AEROBIC CAPACITY AND BILE ACID METABOLISM

By

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Abstract

High aerobic capacity is strongly correlated to protection from a wide range of metabolic diseases. Bile acids are now recognized as potential players in protection against metabolic disease. We tested the hypothesis that human subjects with high aerobic fitness (≥45 ml/kg/min) display differences in fecal bile acid species and total bile acid excretion compared to those with low or average aerobic fitness (≤35 ml/kg/min) (matched for age and body weight). We also evaluated if there were differences in circulating bile acid content.

Healthy women ages 18 to 35 were recruited and screened for inclusion criteria by a VO_{2max} treadmill test. A total of 20 women were enrolled in the study, n=10 in the high-fit group, n=10 in the low-fit group. Diet intake, activity level, stool, and blood were measured at baseline before receiving a 1-week standardized diet. After the 1-week standardized diet, stool and blood were again measured, and an Oral Glucose Tolerance Test (OGTT) and body composition scan (Dual-energy X-ray Absorptiometry) were performed. Contrary to our hypothesis, no differences in circulating or fecal bile acid content was found between the high- and low-fit women. Future research should consider using direct measurements of the rate-limiting enzyme in bile acid synthesis, CYP7a1, and also the utilization of a 24+ hour fecal collection in order to account for volume differences.

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Chapter I: Introduction

Low aerobic capacity has been shown to be a major determinant for risk of chronic diseases and early mortality (3-5). Underlying mechanisms that explain the preservative effect of a high aerobic capacity on health are largely unknown (3, 6). Genetic or intrinsic characteristics contribute approximately 50% to an individual's aerobic capacity level, while behavioral influences, such as daily physical activity/exercise, serve as the other significant contributor (7). Separating intrinsic versus behavioral or adapted variables upon aerobic capacity within a human population is nearly impossible. In addition, it would be difficult to study underlying mechanisms in various tissues that may play a role in the links between aerobic capacity and health. Consequentially a rat model was created to examine the intrinsic impact of high and low aerobic capacity on health. The rat model involves two strains of rats, high capacity runners (HCR) and low capacity runners (LCR), bred selectively for divergent endurance running capabilities (8). As a product of this continuous selective breeding, the HCR/LCR rats began to develop notable differences not only aerobically, but also metabolically, regardless of being held in a sedentary condition (8-10). LCR rats have shown to be more susceptible to obesity, insulin resistance, and non-alcoholic fatty liver disease (NAFLD) while HCR rats have demonstrated a protection against these conditions, even when fed an obesogenic, high fat/high sucrose diet (11-13). The HCR/LCR rats have proven to be an excellent model because many of the outcomes are similar to the effects of high and low aerobic capacity in humans (14, 15). Other notable similarities are differences in mortality rate and susceptibility for neurological and cardiovascular disease conditions (9, 16, 17), events that have also been strongly linked to aerobic capacity in humans. All told these results strongly validate the model as a useful tool in understanding links between aerobic capacity and health.

A primary difference between the HCR/LCR rats is the quantity and functional capacity of mitochondria within the muscle and liver tissues (9, 11, 13). This crucial difference between the HCR/LCR rats likely impacts susceptibility for metabolic disease as it is the reduction of liver mitochondrial content and fatty acid oxidation within the LCR rats that may significantly increase their risk for NAFLD (11, 13). Meanwhile, the HCR's protection from NAFLD is likely because they have an increase in hepatic mitochondrial content and fatty acid oxidation (11, 13). Results from a gene array in the livers of the HCR/LCR rat model (unpublished) also suggests an increase in the expression of CYP7a1, the rate limiting step for converting hepatic cholesterol to bile acids, in the HCR, compared to the LCR rats. This was also tied to other genes being upregulated in the path between citrate, which can accumulate within the cell during conditions of energy excess, and bile acid production. Similar to the HCR rats, transgenic over-expression of hepatic CYP7a1 in mice is protective from an obesity and insulin resistant inducing diet, suggesting a connection between bile acid metabolism and susceptibility for NAFLD and insulin resistance (18). Preliminary data by the Thyfault lab has found the HCR rats excrete more fecal bile acids than the LCR. Thus a potential mechanism for the HCR rat's protection against the development of NAFLD could be an ability to excrete a greater amount of bile acids and cholesterol within their feces. This is a potential "energy dumping" mechanism that would protect them from fatty liver during hypercaloric conditions. The Thyfault lab also found that the HCR maintain a different microbiota profile then LCR, which can impact the processing and species of bile acids that emerge from the intestinal system (19). Interestingly, it is well known that microbiota metabolize and alter bile acid species. Given the similar effects of aerobic capacity to impact longevity and risk for disease in both rats and humans, we are curious

to see if fecal bile acid content and species are also different in high versus average or low fit human subjects.

It is currently unknown if humans with high or low fitness display differences in total bile acid excretion or in the type of bile acids that are excreted. It is also unknown if bile acid total content and species are different in circulation. We tested the hypothesis that human subjects with high aerobic fitness (≥45 ml/kg/min) display differences in fecal bile acid species and greater total bile acid and cholesterol excretion compared to those with low or average aerobic fitness (≤35 ml/kg/min) (matched for age and body weight). We also evaluated differences in circulating bile acid content and species.

Chapter II: Literature Review

Aerobic Fitness And Links To Health

Aerobic capacity, also known as cardiorespiratory fitness or aerobic fitness, is a term used to describe the maximal rate at which oxygen can be consumed and utilized in the body while performing maximal physiological work, and is measured as VO_{2max} or estimated by time to exhaustion during a graded exercise test (3). Aerobic capacity is important because it serves as a useful tool for determining disease susceptibility in humans, as it is highly dependent on an array of physiological factors such as pulmonary function, nutritional status, and overall muscle, joint, and organ function (4, 5). Having a high aerobic capacity increases the ability of the human body to maintain homeostasis under stress (4). Ultimately, aerobic capacity has shown to be a major determinant of health. In fact, there are many studies that illustrate how having a higher aerobic capacity is connected to lower risks for chronic disease and all-cause mortality (20-23). In contrast, having a low aerobic capacity is predictive of higher disease incidence and early mortality (20-22).

Aerobic Capacity and Risk for Disease: Mechanisms Are Mostly Unknown

Although the effects of aerobic capacity on health outcomes are well established, mechanisms that help explain this connection remain unknown and largely unexplored. Specifically, limited data exists to explain the differences in whole-body energy metabolism between a high and low fit population, especially within non-muscle tissues, and how these differences ultimately determine disease susceptibility (11). Currently there are three known factors that influence aerobic capacity. They are genetics, age, and daily behavior patterns in the form of physical activity or exercise training (3). In general, genetics contribute approximately

50% to total aerobic capacity, although some studies have suggested up to a 70% contribution (7). A positive relationship exists between regular physical activity and aerobic capacity, whereas a negative relationship exists between physical inactivity and aerobic capacity (24). Given these relationships, one can deduce that regular physical activity can help improve or maintain aerobic capacity, while physical inactivity or a sedentary lifestyle decreases aerobic capacity. Current evidence suggests an optimal window for achieving peak aerobic capacity across a lifespan. This window for peak aerobic capacity is thought to occur within the second- to third-decade of human life, and then gradually decrease with successive decades (Figure 1) (24). Although peak aerobic capacity can be limited by aging, significant improvements in aerobic capacity can still be made in a relatively short amount of time through exercise intervention (3) and in converse, extreme forms of inactivity can lead to dramatic declines in aerobic capacity (25, 26).

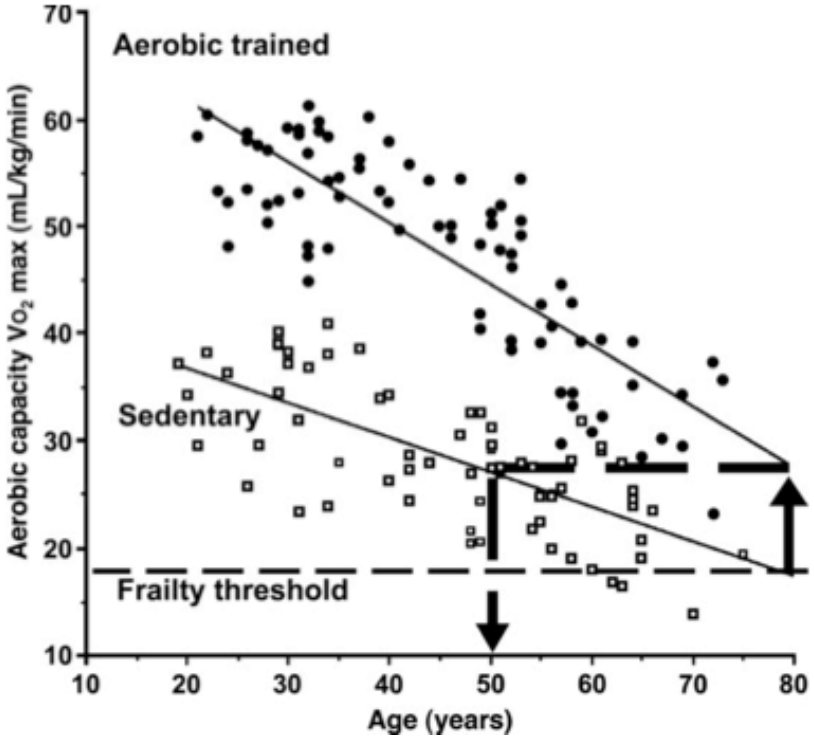


Figure 1: Fitness, Age, and Activity (Tanaka and Seals (27))

There are many practical reasons as to why the mechanistic relationship between aerobic capacity and disease susceptibility are unexplored, especially within non-muscle tissues (3). First and foremost, the logistics of performing such an experiment on humans is extremely difficult, if not impossible. Samples from skeletal muscle are more easily obtained, whereas samples from other organs, such as heart, lungs, liver, kidney, etc. are more difficult to collect, and bring ethical concerns (3, 6). Another major dilemma encountered in studying aerobic capacity is being able to separate genetic (intrinsic) influences from behavioral (exercise) influences (3). Inherently, a population who is more intrinsically fit may also exercise regularly, therefore it becomes very difficult to identify whether a person's aerobic capacity is attributed to their exercise program or preexisting genetic variables. In addition, any physiological outcome measure could be influenced by the subject's last bout of exercise, requiring that measures be made after a defined time of exercise cessation. Thus, in order to practically study the connection between aerobic capacity and disease susceptibility, a model was needed.

The HCR/LCR Model

To fill this need for a model which demonstrates the mechanistic differences between high and low aerobic capacity and disease susceptibility, Britton and Koch (2001) began breeding rats for intrinsic high and low exercise capacity (8). The founder population began with a treadmill run to exhaustion with the top 10% of performers selectively bred for high intrinsic exercise capacity (HCR) and the bottom 10% of performers selectively bred for low exercise capacity (LCR) (8). Outside of being regularly maintained in a cage, the rats had no other exposure to regular activity. Control of exposure to regular exercise between the groups created differences that were solely attributable to genetic variation. After six generations of selective breeding, running capacity differed between the two groups by 171%. After eleven generations,

signs of cardiovascular and metabolic disease became more prevalent among the LCR group, with the HCR group demonstrating no effect and a 60% higher VO_{2max} (8, 9). From this generation up to generation 28, the health effects of the HCR/LCR breeding are more pronounced, and there stands a 10-fold difference in running capacity between the two groups (10). LCR rats show a greater susceptibility to obesity, insulin resistance, non-alcoholic fatty liver disease (NAFLD), neurological disease conditions, and in general, the LCR rats have a higher mortality rate (11, 13, 16, 17). These findings are important, as well as relevant, because they closely resemble the disease outcomes in humans who also display low fitness levels (4, 15).

