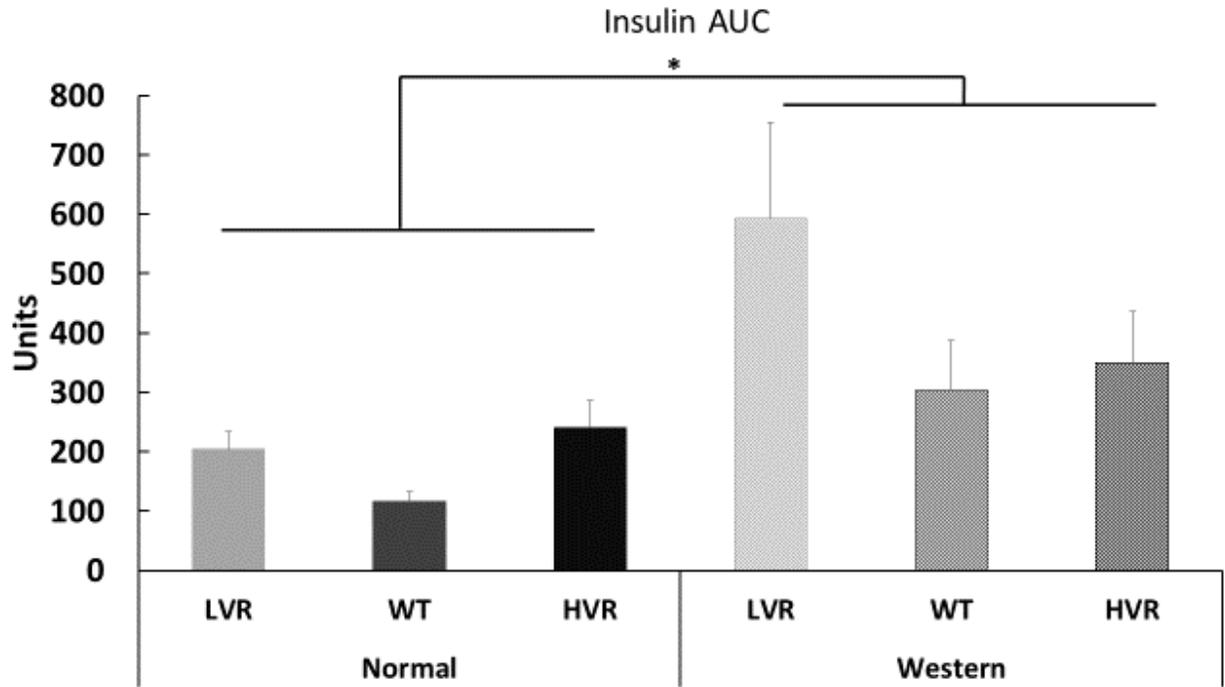


Figure 10: Area under the curve for insulin.

Bars represent the average of the summed insulin concentrations collected during each time point of the GTT. The asterisk represents the Western diet had a higher mean than the normal chow diet ($p=0.0135$). LVR normal $n = 7$, WT normal $n = 4$, HVR normal $n = 7$, LVR Western $n = 5$, WT Western $n = 6$, HVR Western $n = 8$.

Quantitative insulin sensitivity check index (QUICKI)