HCRs Display A Protective Effect

Clear differences are seen in the running capacity between the HCR/LCR groups and their consequential susceptibility for developing disease. Understanding that intrinsic aerobic capacity clearly plays a role in health outcomes, Noland et al. (12) wanted to evaluate if intrinsic aerobic capacity impacts diet induced obesity and insulin resistance. After an 8-week high fat diet (HFD; 50% of energy from fat) in male HCR/LCR rats, Noland et al. (12) found the LCR rats to show a distinct amount of weight gain, accompanied with a deteriorating level of insulin sensitivity. In contrast, the HCR rats maintained insulin sensitivity and did not gain weight on the HFD (12). Many studies have since confirmed this apparent protective response within the HCR rats, and early deterioration of health in the LCR rats for both males and females (17, 28-31).

Further evaluation by Wisloff et al. (9) describes a primary feature of the skeletal muscle of HCR rats as having higher mitochondrial content and oxidative capacity than the LCR rats. These findings are also in agreement with studies that examined skeletal muscle mitochondrial

differences among aerobically trained vs. untrained humans (14, 32-35). This increase in the amount of skeletal muscle mitochondrial content and oxidative capacity in the highly fit population was initially thought to serve as an explanation for the lower degree of disease susceptibility in the high fit population. But it wasn't until 2006 when Church et al. (15) brought to light the higher prevalence of NAFLD among low-fit men, independent of body weight, that the focus of metabolic differences between the two groups started to shift from skeletal muscle to other tissues like the liver.

HCR Protection: Role of the Liver

NAFLD, or hepatic steatosis, is a disease characterized by the accumulation of fat (>5% by weight) within the liver and is strongly associated with the development of obesity, insulin resistance, and type II diabetes (36). Under normal physiological conditions, the liver is ill suited to store excessive levels of fat. Most of the fatty acids that enter the liver are generally utilized for energy through beta-oxidation in the mitochondria, or repackaged into lipoproteins to be secreted and delivered to systemic circulation. There are many factors that regulate the trafficking of fatty acids in the liver, although in the case of NAFLD, Thyfault and colleagues have found mitochondrial content and oxidative capacity to have a significant impact (13). In fact, Thyfault and colleagues found that the reduction of mitochondrial content and oxidative capacity within the LCR rats was associated with the development NAFLD (13). Further investigation within the Thyfault laboratory revealed that when both HCR and LCR rats were fed a 3-day HFD, the LCR rats showed a greater increase in body fat, lower resting energy expenditure, and a significantly higher respiratory quotient, suggesting a lower amount of whole-body fatty acid oxidation (11). Interestingly, the HCR group remained protected against the development of NAFLD despite having increased their energy intake while the LCR developed

even worse steatosis (11). Additional work by the Thyfault laboratory shows the same relationship also occurs after chronic HFD feeding (6).

The liver serves as a critical energy metabolism hub as it is responsible for converting glucose to fat (de novo lipogenesis), secreting lipids into circulation, and it is the only major site for glucose output, and thus maintenance of normoglycemia. In the case of the HCR/LCR rats, there appears to be a distinct metabolic difference between the two groups with regards to their ability to digest, absorb, and metabolize fat, particularly within the liver. Bile acids play a major role in the digestion and absorption of dietary fat and have a strong connection to liver, as it is the primary site of bile acid synthesis and facilitator of enterohepatic circulation (the recycling of bile acids). Given the important role bile acids serve in fat metabolism, the Thyfault lab continued to hypothesize a potential difference in bile acid metabolism among the HCR/LCR rat model.

Bile Acids: The Connection Between Fitness and Health

The Thyfault lab used a gene array (unbiased analysis of mRNA) to search for other metabolic differences between the HCR/LCR rats that may impact their susceptibility for NAFLD. The results suggest a difference in the regulation of cholesterol and bile acid metabolism. Specifically, CYP7a1 gene expression was found to be higher in the livers of HCR rats fed a HFD compared to LCR rats. This is noteworthy as CYP7a1 (CYP7a1 gene encodes the CYP7a1 enzyme) is the rate-limiting step in converting cholesterol into bile acids (37). Another striking difference seen in the HFD-fed HCR gene expression was the additional up-regulation of HMG-CoA reductase and ATP Citrate Lyase, two enzymes important for cholesterol production. During conditions of energy excess citrate can accumulate in the mitochondria and cross the mitochondrial membrane into the cytosol. Citrate lyase converts

citrate back to acetyl CoA. Together, these findings suggest that the HCR rats have a way of diverting excess energy (Acetyl-CoA) obtained from the HFD into cholesterol, and eventually bile acid production, rather than allowing unused energy to accumulate in the liver (mechanism shown in Figure 2). Enzymes marked with yellow asterisk are higher in the HCR than the LCR.

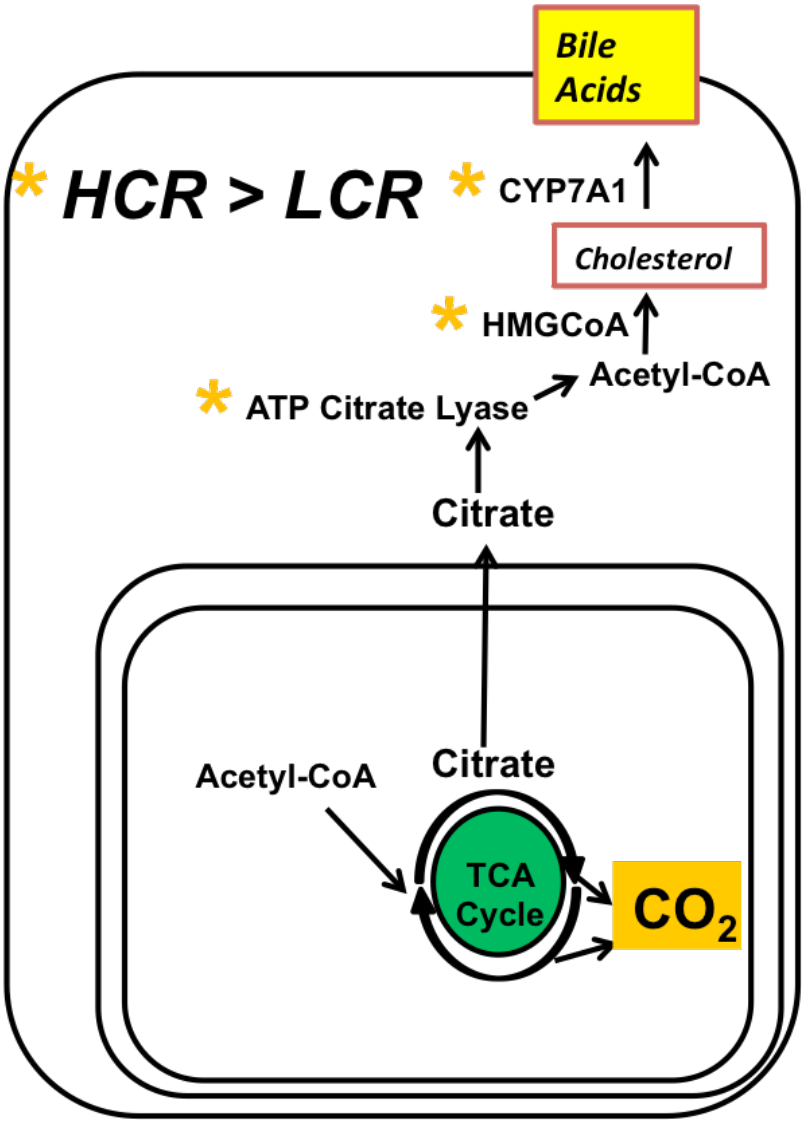


Figure 2: Proposed Bile Acid "Energy Dumping" Mechanism

The Thyfault lab has also measured direct bile acid concentration in serum, liver, intestines, and feces among the HCR/LCR groups when fed a HFD. Interestingly, they found a general increase in the amount of bile acid present in serum, liver, and intestines within the LCR, but a higher amount of total bile acids and cholesterol in the HCR feces (~20-30% higher). It is known that about 5% of bile acids produced by the body are excreted in the feces daily, while 95% of bile acids are recycled back to the liver (38). In addition, approximately 50% of cholesterol is secreted while the other 50% is excreted in feces (39). The Thyfault lab theorizes that the HCR's protection against the development of NAFLD may be partially attributed to their differential bile acid metabolism, and their ability to excrete a greater amount of bile acids and cholesterol in feces (Figure 2). This is potentially a way that the HCR rats are able to avoid developing fatty liver, even when exposed to a hypercaloric diet, by demonstrating an ability to "dump energy".

Another interesting difference between the HCR/LCR rats is their microbiota profile. Through recent discoveries, intestinal microbiota has been shown to influence disease conditions as well as whole body metabolism. In particular, intestinal microbiota has a close relationship with the liver, as about 70% of the liver's blood supply comes from the intestine via portal circulation (40). This close connection provides opportunity for microbiota to influence gut-signaling pathways, which can in turn affect insulin sensitivity, lipid metabolism, energy harvest and storage, and inflammation (41-44). Gut microbiota have proven the ability to induce obesity-related factors. Such an example comes from Turnbaugh et al. (42), who used sterile mice and colonized them with "obese bacteria" finding a significantly greater increase in body fat than when they were colonized with a "lean bacteria". As previous studies of the LCR rats show, they are at a greater risk for poor metabolic outcomes, especially fatty liver, and part of

this reason may be because of their microbiome differences. Again, the Thyfault lab has tested this theory by using the HCR/LCR rats that were exposed to a 3-day HFD and obtained cecal microbiota samples (19). Results showed the LCR rats to contain a different microbiota profile that is favorable to a more efficient metabolism of carbohydrates and energy than the HCR rats (19). This difference in microbiota profile between the HCR/LCR rats is important to note, as it is the intestinal bacteria that is responsible for converting bile acids into their secondary form (38). Consequently intestinal microbiota then has the ability to largely dictate bile acid processing and species population, which is part of a feedback mechanism, and has the potential to disrupt a number of metabolic processes. It is currently unknown if the differences in microbiota species between the HCR/LCR rats is influencing bile acid species, but studies are ongoing to examine these links. A recent study reported that high and low fit humans also display differences in fecal microbiota profiles (45) however, the study did not examine fecal or serum bile acids.

Bile Acid Overview

The Formation of Bile Acids

Traditionally known to aid in lipid digestion and absorption, the primary role of bile acids is to serve as a fat emulsifier in the small intestine. Bile acids are formed from cholesterol through a series of enzymatic reactions in the liver, and have a unique amphipathic structure that makes it optimal for facilitating fat digestion (2). Bile acids are responsible for a large portion of daily cholesterol turnover as they are the products of cholesterol breakdown (2, 46-48). Once made, bile acids are conjugated in the liver with either glycine or taurine and converted to primary bile acids, or bile salts (1). (Bile acids become bile salts to increase their solubility

through the attachment of a cation, usually sodium.) Conjugation of bile acids with glycine or taurine occurs at a ratio of about 3 to 1 in humans, respectively, and is predominantly conjugated (>95%) by taurine in mice (2, 38). These primary bile acids are referred to as either cholic acid (CA) or chenodeoxycholic acid (CDCA) and are produced in relatively equal amounts via the neutral pathway (49).

There are two main pathways in which primary bile acids are formed, the neutral (classical) and the acidic (alternative) pathway (Figure 3) (2). Under normal physiological conditions, the "neutral", or "classical" pathway is the main pathway for bile acid conjugation and is initiated by the only rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase (CYP7a1) (1, 2, 49). Because CYP7a1 is regulated through feedback inhibition (bile acids) and plays such a vital role in primary bile acid synthesis, its activity is vulnerable to a wide range of influences (50).

The second pathway in which primary bile acids are formed is the "acidic" or "alternative" pathway. Differing from the neutral pathway, the acidic pathway is driven by the enzymatic activity of sterol 27-hydroxylase (CYP27a1) which is not regulated by bile acids (1, 2, 50). Under normal physiologic conditions, this pathway may only contribute up to 9% of bile acid synthesis (51). But when under inflammatory conditions, such as in the case of liver disease, or during neonatal development, the acidic pathway can become a more dominant contributor to bile acid synthesis because the neutral pathway becomes depressed (1, 2).

In the acidic pathway, the main primary bile acid produced is CDCA, whereas in the neutral pathway, both CA and CDCA are produced, but in a ratio determined by the sterol activity of 12α-hydroxylase (CYP8B1) (1). With CYP8B1, the classical pathway is able to produce hydrophilic CA, but without this sterol, the resulting product is hydrophobic CDCA (2).

Thus, the action of CYP8B1 is able to control the amphipathic properties of bile acids needed for fat digestion through its production of CA or CDCA (50).

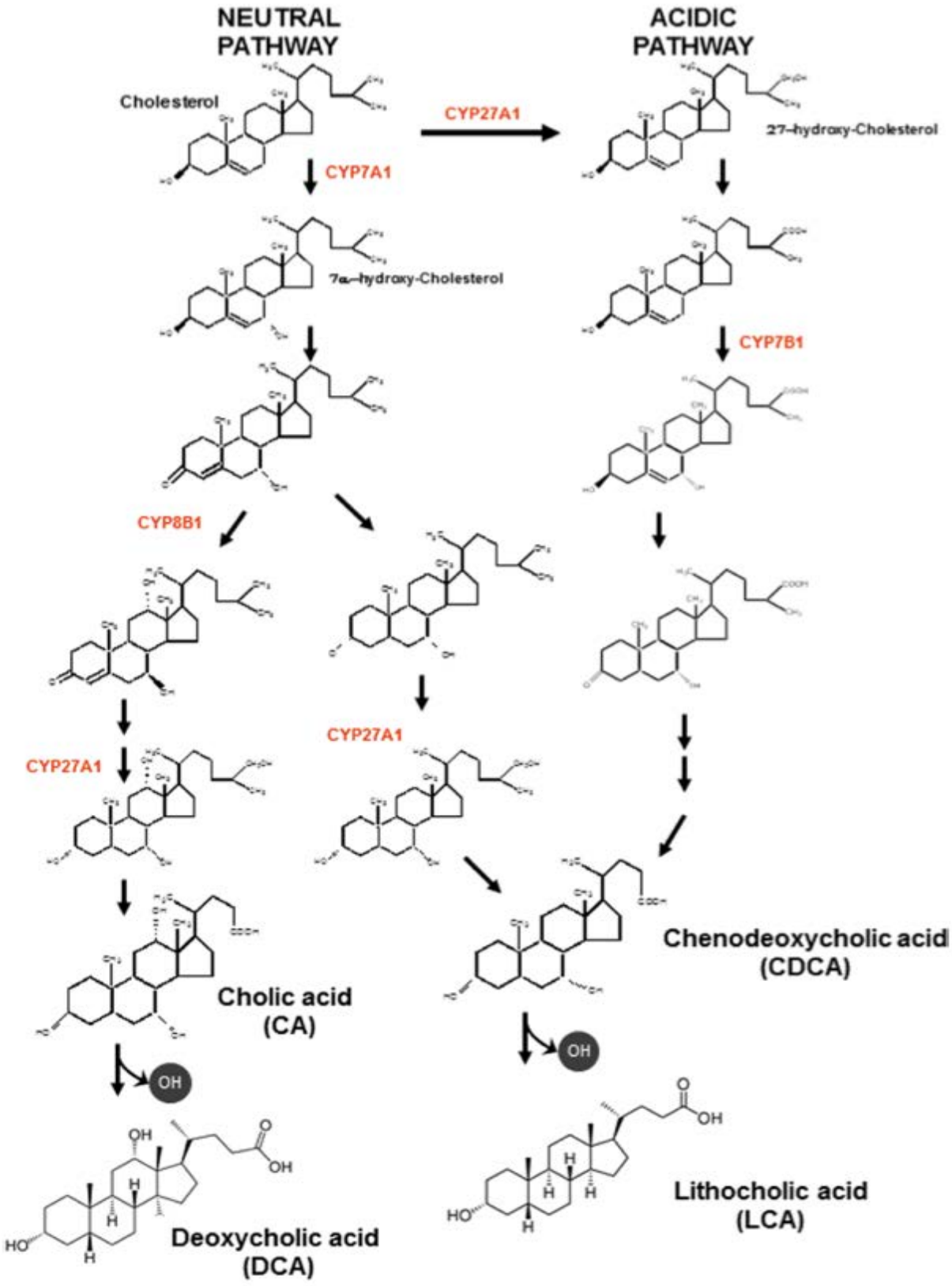
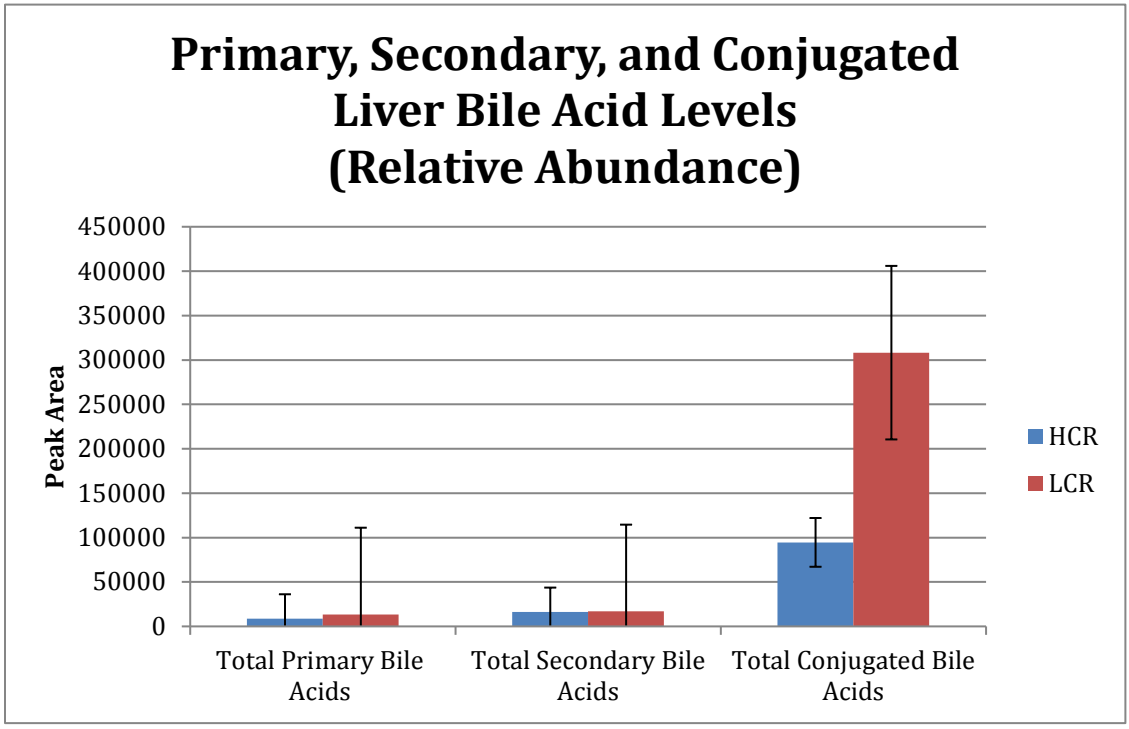


Figure 3: Pathways of Bile Acid Synthesis (Zhou and Hylemon (1))

Enterohepatic Circulation

After being formed, bile salts are secreted by the liver and stored in the gallbladder as the main ingredient of bile. After the ingestion of a meal, the gallbladder contracts and bile salts are secreted into the upper small intestine to aid in digestion and absorption. After their secretion into the upper small intestine, bile acids are reabsorbed in the distal ileum and transported back to the liver via portal circulation where they are reabsorbed by the liver so that they can be secreted again (2). This bile acid recycling process is referred to as enterohepatic circulation (Figure 4). The recycling of bile acids within the gastrointestinal tract is extremely efficient as was previously stated, approximately 95% of the bile salts secreted are reabsorbed (52). The literature suggests that conjugated bile acids are the species primarily reabsorbed by the intestines. Interestingly, the LCR have a higher concentration of conjugated bile acids in the liver than the HCR. This further supports that the HCR reabsorb less and excrete more bile acids than the LCR. Analysis of bile acid species in the feces is ongoing (Table 1)

Table 1: HCR/LCR Liver Bile Acid Composition



The remaining 5% that is not absorbed through enterohepatic circulation is excreted in the feces and replaced by de novo synthesis of bile acids in the liver (38, 49). Bile acids that do not get reabsorbed through enterohepatic circulation, also become susceptible to the actions of intestinal bacteria which turn the primary bile acids into secondary bile acids (damaged bile acids), deoxycholic and lithocholic acid, derived from CA and CDCA respectively (53). Depending on the amount of food ingested, the bile acid pool can be recycled many times throughout a day, but their production is tightly regulated through a feedback mechanism which is used to prevent hepatotoxic levels and control cholesterol metabolism (49, 52). Enterohepatic circulation is an important process not only for assuring proper nutrient absorption but also for

maintaining metabolic homeostasis (2). The underlying feedback mechanisms that regulate enterohepatic circulation, and therefore bile acid synthesis, have yet to be fully understood.