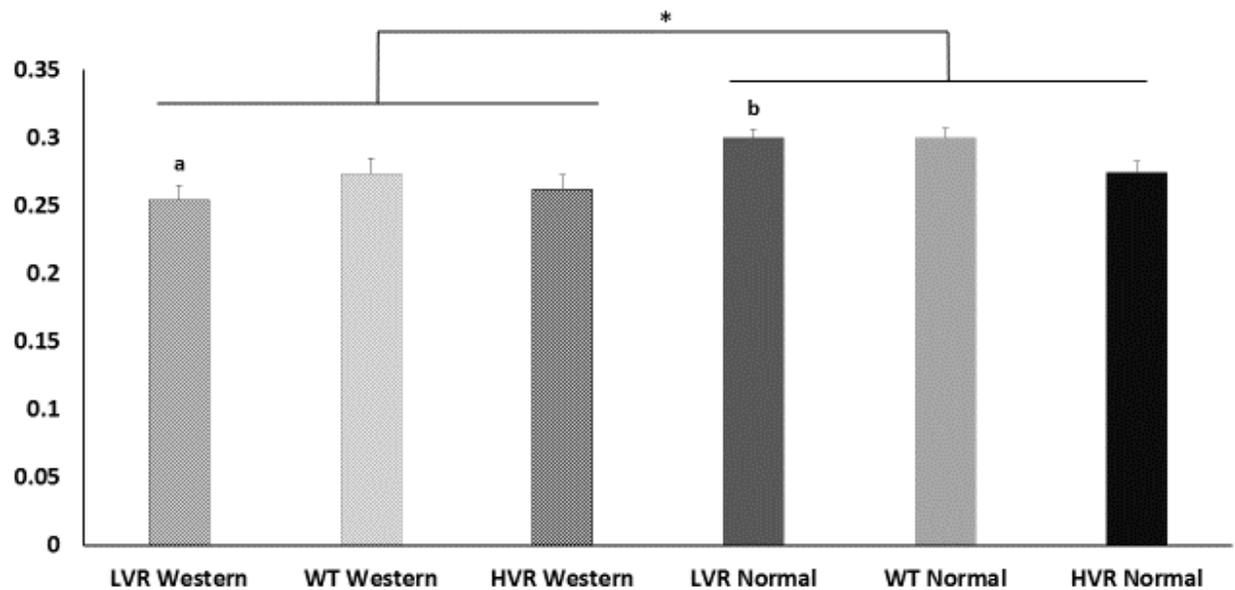


Figure 11: Quantitative insulin sensitivity check index.

Bars represent the average QUICKI value for each rat line. The asterisk indicates a main effect of diet between the Western diet rats and the normal chow rats ($p = 0.001$). The letters represent a difference between the LVR normal chow diet and the LVR Western diet ($p = 0.003$). LVR Western $n = 6$, WT Western $n = 7$, HVR Western $n = 8$, LVR normal $n = 7$, WT normal $n = 6$, HVR normal = 7.

Body Weights/DEXA/ Heart Weight

	LVR ND	WT ND	HVR ND	LVR WD	WT WD	HVR WD
BW wk 0	216.0 ±12.4 ^a	261.9 ±7.8 ^b	207.7 ±8.5 ^a	211.5 ±11.1 ^a	257.6 ±8.7 ^b	207.2 ±8.3 ^a
BW wk 7	498.3 ±12.4 ²	487.8 ±12.8 ²	480.1 ±8.5 ²	579.2 ±11.0 ¹	531.4 ±8.4 ¹	539.2 ±8.3 ¹
DEXA-wk 0	9.7 ±0.8	ND	7.9 ±0.6	8.5 ±0.9	ND	8.6 ±0.6
DEXA- wk 4	14.5 ±0.9	12.9 ±0.8	12.1±1.0	ND	ND	ND
DEXA- wk 8	19.5 ±1.2 ²	19.13 ±1.5 ²	17.0 ±1.6 ²	35.2 ±2.7 ¹	28.4 ±2.1 ¹	33.2 ±2.1 ¹

Diet – Numbers
Line – Letters

Table 2: Body Weight, DEXA, and Heart Weights.

Columns indicate lines on a specific diet and the rows indicate the particular measurement.

Body Weight (BW) is measured in grams. DEXA is measured in percent body fat. Diet differences are indicated by numbers. Line differences are indicated by letters.

The WT line had significantly heavier body weights at week 0. The rats on a Western diet had significantly heavier body weights at week 7 than the rats on a normal chow diet. There were no differences between DEXA results in week 0 or week 4. The rats on a Western diet had significantly more body fat at week 8 than the rats on a normal chow diet.

For BW: LVR normal n = 7, WT normal n = 6, HVR normal n = 8, LVR Western n = 7, WT Western n = 7, HVR Western n = 8. For DEXA-wk 0: LVR normal n = 7, HVR normal n = 8, LVR Western n = 7, HVR Western n = 8. For DEXA- wk 4: LVR normal n = 7, WT normal n = 6, HVR normal n = 7. For DEXA-wk 8: LVR normal n = 7, WT normal n = 6, HVR normal n = 8, LVR Western n = 6, WT Western n = 7, HVR Western n = 8.

Rank order of values from highest (1) to lowest (3)				
	HVR	LVR	WT	Western > normal
Glucose tolerance test (AUC)	1	2	2	yes
Body weight (wk 7)	1	1	1	yes
Body fat percent (wk 8)	1	1	1	yes
Heart weight	2	1	2	HVR only
Liver triglycerides	2*	1*	2*	yes
Plasma insulin (AUC)	1	1	1	yes
Plasma corticosterone	1	1	1	no
Total energy intake	1*	2*	3*	yes**
Insulin sensitivity Index	1	1	1	yes

Table 3: Rank Order of Metabolic Markers.

This table gives the rank order of the three different rat-lines in regards to different metabolic and physiologic markers. The rank ranges from 1-3 with 1 being the highest and 3 being the lowest in terms of concentration. If numbers are the same then there was no significant difference in concentration. An asterisk (*) indicates a group with trending values that are not significant (or at least partially trending due to multiple time-point measurements not all being significant). Double asterisk (**) indicates that total energy intake was taken over multiple time points (repeated measures) and that many of the results, but not all, showed significant values for Western > normal diet.

Rank order of values from highest (1) to lowest (3)			
	HVR	LVR	WT
Western Diet: Total cholesterol	2	1	3
Western Diet: Triglycerides	2	1	3
Western Diet: NEFA	1	1	2
Normal Diet: Total cholesterol	1	1	2
Normal Diet: Triglycerides	2	1	2
Normal Diet: NEFA	2	1	2
HDL	1	2	2
LDL	2	1	3
Total number of 1's	3	7	0

Table 4: Rank Order of Lipid Panel Results.

This table gives the rank order of the three different rat-lines in regards to their blood lipid panel results. The rank ranges from 1-3 with 1 being the highest and 3 being the lowest in terms of concentration. If numbers are the same then there was no significant difference in concentration. HDL and LDL do not have an indicated diet because interactions were not significant and only main effects were analyzed. For Total cholesterol, Triglycerides, and NEFA, significant interactions occurred so the interpretation of results for one factor (line) must be done for each level of the other factors (diet). Therefore, both diets are indicated.

Rank order of values from highest (1) to lowest (3)			
	HVR	LVR	WT
Body Weight Body Fat Percent Plasma Insulin Plasma Corticosterone Insulin Sensitivity	1	1	1
Glucose Tolerance	1	2	2
Total Energy Intake*	1	2	3
Heart Weight Liver TAGs*	2	1	2

Table 5: Grouping of Different Metabolic Markers.

This table offers a different view of the metabolic markers measured. The rank ranges from 1-3 with 1 being the highest and 3 being the lowest in terms of concentration. An asterisk indicates a group with trending values that are not significant (or at least partially trending due to multiple time-point measurements not all being significant).