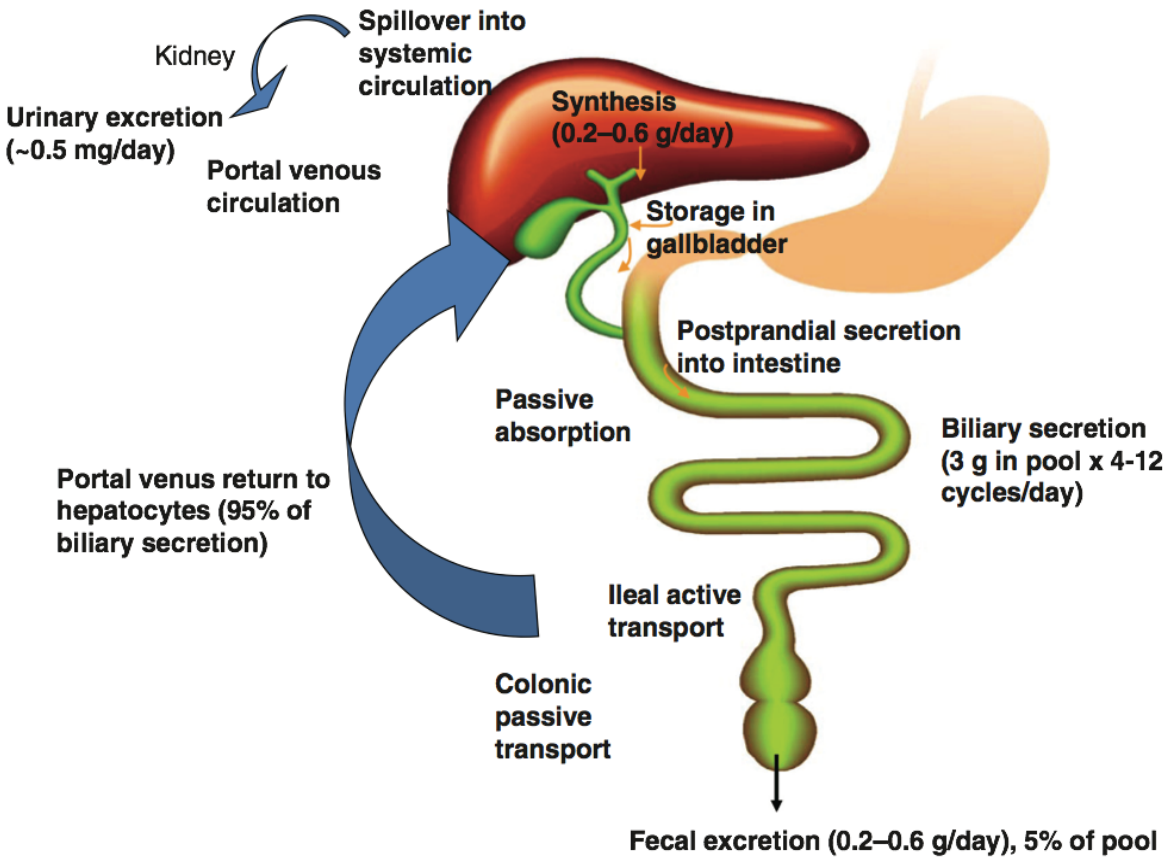


Figure 4: Enterohepatic Circulation (Chiang (2))

Emerging Role of Bile Acids in Metabolism

About 20 years ago, bile acids were once thought to act exclusively as fat emulsifiers. Their function, by way of secretion from the liver into our upper intestine, is to breakdown dietary fat into tiny lipid droplets in order to be absorbed more efficiently. Bile acids are now believed to serve as signaling molecules, creating metabolic effects that extend far beyond their role in lipid emulsion (54). Bile acid signaling is thought to influence glucose homeostasis, energy expenditure, and potentially "energy dumping". One of the main regulatory mechanisms involved with controlling the signaling potential of bile acids is enterohepatic circulation. Enterohepatic circulation is the highly efficient process of bile acid recycling within the gastrointestinal tract. An adult body maintains a bile acid pool of about 3 grams, and it is through enterohepatic circulation that this pool is conserved and reused with each eating occasion, about 4-12 times a day (2). Disruptions in the enterohepatic circulation can alter the bile acid pool and can therefore affect bile acid signaling, leading to metabolic consequences (54). These metabolic consequences are, in part, due to bile acids serving as signaling molecules for both nuclear receptor farnesoid X receptor (FXR) (expressed in the liver, intestine, adipose tissue, vascular wall, pancreas and kidney) and membrane G protein-couple receptor, TGR5, (aka Gpbar-1, G-protein- coupled bile acid receptor) (expressed in the gallbladder, spleen, liver, intestine, kidney, skeletal muscle, pancreas, adipocytes, and macrophages) (2, 55). Both receptors have proven to serve multiple purposes in maintaining metabolic homeostasis, TGR5 specifically has shown to play a role in improving insulin sensitivity, stimulating energy metabolism, and serving to defend the liver against steatosis (53, 56-60).

Bile Acids Improve Insulin Sensitivity

In many studies, the nuclear receptor FXR has been recognized as a regulator in hepatic glucose metabolism (2). The digestion and absorption of nutrients in humans are highly dependent on the fasting-refeeding cycles experienced every day. Gluconeogenesis, a process that primarily takes place during the fasting phase, is inhibited by bile acids through activation of FXR (61, 62). Additionally, FXR has been shown to stimulate the insulin/AKT pathway, a process primarily found in the postprandial period, resulting in glycogen synthesis (18). FXR increases insulin sensitivity by increasing insulin production and secretion from pancreatic beta cells (63). In high concentrations, insulin can inhibit FXR, while glucose increases FXR expression (64). Thus, FXR inhibits gluconeogenesis, but stimulates glycolysis and glycogenesis (2).

Although many studies may agree that FXR plays a role in glucose metabolism, conflicting results have been reported when studying the role of FXR in mice (65, 66). Some studies using FXR null mice showed the development of severe fatty liver accompanied with high glucose and insulin levels, indicating decreased insulin sensitivity (65, 67). In contrast, another study reports FXR deficient mice as having reduced body weight and improved insulin sensitivity (68). FXR exhibits influence on the bile acid pool by inhibiting the rate-limiting enzyme in bile acid synthesis, CYP7a1. This negative influence of FXR on CYP7a1 should theoretically reduce bile acid synthesis, the bile acid pool, and therefore reduce energy expenditure (2). These conflicting findings regarding the true role of FXR in glucose metabolism may be in part explained by TGR5 signaling (2). In vivo studies looking at the signaling effects of TGR5 show that it induces intestinal glucagon-like peptide-1 (GLP-1)

release, leading to improved liver and pancreatic function and enhanced glucose tolerance in obese mice (69).

Bile Acids Increase Energy Expenditure

Bile acids are now being recognized as having a major impact in metabolic regulation not only because of their role in both lipid and glucose metabolism, but also because of their ability to increase energy expenditure. The effect that bile acids have on energy expenditure seems to be facilitated through the modulation of thermogenesis (50). When supplied with bile acids, mice fed a high-fat diet increased energy expenditure in brown adipose tissue, preventing obesity and insulin resistance (70). This protective effect is believed to be attributed to the binding of bile acids to TGR5, which stimulate cyclic AMP-dependent thyroid hormone (cAMP) to activate the enzyme, type 2 iodothyronine deiodinase (D2). This cascade of events ultimately leads to bile acids increasing D2 activity and oxygen consumption in brown adipose tissue, independent of FXR activation (70).

Although bile acid activation of FXR may not play a direct role in thermogenesis, studies of FXR deficient mice have still shown an influence on energy expenditure. FXR deficient mice demonstrate an extreme intolerance to cold temperatures, and when fasted, they also present with an accelerated entry into torpor (71). These altered responses may be associated with an unusually rapid decrease of plasma energy substrates (lipid and glucose) and an impaired ability to mobilize and store these substrates (71). These findings support the idea that FXR plays a role in energy expenditure and overall energy homeostasis.

Bile Acids May Play A Role In Getting Rid Of Excess Energy

Research regarding the potential for bile acids to serve as therapeutic agents for various metabolic diseases such as NAFLD and metabolic syndrome are starting to become more prevalent (60). Upregulation of the rate-limiting enzyme in bile acid synthesis, CYP7a1, has become a new focal point in the prevention of these types of diseases and is best exemplified in studies using transgenic mice. Multiple studies have showed the over-expression of CYP7a1 in mice to maintain cholesterol homeostasis and be protective of a high fat diet-induced obesity, fatty liver, and insulin resistance (18, 72-74). In one particular study by Li et al (18), CYP7a1 transgenic mice had decreased expression of lipogenic and gluconeogenic genes, but had increased whole body energy expenditure and induction of fatty acid oxidation genes in brown adipose tissue. Interestingly, when paired with an LDL-receptor deficiency, CYP7a1 transgenic mice continued to show protective effects on the liver regardless of a cholesterol-enriched diet (72). Using the CYP7a1 transgenic mouse model as a guide, increasing bile acid synthesis, specifically the hydrophobic version, may be a strategy for preventing high-fat diet-induced NAFLD, obesity, and diabetes through activating FXR and TGR5 signaling to manage energy metabolism and glucose homeostasis (2, 18, 72, 73).

Another proposed mechanism, as suggested by the preliminary Thyfault data, is that an increase in the bile acid pool may also induce “energy dumping”. Similar to the transgenic mice, a gene array of the HCR rats (unpublished) showed a greater expression of CYP7a1, along with increased expression of HMG-CoA reductase and ATP Citrate Lyase. These results indicate both the HCR rats and transgenic mice experience an increase in the bile acid pool. HCR rats also demonstrate a greater amount of total bile acids in their feces, whereas the LCR rats had higher amounts of bile acid present in serum, liver, and intestines. Interestingly, in another study

by the Thyfault lab (unpublished), 4 weeks of varying levels of exercise intensity and food exposure also showed a significant upregulation of genes controlling cholesterol and bile acid synthesis in the livers of wild-type rats. Endurance training, interval sprint training, and voluntary wheel running with an overnight fast all showed an upregulation of CYP7a1, HMG-CoA reductase, ATP-Citrate lyase, and squalene epoxidase when compared to a sedentary control. These findings suggest that exercise training can provide the same type of effects on energy metabolism as intrinsic, or genetic factors, and may provide an explanation as to how fitness is able to help prevent the development of fatty liver, even when fed a hypercaloric, high-fat diet. As suggested by the data, excess energy that enters into the mitochondria as acetyl CoA could be converted to citrate by way of the TCA cycle. This build-up of citrate within the mitochondria would then leak into the cytoplasm and be converted to bile acids through a series of reactions involving the upregulation of the aforementioned enzymes (Figure 2). The bile acids can then be excreted, essentially "dumping" the unneeded energy as feces. The same could also be true for humans who also possess a high aerobic capacity and demonstrate a protective effect against NAFLD. Revelations regarding the versatility of bile acids has been promising, although further research is needed in order to fully understand the role of fitness with respect to bile acid excretion, and whether or not this is a potential mechanism for protection against fatty liver.

Chapter III: Methods

Overview

High aerobic capacity is strongly correlated to protection from a wide range of metabolic diseases. Bile acids are now recognized as potential players in protection against metabolic disease. We tested the hypothesis that human subjects with high aerobic fitness (≥45 ml/kg/min) display differences in fecal bile acid species and total bile acid excretion compared to those with low or average aerobic fitness (≤35 ml/kg/min) (matched for age and body weight). We also evaluated if there were differences in circulating bile acid content.

Setting

Informed consent and participant screening took place at the University of Kansas Medical Center main campus in the Research in Exercise and Cardiovascular Health (REACH) Laboratory lab of Dr. Sandy Billinger at Hemenway Life Sciences Center. All other visits took place at the University of Kansas Clinical Research Center located in Fairway, KS.

Procedure

Participants were recruited from the KUMC campus and nearby communities through the use of flyers, email, and word of mouth. Prior to coming in the lab for a more formal screening, participants were asked to answer some basic questions related to health and behavior to check inclusion/exclusion criteria (see Appendix A). Participants eligible for the formal screening (visit 1) were female between the ages of 18 and 35, non-smoker, weight stable (no greater than 5% change ≤3 months), did not follow a restrictive diet, and had no history of chronic diseases or surgeries that would interfere with bile acid metabolism. Formal screening included a VO_{2max}

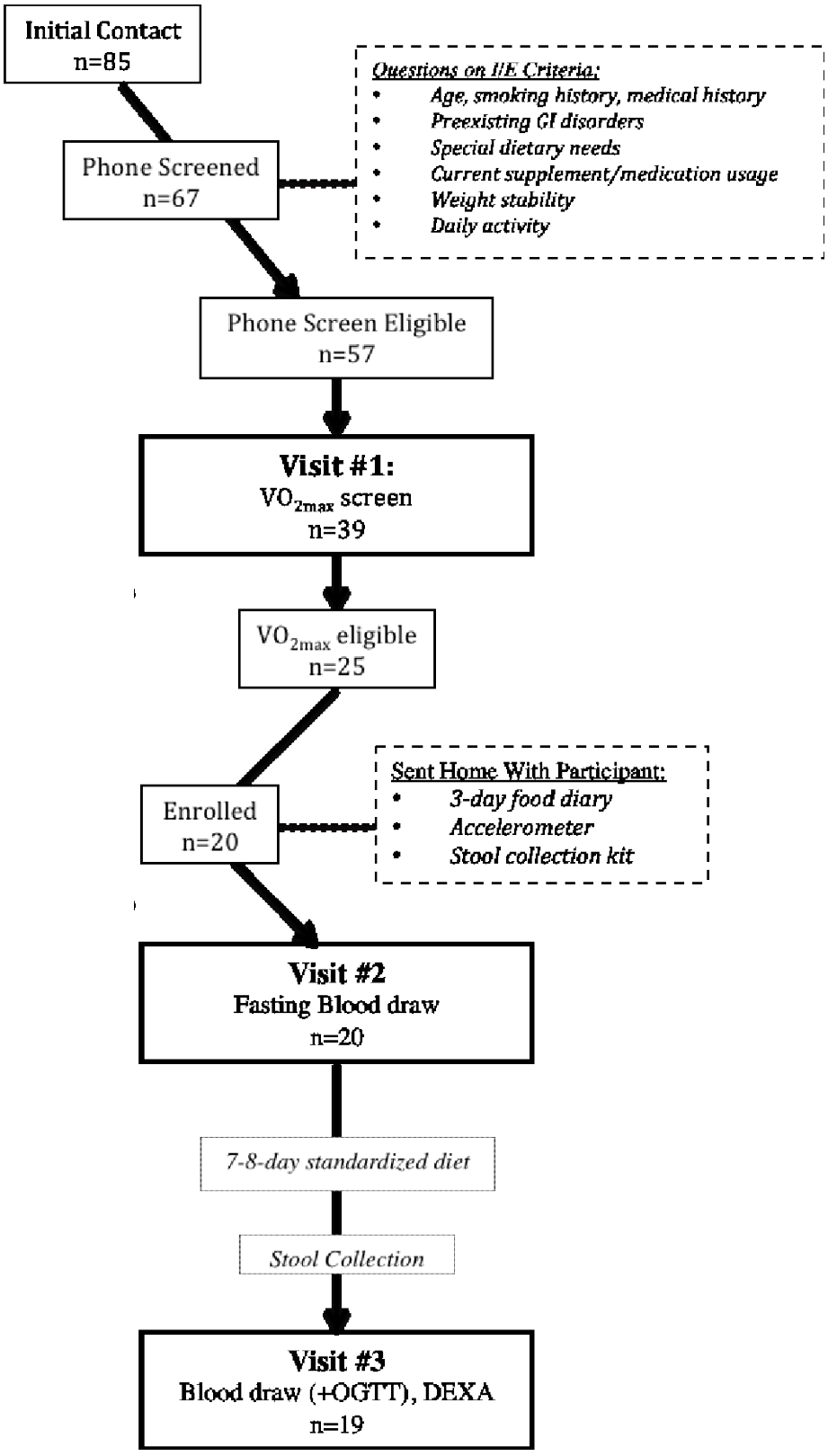
exercise test to exhaustion and health history questionnaire (see Appendix C). In order to be enrolled in the study, participants' VO_{2max} results had to either be ≥45 ml/kg/min to be in the "high-fit" group or ≤35 ml/kg/min to be in the "low-fit" group.

Once determined eligible for enrollment, study subjects were then sent home with a 3-day food diary, accelerometer, and stool collection kit. About 1 week after visit 1, subjects returned for a fasting blood draw (visit 2), bringing back with them the completed 3-day food diary, stool sample kit, and accelerometer. After visit 2, subjects were then supplied with a standardized diet for the next 7-8 days. During the last 3-4 days of the standardized diet, subjects provided a second stool sample and brought this to visit 3. On visit 3, subjects completed another fasting blood draw paired with an oral glucose tolerance test and also a DEXA body composition scan.

Participants

A total of 20 subjects were enrolled in the study, n=10 with high aerobic fitness (≥45 ml/kg/min) and n=10 with low or average aerobic fitness (≤35 ml/kg/min). Of the 20 subjects enrolled, 19 made it to the final visit. One subject was discontinued from participation at the final visit due to a vasovagal response during the oral glucose tolerance test, leaving a total of 18 to fully complete the study, 9 from each cohort.

Study Design Timeline



Demographic Measures

Height was measured during formal screening (visit 1) using stadiometer in the REACH lab. Weight was measured during screening (visit 1), before intervention (Visit 2) and post intervention (Visit 3) using a body weight scale in REACH lab and at KU MED CRC in Fairway, KS. Participants were responsible for recording daily food intake for 3 days (2 weekdays and 1 weekend) (Appendix D). Diet records were analyzed for micro- and macronutrient content using Nutrient Data System for Research (NDSR, version 2016). Current activity level of participants was tracked and measured using individual accelerometers. Maximal oxygen consumption (VO_{2max}) was determined by indirect calorimetry using the standard Bruce protocol (75). VO_{2max} testing was performed in the REACH lab using a Bruce exercise test protocol on a motor driven treadmill as performed by the Thyfault lab previously (76). Body composition via dual energy x-ray absorptiometry was measured post intervention (Visit 3) as a health measure to help phenotype participants at KU MED CRC in Fairway, KS. Participants also provided information on general health history, medication usage (including birth control), and lifestyle habits through the use of questionnaires.

Stool Collection

Two stool samples were collected on two separate occasions, once before initiation of 7-day standardized diet, between visit 1 and 2, and again, right before visit 3 within the last few days of standardized diet. Collection occurred at two time points to determine if non-standardized diet conditions impact fecal bile acids in either group. Protocols were given to participants instructing them on the proper handling of stool specimen (Appendix E). Stools

were then transported to the Thyfault lab and stored at -80 degrees Celsius until ready for analysis.

Serum Measures

Serum bile acid, glucose, and lipid measurements were taken before intervention (visit 2) and post intervention (visit 3). Measurements were taken at two different time points to determine if non-standardized diet conditions impact metabolic measures in either group or in both groups. To measure serum metabolites, participants fasted overnight (~10h). A small in-line catheter was placed in an arm vein, and a baseline blood sample (≤100 ml) was collected.

An Oral Glucose Tolerance Test (OGTT) was performed at visit 3 to assess metabolic health via glucose tolerance and insulin sensitivity. Participants fasted overnight (~10h). A small in-line catheter was placed in an arm vein, and a baseline blood sample (≤100 ml) was collected. Participants then consumed a sugary drink containing 75 grams of glucose. For the next 2 hours, they were asked to lie still on a bed while a small blood sample was collected (≤100 ml mls) every 30 minutes to measure glucose, insulin, and bile acids. Blood samples were collected into serum separator or EDTA tubes at 0, 30, 60, 90 and 120 min. Serum samples were allowed to clot for 30 min, and all samples were centrifuged at 3000 *g* for 10 min at 4°C. Serum and plasma were frozen at -80°C for subsequent analysis. Serum glucose was determined using the glucose oxidase method (Sigma, St. Louis, MO) or a glucose analyzer (YSI), and serum insulin was measured by ELISA kit (Alpco, Salem, NH). The area under curve (AUC) for glucose, insulin, and bile acids was calculated using the trapezoidal method. Surrogate markers of insulin sensitivity and insulin resistance was calculated from the glucose and insulin responses to the OGTT using the Matsuda composite insulin sensitivity index (ISI) (77), and from fasting glucose and insulin values using the homeostasis model assessment of insulin resistance (HOMA-IR)

(78), respectively. Oral glucose tolerance tests were performed at the KU MED CRC in Fairway, KS.

Standardized Diet

Diet was prepared and packed-out at the KU MED CRC in Fairway, KS. Meals were designed to be eucaloric for each participant (based on Harris Benedict prediction) and consisted of the following average macronutrient distribution per day: 30-35% Fat, 15-20% Protein, 45-50% CHO, along with 15-25 grams/day of fiber. Before obtaining meals, participants were provided with menu for the 7-8 day standardized diet and asked if there are any foods they do not tolerate or like. Any foods identified by the participant were swapped out with another food item of approximately equal nutritive value. Depending on whether or not the participant was on any supplements that would interfere with bile acid or lipid metabolism determined the start time of the standardized diet. Participants started the standardized diet immediately after visit 2 if they were not taking supplements that could impact bile or lipid metabolism. Participants who were on supplements that could potentially confound results, stopped taking the supplements for a 1-week washout period after visit 2 prior to starting the standardized diet. During the standardized diet, participants were given directions to only consume the food given to them, and to eat all the food provided (Appendix F). Participants were allowed to consume additional caloric free beverages. A food record was given to the participant in order to record their food intake and any additional beverages consumed (Appendix F). Participants arrived at KU MED CRC in Fairway, KS about 1-2 times during the 7-8 day feed to receive pack-out meals.

Bile Acid Analysis

Serum Biochemistry

Total serum bile acids were measured by the DaytonaRX clinical analyser (Randox, Kearnesyville, WV) using commercially available enzymatic colorimetric assay (BI3863, Randox, Kearneysville, WV).

UHPLC-MS Analysis of Bile Acid Concentrations

Primary, secondary and sulfated bile acids were purchased from Steraoloids (Newport, RI) or Sigma-Aldrich (St.Louis, MO). Deuterated bile acids were purchased from Cambridge isotope laboratories (Tewksbury, MA). All solvents used were of optima grade and purchased from Fisher Scientific (Pittsburgh, PA). For each sample, feces (250 mg) was homogenized in 1.5 ml of LC/MS grade water using a PowerGen 1000 homogenizer (Fisher Scientific, Pittsburgh, PA), then centrifuged 3,000 g for 10 minutes at 4^{o}C. Fecal water was collected. To 100 µl of fecal water, 600 µl of cold MetOH plus 200 µl of the internal standard solution (CA-D4, DCA-D4, GCDCA-D9, LCA-D4, 50% MetOH, final concentration 5 µg/ml) was added. All extractions were homogenized by vortexing, and then centrifuged at 3,000 g, 4^{o}C for 10 minutes. The supernatant was collected into a clean tube, evaporated to dryness under a nitrogen stream and then reconstituted in 100 µl of 50% MetOH plus calibration standard, 100 ng/ml lorazepam, final concentration. Chromatic separation of individual primary and secondary bile acids was performed on an Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA) fitted with a Hypersil GOLD C18 reversed-phase column (50 X 2.1 mm, 1.9 µ column) as previously described (79). Detection was carried out on a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer with data acquisition executed using *Xcalibur 4.0* software (Thermo Fisher

Scientific, Waltham, MA). All samples were analyzed by ESI-Full-MS scan mode. Nitrogen as sheath, auxiliary, and sweep gas was set at 50, 13, and 3 units, respectively. Other conditions included: resolution, 70,000 FWHM; AGC target, 3e6 ions; maximum injection time, 200 ms; scan range, 50-750 m/z; spray voltage, 3.50 kV; and capillary temperature, 320°C. All data was processed using *TraceFinder 3.3* software. Individual bile acids were identified by exact mass and a retention time as shown in Table 2. Calibration curves, with concentrations in the 5-2000 ng/ml range, were generated by plotting peak area ratio of the corresponding internal standard versus the nominal value. The line of best fit was determined by linear-weighted (1/x) least-squares regression, with regression correlation 0.99 or better arc.