DISCUSSION

The purpose of this study was two-fold. One goal, was to determine if rats selectively bred for an inherent trait to voluntarily run long distances express healthy marker phenotypes when they are not permitted to engage in voluntary running. The second direction of my study was to test induction to become prediabetic-like and/or pre-metabolic syndrome-like through dietary challenge among three rat lines with different inherent levels of motivation for voluntary running distances/time. The test challenge was a Western diet. After eight weeks of specific dieting (normal or Western dieting) with no access to running wheels, the rats were then assessed, through various metabolic markers, on their inherent genetic ability to protect against Western dieting. To accomplish this goal, I used the HVR and LVR rat model (Roberts et al., 2013), of extreme high and low motivation for inherent voluntary running distance/time, respectively, as well as an intermediate running distance WT line of the same founder strain – Wistar. The major findings of this experiment were that HVR rats with no access to voluntary running wheels did not inherently protect themselves from the negative effects of a high fat/high sugar (Western) diet, as compared to the rat line with lower levels of motivation. What is remarkable is that the HVR line had higher levels of inherent motivation for voluntary running. This finding was in contrast to my initial hypothesis that the HVR line was co-selected to have genetic factors that would give them some form of protection from the deleterious effects of Western dieting. In other words, the inheritance to be active is ineffective for the normal, positive health benefits associated with exercise at least when the drive is not actually exerted. From here, I will break down the results into sections and then conclude by compounding their interconnections.

Blood lipid panel

The blood lipid results offer intriguing outcomes for the five lipids measured. Some studies have shown that high sugar diets increase lipogenesis and triglyceride accumulation, as well as increase the LDL particles in the liver (Mohamed Salih, Nallasamy, Muniyandi, Periyasami, & Carani Venkatraman, 2009; Woodhead, 1990). Other studies have found that high-sugar diets have no adverse effects on the total cholesterol, LDL, or HDL (Rippe & Angelopoulos, 2013). Thus, the previous research becomes unclear, suggesting further research to clarify this issue regarding the effects of a high-sugar diet on lipid markers. On the other hand, continuous ingestion of a high-fat diet is related to hyperlipidemia in humans (Akiyama, Tachibana, Shirohara, Watanabe, & Otsuki, 1996). Therefore, my initial hypothesis was that the normal-chow diet rats would have much lower lipid concentrations than the Western diet rats in general and, especially, in rats genetically predisposed to voluntarily run long distances/times.

One of the dietary outcomes is cholesterol. Cholesterol is a fatty substance that is important and critical in cell structure. Cholesterol must be transported through the blood by carriers, known as lipoproteins. In regards to this study, there are two major types of lipoproteins, LDL and HDL. Total cholesterol is a measurement of LDL-cholesterol, HDL-cholesterol, as well as triglyceride concentration. LDL-cholesterol is commonly known as the “bad” cholesterol compared to HDL-cholesterol, or the “good” cholesterol. LDL-cholesterol transfers cholesterol to tissues and can build up in the arteries and lead to heart disease. On the other hand, HDL-cholesterol transfers cholesterol away from tissues and antagonizes the effects of LDL-cholesterol by taking cholesterol off of vessel walls, preventing its build up in the arteries (Badimon & Vilahur, 2012). Though the body does require some circulating cholesterol, increased levels, especially LDL-cholesterol, can lead to plaque build-up, coronary heart disease, and eventually death (Scirica & Cannon, 2005).

As predicted in regards to a diet effect, plasma total cholesterol concentration was significantly higher in the HVR and LVR rats on a Western diet compared to those on a normal diet. Interestingly, there was no cholesterol difference between the normal chow diet and Western diet in the WT line. Additionally, the WT line showed lower amounts of total cholesterol than both the HVR and LVR line. This suggests a lessened effect of the Western diet on the WT line compared to the LVR and HVR line. An explanation could be that the WT line had less body fat percentage and a lower body weight after eight weeks of Western dieting when compared to the LVR and HVR lines. Having a lower body fat percentage does not directly impact on levels of blood lipids, however, the activities required to lower body fat percentage, such as increase exercise and proper dieting, do improve cholesterol levels (Phifer, February/March 2009). This cannot be a plausible explanation due to the fact that no animal was given access to voluntary running wheels. Total cholesterol has been shown to increase with age, weight, and fat intake (Brennan, Simpson, Blacket, & McGilchrist, 1980; Scirica & Cannon, 2005). The rats at this point were the same weight and the majority were the same age (minus the few WT rats that were a week older). However, the WT line's total energy intake was significantly lower or trending to be lower than the HVR and LVR total energy intake. Less energy intake in WT than LVR and HVR could explain the lower amount of total cholesterol for the WT group on a Western diet. In summary, the WT line was able to handle the effects of a high fat/high sugar diet better than the LVR and HVR lines in regards to total cholesterol. In other words, I propose that the selective breeding model (HVR and LVR) could be co-selecting for metabolic traits that are undesired or deleterious to the rat, whereas the nonselected model, having a more diverse gene pool, is better able to handle the negative effects of the Western diet on total cholesterol.