Table 2. Mass Spectrometer Set-Up For Identification and Quantification of Selected Bile Acids

Bile Acid	Exact Mass (M-H)	RT (min)	ISTD
aMCA	407.28030	10.89	CA-D4
bMCA	407.28030	11.21	CA-D4
wMCA	407.28030	11.61	CA-D4
CDCA-S	471.2422	13.72	DCA-D4
UDCA-S	471.2422	10.22	DCA-D4
CA	407.28030	11.99	CA-D4
CA-D4	411.30540	11.97	
CDCA	391.28538	15.89	DCA-D4
DCA	391.28538	16.34	DCA-D4
DCA-D4	395.30942	16.32	
HCA	407.28030	11.08	CA-D4
HDCA	391.28538	12.56	DCA-D4
LCA	375.29047	17.58	LCA-D4
LCA-D4	379.31558	17.57	
UDCA	391.28538	12.10	DCA-D4

Fecal Analysis

For each sample, feces (250 mg) was homogenized in 1.5 ml of LC/MS grade water using a PowerGen 1000 homogenizer (Fisher Scientific, Pittsburgh, PA), then centrifuged 3,000 g for 10 minutes at 4ºC. Fecal water was collected. To 100 µl of fecal water, 600 µl of cold MetOH plus 200 µl of the internal standard solution (CA-D4, DCA-D4, GCDCA-D9, LCA-D4, 50% MetOH, final concentration 5 µg/ml) was added. All extractions were homogenized by vortexing, and then centrifuged at 3,000 g, 4ºC for 10 minutes. The supernatant was collected into a clean tube, evaporated to dryness under a nitrogen stream and then reconstituted in 100 µl of 50% MetOH plus calibration standard, 100 ng/ml lorazepam, final concentration. Chromatic separation of individual primary and secondary bile acids was performed on an Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA) fitted with a Hypersil GOLD C18 reversed-phase column (50 X 2.1 mm, 1.9 µ column) as previously described (79). Detection was carried out on a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer with data acquisition executed using *Xcalibur 4.0* software (Thermo Fisher Scientific, Waltham, MA). All samples were analyzed by ESI-Full-MS scan mode. Nitrogen as sheath, auxiliary, and sweep gas was set at 50, 13, and 3 units, respectively. Other conditions included: resolution, 70,000 FWHM; AGC target, 3e6 ions; maximum injection time, 200 ms; scan range, 50-750 m/z; spray voltage, 3.50 kV; and capillary temperature, 320°C. All data was processed using *TraceFinder 3.3* software. Individual bile acids were identified by exact mass and a retention time as shown in Table 2. Calibration curves, with concentrations in the 5-2000 ng/ml range, were generated by plotting peak area ratio of the corresponding internal standard versus the nominal value. The line of best fit was determined by linear-weighted (1/x) least-squares regression, with regression correlation 0.99 or better.

Data Analysis

Differences in fecal and serum total bile acids, and bile acid species were compared between the high fit and low fit groups using a One-way ANOVA. To test if bile acid content or species are altered by the standardized diet we performed a repeated measures two-way ANOVA (time x group). Statistical significance is set at $P < 0.05$, and all data is expressed as means ± SE. Statistical analyses were performed using SPSS (IBM Analytics, New York, USA).

Ethics

This prospective cohort study was approved by the University of Kansas Medical Center Human Subjects Committee (HSC #00140444). All subjects enrolled completed informed consent.

Chapter IV: Results

A total of 85 women inquired about participation in the study, of which 67 were screened for inclusion/exclusion criteria by telephone. About 85% (n=57) of participants who were interviewed by phone were determined eligible for the VO_{2max} screen. Only 64% (n=25) of participants who performed the VO_{2max} screen were determined eligible for enrollment by having a VO_{2max} above 45 ml/kg/min or below 35 ml/kg/min. A total of 20 participants were enrolled in the study, n=10 in the high fit, n=10 in the low fit. After visit 2, 1 participant dropped out due to work related travel requirements. Another participant dropped out during visit 3 due to a vasovagal response during the OGTT, leaving n=9 in each cohort.

VO_{2max} was the greatest differentiating factor between groups ($p<0.0001$), while body fat was also different ($p\leq0.05$) (Table 3). The mean ages and BMI of the high-fit and low-fit cohort were similar. The high-fit group possessed a greater amount of fat free mass, and was assigned ~22% higher daily kcal intake compared to the low-fit. As expected, the high-fit group averaged a greater amount of daily moderate to vigorous physical activity, which greatly contributed to their higher amount of assigned kcals per day. Although the standardized diet was meant to be eucaloric, there was still a change in body weight for both groups. This change in body weight could most likely be attributed to either an underestimation in energy needs or failure to follow the diet protocol, as compliance issues were encountered during the week-long diet. Overall, diet did not have any major effect on the outcome variables as no significant changes were seen after the 7-8 day standardized diet in either group.

Differences in serum fasting glucose and lipid profiles between the high- and low-fit women were unremarkable (Table 4). Serum bile acids were slightly higher in the low-fit group and trending in the direction of our proposed hypothesis. Results from the oral glucose tolerance

test (OGTT) showed the high-fit women secreted less insulin per relatively the same amount of glucose as the low-fit women, indicating a greater amount of insulin sensitivity (Figure 5). The insulin area under the curve (AUC) was almost 2-fold higher in low fit vs. high fit while glucose AUC were similar (Table 5). Both groups experienced a rise in serum bile acids during the OGTT, which was expected as bile acid production goes up during the consumption of a meal to help facilitate nutrient digestion and absorption. The rapid decline in serum bile acids at the 30-minute time point experienced by the high-fit group was unexpected and is unexplainable.

As predicted, the primary bile acid excreted by both groups were unconjugated. Differences in excretion between the high- and low- fit groups trended in the opposite direction of our hypothesis. The low-fit women tended to not excrete as much total bile acid in their feces as the low-fit women both pre- and post-diet (Tables 6-8).

Table 3: Subject Characteristics

Variable	High-fit	Low-fit
Height (cm)	169.0 ± 2.4	163.9 ± 2.6
Weight (kg)	64.5 ± 3.1	58.3 ± 2.3
BMI	22.5 ± 0.8	21.7 ± 0.7
Age (yrs.)	25.8 ± 1.1	24.8 ± 1.3
VO_2max (ml/kg/min)	48.5 ± 0.7	34.0 ± 0.8**
Body Fat (%)	24.2 ± 1.4	32.5 ± 1.8*
Fat Free Mass (kg)	49.0 ± 2.3	39.7 ± 1.6*
Average MVPA (min/day)	48.6 ± 6.7	25.8 ± 4.8*
Assigned Diet (kcal)	2,360 ± 83	1,844 ± 28**
Δ Weight (kg)	-0.5 ± 0.2	-0.1 ± 0.3

Table 3. Values are means ± SE (n=9-10). *A significant difference between groups ($p \leq 0.05$). **The level of significance between groups is <0.0001. BMI = Body Mass Index;
MVPA = Moderate to Vigorous Physical Activity

Table 4: Subject Metabolic and Lipid Profiles Pre- and Post- Standardized Diet

	High-Fit		Low-Fit	
Variable	Pre-Diet	Post-Diet	Pre-Diet	Post-Diet
Glucose (mg/dL)	83.6 ± 1.2	82.0 ± 1.6	84.7 ± 1.3	82.6 ± 2.1
Cholesterol (mg/dL)	173.4 ± 9.3	161.9 ± 7.5	190.8 ± 13.7	162.6 ± 12.7
Triglycerides (mg/dL)	97.7 ± 10.9	75.0 ± 6.2	90.2 ± 15.4	91.6 ± 15.0
HDL (mg/dL)	65.2 ± 4.3	63.3 ± 4.5	73.4 ± 4.1	65.3 ± 4.2
LDL (mg/dL)	88.7 ± 7.4	83.7 ± 5.7	99.4 ± 12.5	78.8 ± 10.7
Bile Acids (μmol/L)	1.8 ± 0.4	2.2 ± 0.4	3.7 ± 1.1	2.4 ± 0.5

Table 4. Values are means ± SE (n=9-10). HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein

Table 5: Oral Glucose Tolerance Test Measurements

Variable	High-fit	Low-fit
Glucose – AUC	12952.21 ± 723.77	13088.19 ± 544.02
Insulin – AUC	3915.86 ± 459.15	7144.52 ± 746.74*
Serum BA – AUC	351.30 ± 56.28	443.17 ± 88.64

Table 5. Values are means ± SE (n=9-10). *A significant difference between groups ($p \leq 0.05$).
AUC = Area Under the Curve; BA = Bile Acid

Table 6: Fecal Unconjugated Bile Acid Content

	High-Fit		Low-Fit	
Species (nmol/g)	**Pre-Diet**	**Post-Diet**	**Pre-Diet**	**Post-Diet**
aMCA	155.3 ± 69.2	182.5 ± 86.8	951.1 ± 622.5	306.3 ± 107.5
bMCA	14.6 ± 14.2	0.3 ± 0.1	0.7 ± 0.4	0.6 ± 0.2
CA	26.3 ± 13.3	10.3 ± 3.7	262.2 ± 153.5	9.0 ± 3.6
CDCA	11.5 ± 8.5	9.7 ± 4.7	106.6 ± 62.3	3.3 ± 1.6
CDCA-S	22.1 ± 19.9	5.9 ± 5.3	50.1 ± 26.0	13.9 ± 10.7
DCA	1,495.2 ± 282.2	1,172.7 ± 441.8	1,794.8 ± 490.3	1,138.6 ± 227.1
HCA	0.5 ± 0.2	0.2 ± 0.1	2.6 ± 1.5	0.5 ± 0.2
HDCA	5.6 ± 1.8	4.0 ± 2.0	13.2 ± 4.4	10.2 ± 2.6
LCA	612.4 ± 105.3	527.8 ± 100.2	802.5 ± 179.5	936.9 ± 233.5
UDCA	132.6 ± 33.9	110.2 ± 33.9	195.8 ± 69.6	155.9 ± 42.1
UDCA-S	116.6 ± 29.6	91.5 ± 31.0	160.6 ± 58.5	121.8 ± 30.0
wMCA	1.9 ± 0.9	2.0 ± 1.1	4.5 ± 1.5	2.3 ± 1.3
Total	**2,594.5 ± 408.7**	**2,116.9 ± 583.2**	**4,344.8 ± 1,506.7**	**2,699.4 ± 514.0**

Table 6. Values are means ± SE (n=9-10).

Table 7: Fecal Conjugated Bile Acid Content

	High-Fit		Low-Fit	
Species (nmol/g)	**Pre-Diet**	**Post-Diet**	**Pre-Diet**	**Post-Diet**
GCA	4.50 ± 1.78	2.23 ± 0.67	3.52 ± 1.00	0.92 ± 0.32
GCDCA	1.61 ± 0.40	1.09 ± 0.27	2.28 ± 0.61	0.80 ± 0.24
GCDCA-S	0.07 ± 0.03	0.03 ± 0.02	0.06 ± 0.04	0.09 ±0.08
GDCA	17.74 ± 4.69	13.49 ± 5.93	16.70 ± 3.19	20.64 ± 4.91
GHDCA	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.12 ± 0.06
GLCA	2.03 ± 0.78	1.92 ± 0.53	2.86 ± 1.12	5.12 ± 1.69
GUDCA	0.22 ± 0.05	0.21 ± 0.08	0.85 ± 0.36	0.35 ± 0.14
TaMCA	0.02 ± 0.02	0.02 ± 0.02	0.27 ± 0.20	0.05 ± 0.03
TbMCA	0.02 ± 0.02	0.02 ± 0.02	0.27 ± 0.20	0.05 ± 0.03
TCA	3.56 ± 2.03	1.53 ± 0.71	2.24 ± 1.01	0.42 ± 0.17
TCDCA	2.55 ± 1.24	1.48 ± 0.72	2.30 ± 0.86	0.62 ± 0.23
TDCA	3.24 ± 1.37	2.09 ± 1.00	19.24 ± 10.66	4.57 ± 3.02
TLCA	0.24 ± 0.15	0.19 ± 0.13	3.05 ± 2.02	0.94 ± 0.55
TUDCA	0.04 ± 0.02	0.04 ± 0.02	0.29 ± 0.13	0.09 ± 0.08
TwMCA	0.03 ± 0.02	0.03 ± 0.03	0.20 ± 0.12	0.03 ± 0.03
Total	**35.90 ± 6.61**	**24.38 ± 7.28**	**54.14 ± 17.88**	**34.83 ± 8.01**

Table 7. Values are means ± SE (n=9-10).

Table 8: Total Fecal Bile Acid Content

	High-Fit		Low-Fit	
Form (nmol/g)	**Pre-Diet**	**Post-Diet**	**Pre-Diet**	**Post-Diet**
Unconjugated	2,594.5 ± 408.7	2,116.9 ± 583.2	4,344.8 ± 1,506.7	2,699.4 ± 514.0
Conjugated	35.9 ± 6.6	24.4 ± 7.3	54.1 ± 17.9	34.8 ± 8.0
Total	**2,630.4 ± 409.3**	**2,141.3 ± 589.5**	**4,399.0 ± 1,508.3**	**2,734.2 ± 517.1**

Table 8. Values are means ± SE (n=9-10).

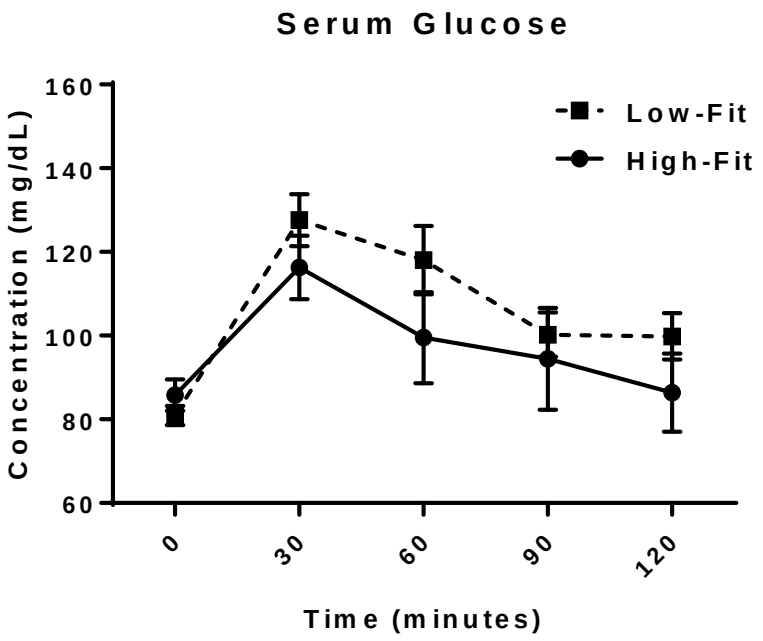


Figure 5: Serum Glucose Measurements During OGTT

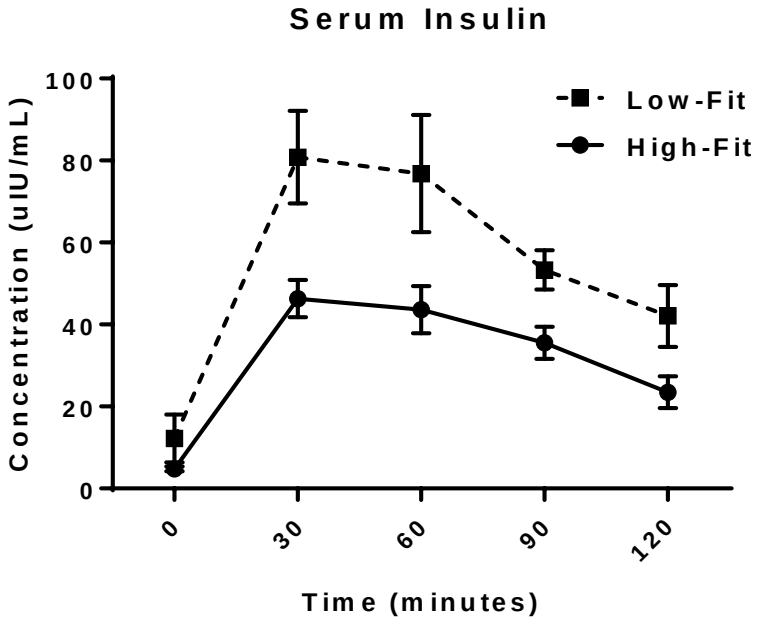


Figure 6: Serum Insulin Measurements During OGTT

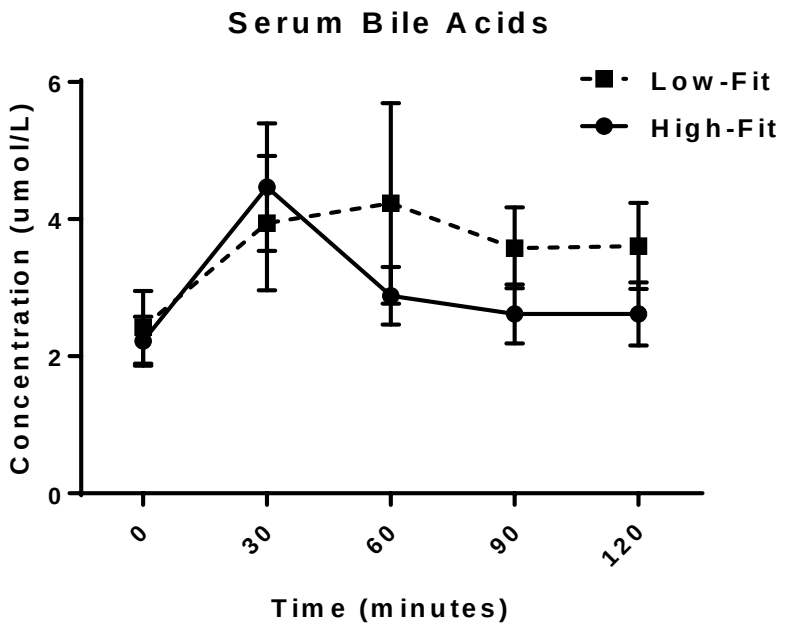


Figure 7: Serum Bile Acids Measurements During OGTT

Chapter V: Discussion

As far as we are aware, this is the first study to directly measure and compare bile acid content between high- and low-fit human populations. The results of this study contradict previous findings in rats with high and low running capacities. In this study, low-fit women were found to excrete more total fecal bile acids than high-fit women. Although these findings were non-significant, this trend is opposite of what was predicted to occur based on the HCR/LCR rat model. There are many potential reasons as to why this difference may have occurred.

A significant limitation of this study is related to extracting and measuring fecal bile acids. During the extraction process, feces were initially homogenized in water and then the fecal water was strained from the fecal pellet and analyzed. This became a problem, as the more hydrophobic bile acids, particularly DCA and LCA, may not have completely partitioned into the water but rather remained in the fecal pellet. Therefore, a significant portion of bile acids may have been left in the fecal pellet and thus not included in our measurement. DCA and LCA represent a major portion of the total bile acids found in feces so this problem could potentially have significant implications. LCA, being the most hydrophobic bile acid tends to be the most effected, as preliminary testing during the analysis suggested that there were equal quantities in the fecal water and fecal pellet in some samples (n=6 samples). The solution to this problem was to extract the leftover feces in methanol so that all hydrophilic bile acids were extracted. The new extraction data are reflected in the tables presented under the results section. Although the re-extraction greatly increased the amount of total bile acids in each group, the difference between groups remained unchanged. The new data obtained from the re-extraction results

trended the same direction as the original extraction results, leaving the general outcomes of the study unchanged.

A second reason as to why the low-fit were found to excrete more in feces could be due to the amount collected. In this study, total bile acid content was expressed as a concentration because the samples provided by participants were relatively small compared to total fecal excretion. Another limitation of this study was that we did not implement a 24+ hour fecal collection so we do not know the total volume of feces excreted by each individual. Although fecal concentration may have been higher in the low-fit group, we do not know if this is truly an indicator that they excrete more bile acids. On average, the high-fit group consumed about 500 kcal more than the low-fit group during the weeklong diet due to their larger body mass and reported activity level. Previous observations have reported energy intake to contribute up to a 28% difference in fecal output, correlating strongly with mean daily stool weight ($p < .001$) (80). Also, because the high-fit individuals were simply more active on a daily basis, this would promote a greater amount of gastrointestinal motility, leading to a greater amount of stool frequency (81). Although the results of this study show a smaller amount of fecal bile acid in the high-fit group, it could be that this is because their feces is just less concentrated. It is possible that the high-fit group excretes a greater volume of feces throughout the day, but because of the limitations of our study, we cannot confirm this.

Finally, although serum and fecal measures do hold some value in understanding bile acid synthesis in an individual, it cannot directly tell us whether or not the pathway involving bile acid production, more specifically the rate-limiting enzyme, cholesterol 7α-hydroxylase (CYP7a1) is regulated differently between groups. An established proxy measure for assessing hepatic CYP7a1 activity and bile acid synthesis is to measure 7α-hydroxy-4-cholesten-3-one

(C4), an intermediate in bile acid synthesis (82). Sometimes when a metabolic pathway is upregulated, there is an increased amount of leakage of intermediates into circulation (83, 84). Although the exact mode of entry of C4 into blood is still to be fully understood, there is a strong correlation to enzymatic activity of CYP7a1 at both steady state conditions and during times of rapid change such as with those associated with the diurnal phases (82). Measuring C4 has been established as a much more reliable and feasible collection method than other intermediates as it is not as readily oxidized and requires less sophisticated methods for analysis (85). C4 measures have been validated in both human and rodent models where C4 serum levels tracked with hepatic CYP7a1 activity and gene expression (82, 84-87). The next step to understanding the synthesis of bile acids in both the high- and low-fit populations would be to measure C4 content in serum before and during postprandial conditions. This is critical because although differences may not be seen during fasting, they could be unmasked during postprandial conditions. In this study, serum bile acids were analyzed during post-prandial-like conditions, the OGTT, and there was a hint of quantitative differences between groups, but nothing significant. In general, it is expected that bile acid production would increase in serum with the consumption of a meal, as its job is to help with digestion and absorption, but the degree to which production is affected appears to vary slightly with fitness level. Obtaining a C4 measurement would not only give a better picture of bile acid production, but also help with the understanding and interpretation of results from the direct measures of bile acids in serum and feces. Future plans for this study include the analysis of C4 in participant serum to better understand differences in bile acid flux between the two groups.