LDL- and HDL-cholesterol offered intriguing results. As predicted, the three rat lines on a Western diet had higher concentrations of LDL-cholesterol than the rats on a normal chow diet. This could be explained because the Western diet rat groups had much higher fat and sugar intake over the eight weeks of feeding which lead to higher body weights and fat percentages, on average. These characteristics have been shown to increase cholesterol, specifically LDL-cholesterol, concentrations (Ali et al., 2014; Scirica & Cannon, 2005). Interestingly, the HVR line had higher concentrations of LDL-cholesterol than both the LVR and WT line. LDL-cholesterol is regarded as the “bad” cholesterol that leads to coronary artery pathology. One possible explanation for this result could be that none of the rats were given access to voluntary exercise, besides normal cage movement. Since the HVR line is known to be significantly more active than the other lines, it is possible that the motivation or desire for this high level of activity is due, at least in part, to maintain normal body function. Restricting this highly active rat line from wheel running could result in deleterious effects to body functions, for example, normal metabolic markers. On the other hand, the HDL-cholesterol levels showed no difference between diets but had differing concentrations between lines, specifically $LVR > HVR > WT$. Here, the HDL-cholesterol, or “good” cholesterol, showed to be highest in the rat line that is the least motivated for activity. This could be speculated to be due to the restriction of wheel running. The LVR line could have co-selected genes that program them with a low desire to exercise and, thus, I speculate could have compensated metabolically co-selecting for a low level of exercise needed to maintain normal bodily function of HDL- and LDL-cholesterol. Though higher levels of HDL are beneficial, it could be that the WT line does not need elevated HDL levels to maintain a normal, healthy lipid profile where the LVR line needs an elevated level to

attempt to maintain a normal lipid profile. This being said, it would have been helpful to obtain a blood lipid panel before the diets began to see a starting point for each rat line.

Exercise has been shown to raise HDL levels and lower LDL levels in humans (Durstine, Miller, Farrell, Sherman, & Ivy, 1983; Scirica & Cannon, 2005). This can be explained in part because exercise reduces body weight which lowers LDL (Scirica & Cannon, 2005). Also, exercise stimulates HDL movement of cholesterol to the liver where the cholesterol is converted to bile and excreted or used in digestion. Interestingly, the WT line had lower levels of both HDL, LDL, and cholesterol suggesting that the nonselected group may be better equipped to protect against a Western diet.

Body weight and body fat

Body weights were measured weekly. Pre- and post-body fat percentage were measured using a DEXA machine. The starting body weights were similar among the LVR and HVR lines, but the WT line, on average, started at a higher body weight. This can be attributed to an inherent difference between the Wistar rat lines. After seven weeks of specific dieting, all rats showed an increase in body weight. As expected, the Western diet rats, on average, had significantly higher body weights than the rats on a normal chow diet. Specifically, the HVR and LVR rat lines demonstrated significant increases in body weight on a Western diet compared to their normal chow diet counterpart. This was expected because of the increased intake of fat and sugar. Interestingly, the WT rat line had no significant difference in body weights between the normal chow and Western diet. In fact, though the WT line started the heaviest, they gained the least percent increase in body weight from start to finish for both diets followed by HVR line and finally by the LVR line which gained the most (data not shown). Similar findings occurred when measuring the body fat percentage of each rat. Here, no differences were noted at the

beginning of specific dieting. However, the final DEXA revealed that the Western diet rats had significantly more body fat percentage than the normal chow diet rats in all three lines. The LVR line had the highest body fat percent in each specific diet group. This could be explained because the LVR line has previously been shown to have less cage locomotor activity than the HVR line, therefore, inferring burning less and storing more calories as triglycerides. One possible explanation for these results is by the inherent genes of our selective breeding model. The LVR line has the sedentary motivation genes for voluntary running that co-selects for genes that produce obese phenotypes with or without access to wheels. On the contrary, the HVR line has genes that motivate it to have more voluntary running than the LVR line, but when it is not allowed to run, it cannot cope with the effects of a Western diet. The addition of the WT was for a nonselected control group. Interestingly, the WT rats had the smallest percentage difference in body fat between normal chow and Western diet, just as the body weights indicated. What is odd is that the WT rats did not show any difference between body weights, yet the WT line on a Western diet had a significantly higher body fat percentage. One possible explanation for this is that the seven rats in the WT group that were a week older than the rest of the group caused the body weights to not be significant. When the older rats were excluded the n value for the group was very low and resulted in a trending difference between the two diets ($p = 0.097$). Therefore, I propose that if there were more age matched rats in the WT group then there would have been a significant difference in body weights which would correlate with the increase in body fat percentage seen in the Western diet rats.

Energy balance

According to the National Heart, Lung, and Blood Institute, energy balance is the balance of calories consumed through eating and drinking compared to calories burned through physical

activity (Health). In other words, the energy in minus the energy out. If more is going in than out, then energy is stored and causes weight gain. The opposite occurs if more energy is spent than consumed. The rats in this study were limited to voluntary exercise because they had no access to a running wheel. However, they were free to have normal cage activity, but this was not measured. Therefore, I was unable to measure their energy balance because only the energy intake was obtained. If I were to speculate, the HVR rats have been shown to have higher spontaneous cage activity than the LVR rats which could help explain the difference in body composition (Brown, Green, Arthur, Booth, & Miller, 2015). Interestingly, the WT rats showed a lower body weight and body fat percentage. If the WT energy expenditure via spontaneous cage activity was greater than the HVR and LVR rats then this would be a possible explanation for the lower body weights and metabolic markers. However, if this was not the case, another possible explanation would be that the WT line is predisposed with some genes that program it to better handle a Western diet internally than the other two lines. For example, maybe the WT line has a higher density of mitochondria that allow for a more effective use of energy and less storage as fat.

Circulating and hepatic triacylglycerides and circulating free fatty acids

Triacylglycerides (TAG) are another lipid that circulates in the blood-bound lipoproteins, such as HDL and LDL, and are the main source of dietary fat and fat depots within animals (*Clinical Methods: The History, Physical, and Laboratory Examinations.*, 1990). TAGs are obtained either from the diet or synthesized in the liver from free fatty acids and carbohydrates. High amounts of TAG concentrations are directly related to an increased risk of atherosclerotic coronary artery disease (*Clinical Methods: The History, Physical, and Laboratory Examinations.*, 1990; Hokanson & Austin, 1996). Low amounts of exercise and poor dieting

both increase the amount of TAG in circulation and, therefore, the risk of disease (Scirica & Cannon, 2005). With this being said, my expectation was for all three lines to have higher hepatic and circulating TAG concentrations on a Western diet than on a normal chow diet. As expected, the HVR and LVR lines had significantly higher circulating and hepatic TAG concentrations when on a Western diet compared to normal chow diet. Also, the LVR line had significantly higher circulating TAG on both the Western diet and the normal chow diet than the HVR and WT group and tended to have higher hepatic TAG as well. This could be explained because the LVR line had the highest percent body fat and the highest body weights for each of the two diets after the eight weeks of specific dieting. Interestingly, the WT line showed no difference in circulating TAG between the two diets. Again, the WT data could possibly be explained by the selective breeding model, which co-selected deleterious traits that diminish the HVR and LVR lines from being able to handle a Western diet with no exercise.

NEFA, also known as non-esterified free fatty acids, arise from adipose tissue and the breakdown of TAG. Insulin is a major suppressor of NEFA release, therefore, they tend to drop after any carbohydrate meal (Karpe, Dickmann, & Frayn, 2011). However, when the blood samples were taken, the rats were fasted. Thus, I expected to see higher values of NEFA in the Western diet rats due to the increased fat/sugar intake over eight weeks. As predicted, all three lines on a Western diet had significantly elevated NEFA values. This could be explained by the increased body weight and percent body fat in the Western diet rats compared to normal chow diet rats. In other words, the larger the adipose tissue is, the more NEFA it can release (Boden, 2008). On the contrary, one study found that as adipose tissue expands, NEFA release per kilogram adipose tissue is downregulated, not increased (Karpe et al., 2011). These contrasting studies demonstrate the need for further study of the relationship between NEFA and obesity.

Another interesting finding was that the LVR line on a normal chow diet had significantly more circulating NEFA than the HVR and WT lines on the same diet. Possible inherent effects of the low motivation to exercise is my suggested explanation. Though there was no access to running wheels, I would presume that there is less cage activity in the LVR line than any other two lines, and thus utilizing less NEFA during cage locomotion. Taken together, this leads to a higher caloric intake to output ratio and to a higher NEFA concentration. Interestingly, the WT line on a Western diet showed lower NEFA values than both the HVR and LVR line. This could be attributed, in my opinion, to the selective breeding model co-selecting for deleterious traits.

Insulin sensitivity/glucose tolerance

In order to establish a better idea of the metabolic state of these rats, insulin and glucose were measured after seven weeks of specific dieting. As previously stated, insulin resistance (metabolic syndrome) and elevated glucose/glucose intolerance (prediabetes) are clear and prominent symptoms of a large portion of today's population that leads in a direct path to chronic disease and death. Supporting the previous sentence, La Fleur et al. found that after four weeks of a high fat/high sugar diet, rats developed hyperglycemia, hyperinsulinemia, glucose intolerance, and a diminished insulin response to a glucose load (La Fleur, Luijendijk, van Rozen, Kalsbeek, & Adan, 2011).

Based upon the above information, I predicted that the Western diet would induce similar results on the Wistar rats after eight weeks of dieting. Due to the small sample size and high variability, there was unfortunately not much that could be drawn from the insulin curve results. However, it should be noted that the basal insulin levels were elevated in all lines on a Western diet, possibly indicating some insulin resistance. The AUC of the insulin results revealed a diet effect which was to be expected. This can be explained because high sugar diets have been

repeatedly shown to cause hyperinsulinemia (Hwang, Ho, Hoffman, & Reaven, 1987; la Fleur et al., 2011; Zavaroni, Sander, Scott, & Reaven, 1980). Also, high fat diets are associated with weight gain which is in turn associated with insulin resistance (Riccardi, Giacco, & Rivellese, 2004). On top of correct diet, exercise has been shown to increase insulin sensitivity, i.e. make insulin work more efficiently (Borghouts & Keizer, 2000). Here in my study, all rats were not given access to voluntary running wheels, thus I predict that if the experiment were to be carried out for a longer duration and with more subjects, then greater significance could have been revealed regarding the insulin levels of the two diet groups, without access to running wheels.

The AUC of glucose concentration were as predicted in regards to diet. The Western diet rats showed significantly higher levels of circulating glucose compared to the rats on a normal chow diet. Similarly, each individual time point of the GTT revealed either significantly higher or trending to be higher glucose values for the Western diet. Interestingly and contradicting my initial hypothesis, the HVR line had significantly higher AUC glucose levels than the LVR and WT line. Multiple time points of the GTT revealed this same finding. One possible explanation for this result could be explained by the inherent genes within the highly motivated line. It is known that exercise increases the amount of glucose uptake into contracting skeletal muscle (Goodyear & Kahn, 1998); however, my rats had no access to voluntary running wheels. Therefore, instead of a protective effect gained by high motivation to exercise, it is possible that the HVR line has co-selected genes that actually require this high amount of inherent activity to maintain a normal euglycemic state. Not allowing this highly motivated to run rat line to actually exercise seems to reveal deleterious effects its inherited glucose response. Another validity issue was that all three rat-lines, regardless of diet, started at the same basal level of glucose. This fact means that the rats were still able to regulate, with variable amounts of

insulin, the amount of circulating glucose. In other words, the rats did not appear to have developed as severe insulin resistance as seen in many human cases of type 2 diabetes (Skovso, 2014).

The QUICKI measurement of insulin sensitivity offered some results, but was limited, at least in part, by the small and variable sample size of the insulin data. The QUICKI outcome correlated with our initial hypothesis that the Western diet rats would be less insulin sensitive compared to normal chow diet rats. More data would be necessary to solidify this finding, but it does indicate that these rats on a Western diet were developing insulin resistance or metabolic-like disorder.

Corticosterone

Corticosterone is the dominant glucocorticoid in rodents and similar to the dominant human glucocorticoid, cortisol. The corticosterone concentration was measured from the blood plasma obtained from the basal time point and 30 minute time point of the GTT in order to obtain a measurement of stress levels in the rats. Stress increases blood corticosterone, which in turn, increases blood glucose. Increases in blood corticosterone and glucose were seen in all groups between pre- and post-blood sampling, which makes sense because they had been handled multiple times by the second marker of the GTT. Since no differences were seen between lines or diets, this leads me to believe that no one line was more stressed than the other during the GTT. Thus, my inference is that differences in blood corticosterone may not be differentially contributing to differences in skeletal muscle removal of blood glucose.

Limitations

One limitation is that half of the WT rats were approximately a week older than the rest of the rats in the study. Though a week is not a very long time, this could have altered metabolic markers due to the age difference. For example, cholesterol is shown to increase with age (Scirica & Cannon, 2005). Another limitation to the blood lipid panel is that basal levels were not taken prior to the eight weeks of dieting. This would have allowed for an analysis over time instead of just an end result comparing the different groups. Also, a limitation is that the euglycemic-hyperinsulinemic clamp technique was not performed which is the gold standard method for assessing whole-body insulin sensitivity (DeFronzo, Tobin, & Andres, 1979). This would have given a more accurate idea of the level of insulin resistance compared to other indices. Another limitation would be that cage locomotor activity was not measured, which could have helped in explain some metabolic differences between the lines, such as body weight and total energy consumed. Finally, the relatively small sample size, especially for the measured GTT values, limited conclusive determination for whether significance of the results would exist.

Conclusion

The experimental focus of this study was to induce a pre-metabolic-like syndrome and prediabetic-like state into three genetically different rat lines, two of which were bred for minimal or excessive amounts of voluntary exercise. The purpose of this was to see if different levels of inherent genetics for voluntary running in wheels would play a role in a rat's innate ability to defend itself against an eight-week Westernized diet. The results lead to the conclusion that the rats fed a Western diet did develop more of a metabolic-like syndrome and prediabetic-like state than the rats on a normal chow diet through the observation of hyperglycemia, dyslipidemia, and insulin desensitization. Interestingly, the WT line, in nearly every marker measure, showed to have been affected less by the Western diet than the LVR and HVR lines

(Table 3, 4, 5; pages 46, 47, 48). Often in various determinations, there were not significant differences between the normal chow and Western diet of the WT line. Here, I hypothesize that the selective breeding model of high and low voluntary exercise, when not allowed voluntary wheel running, seemingly co-select for undesired deleterious metabolic genes, regardless of the main selection of putative genes for high or low distances of voluntary running when provided wheels. The WT group was added as a nonselected control group and showed it to be able to handle a high fat/sugar diet better when not allowed access to a voluntary running wheel. The human relevance is that even if you have a high genetic drive for voluntary running, unless you actually exert this drive, many of the positive benefits to being highly motivated for voluntary physical activity are negated and possibly even deleterious without the physical activity.

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