Aside from the conflicting bile acid findings in feces, a few results that were expected were those associated with the oral glucose tolerance test (OGTT). The high-fit women

demonstrated a lower level of insulin secretion than the low-fit women per relatively the same amount of glucose, alluding to a greater amount of insulin sensitivity. Although the high-fit women on average did possess a higher BMI than the low-fit, they were much leaner, and demonstrated a greater aerobic threshold, two factors that greatly influence a person's metabolic flexibility. This metabolic flexibility was also demonstrated in the serum bile acid measurements during the OGTT. As expected, in both groups, serum bile acids increased with the consumption of a meal. Interestingly though, in the early stages of the OGTT, the high- and low-fit groups each showed slightly different reactions in regards to their serum bile acid content. This observation is unexplainable but interesting to note nonetheless. The next step in assessing bile acid content in serum would be to measure the different species present and compare to pre- versus post-prandial conditions.

Baseline dietary consumption for each group has yet to be analyzed and could also be useful in understanding the variance in bile acid excretion between and within groups. Although bile acid fecal content was not significantly different between groups, we see a general decrease within both groups, especially in the low-fit. It would be interesting to note from this analysis the average baseline caloric intake, as well as macronutrient distribution, since bile acid content seems to be most different before consumption of the standardized diet.

Conclusion

No difference in fecal bile acid was found between high- and low-fit women. Not only was our hypothesis was incorrect, but the data trended in the opposite direction of what was predicted to occur based on previous findings in rats. A limitation of this study was the ability to accurately extract bile acids from feces in order to obtain reliable results. Initial extraction was performed with water, which preferentially favored the more hydrophilic bile acids. Future studies should focus on primarily using a less bias means of extraction, such as with the use of methanol. Another factor to consider in future analysis is to measure serum C4 both pre- and post-meal consumption. C4 measurements correlate strongly with hepatic CYP7a1 activity and have been validated in both human and animal models. Ideally fecal and serum bile acid measures should reflect bile acid production, but in order to truly understand bile acid flux, C4 would be an important measure to obtain in order to assess hepatic CYP7a1 activity. Finally, this study was only performed in women and the fecal samples provided were relatively small compared to total excretion. Future studies should also consider the inclusion of males as well as performing a 24+ hour fecal collection in order to obtain a true bile acid volume measurement.

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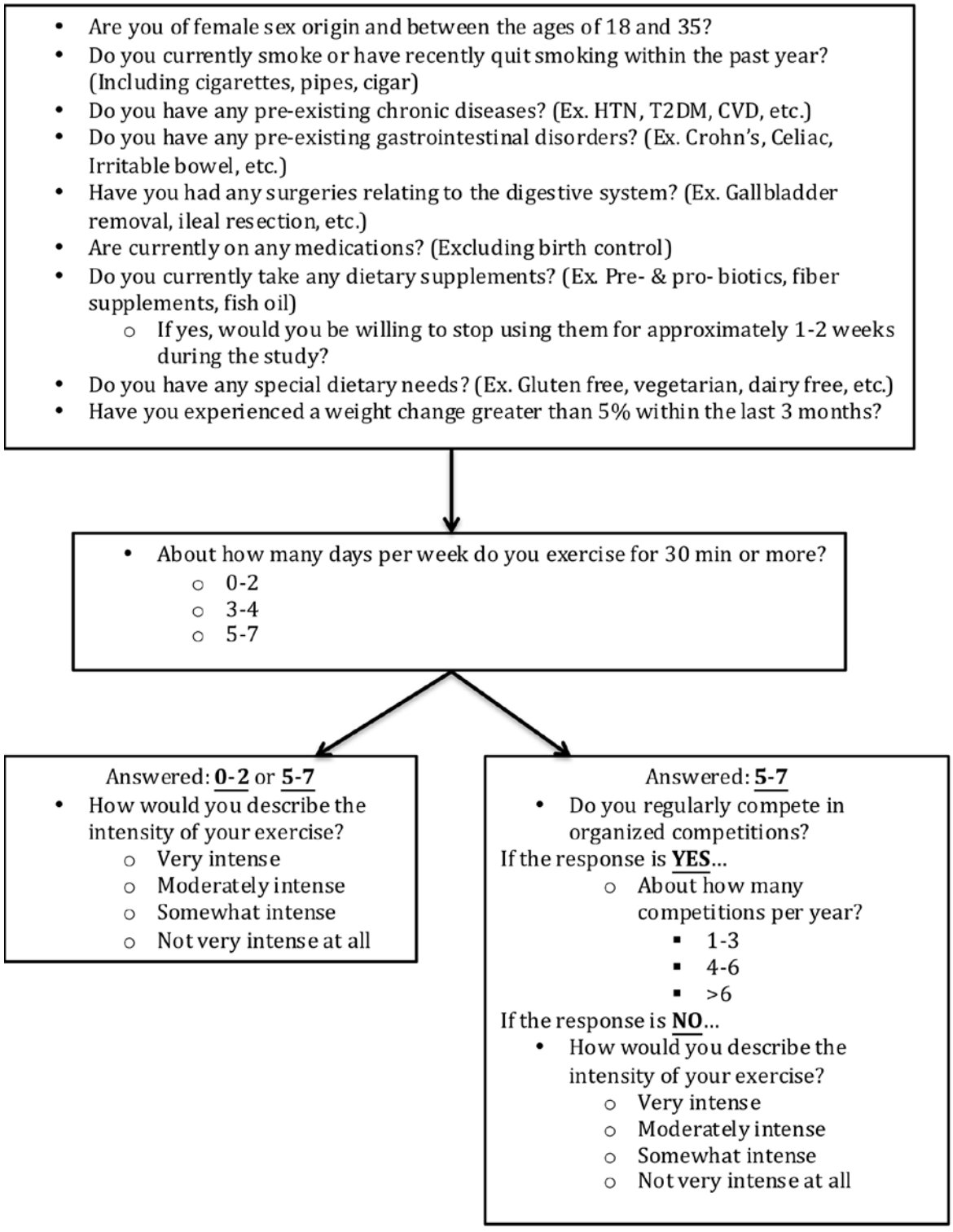
Appendices

Appendix A: Phone Activity Questionnaire



Appendix B: Informed Consent

RESEARCH CONSENT FORM
Aerobic Capacity and Bile Acid Metabolism

Principal Investigator: John Thyfault
University of Kansas Medical Center
913-588-1790

You are being asked to participate in a research study examining how bile acid metabolism relates to aerobic fitness in men and women. Dr. John Thyfault is conducting this research study with other research associates at the University of Kansas Medical Center. About 40 participants will be enrolled at KUMC.

You do not have to participate in this research study. The main purpose of research is to create new knowledge for the benefit of future patients and society in general. Research studies may or may not benefit the people who participate. It is important that you read this form and ask as many questions as you need to before deciding whether or not to participate in this study.

Research is voluntary, and you may change your mind at any time. There will be no penalty to you if you decide not to participate, or if you start the study and decide to stop early. Either way, you can still get medical care and services at the University of Kansas Medical Center (KUMC).

This consent form explains what you have to do if you are in the study. It also describes the possible risks and benefits. Please read the form carefully and ask as many questions as you need to, before deciding about this research.

You can ask questions now or anytime during the study. The researchers will tell you if they receive any new information that might cause you to change your mind about participating.

BACKGROUND

Aerobic fitness (the body's ability to transport and use oxygen) is a powerful predictor of chronic disease risk and early death, but the underlying reasons why fitness has such a big impact on health is largely unknown.

Aerobic fitness is a unique measure because it comes both from your genes (inherited from your parents) and from your own physical activity and exercise patterns. Differences in fitness affect how the body digests food and uses the energy. Our researchers tested two kinds of rats, one strain bred to have high aerobic fitness and another bred to have low aerobic fitness. Results from these rats showed that a higher fitness level may have a major impact on bile acid production. Bile acids are produced by the liver and are used by the body to break down and digest fats.

Our researchers have also found differences in the bacteria that live in the guts of the high-fitness and low-fitness rats. Bacteria are known to change bile acid in the gut. Both

bile acids and the bacteria can be measured in stool samples. Aerobic fitness may impact health and the likelihood of developing metabolic diseases like diabetes through differences in bile acid metabolism and bacteria, but this has never been studied in human subjects.

PURPOSE

By doing this study, researchers hope to learn whether or not individual fitness level impacts both bile acid metabolism and microbiota profiles in human participants.

PROCEDURES

If you are eligible and decide to participate in this study, your participation will last about 3 weeks and will involve up to 5 visits.

Men and women who have high or average aerobic fitness but that are similar ages and body weights will be recruited to test if fitness truly impacts bile acid metabolism and microbiota. Subjects will be provided a study diet while being asked not to eat anything else. Stool samples will also be collected and subjects will be asked to keep the samples in their home freezer until the next study visit. This study will also assess metabolic health by measuring glucose and insulin responses during an oral glucose tolerance test.

The first visit in the study is a screening visit to see if you qualify to participate. This visit will take place at the REACH lab within Hemenway Life Sciences Center at KUMC. You will be asked not to exercise for 24 hours before this visit, and to not eat any food or drink anything containing caffeine for at least 2 hours before this visit. A maximal exercise test will be performed to see if you can be part of the study. If you do not meet the criteria you won't be included in the study and the rest of the procedures in that visit won't be performed. You will be given information on your aerobic fitness, height, and weight.

If you are still eligible to participate after the first visit you will continue on in the study. Visits 2 and 5 will take place at the KUMC Clinical Research Center, 4350 Shawnee Mission Parkway, Fairway, KS 66205.

Below, you will find a schedule of events listing all procedures that will occur at each study visit. Following the table, you will find a description of the study procedures.

	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5
Maximal exercise test	X				
Height/Weight/Vital signs	X	X			X
Health history questionnaire	X				
Pedometer	X				
Food and activity diary	X				
Receive study food	X	X	X	X	
Return leftover study food			X	X	
Blood draw		X			
Stool sample		X			X
Pregnancy test					X
DEXA scan					X
Glucose tolerance test					X
Length of Visit	60-90 minutes	30-60 minutes	30 minutes	30 minutes	3 hours

Maximal Exercise Test: You will be asked to not eat any food or drink anything containing caffeine for 2 hours before the test and to avoid intense exercise for 24 hours prior to the session. You may drink water as needed before and after the test. You will be asked to walk on a treadmill while wearing a breathing mask and sensors attached to your body with sticky patches. As you walk on the treadmill the speed and incline will gradually increase. This test takes about 7-15 minutes depending on your aerobic fitness level. If you have chest pain, shortness of breath, are unable to continue exercising, or have changes in your blood pressure or heart rhythm the test will be stopped. You will feel tired at the end of this test but you should completely recover in about 3-5 minutes.

Height/Weight/Vital Signs: You will be weighed and your height measured. Your blood pressure, heart rate, and breathing rate will also be recorded.

Health History Questionnaire: You will be asked to complete a short questionnaire about your health history (medicines you take, previous surgeries or procedures, and any illnesses or conditions you have).

Pedometer: You will be given a small device to measure how many steps you take and

the study team will instruct you how to wear it. You will be asked to wear the pedometer from the time you leave visit 1 until you return for visit 2, and to bring it with you to visit 2.

Food and Activity Diary: You will be given a diary to record everything you eat and drink, as well as your exercise or other physical activity at the end of visit 1. A member of the study team will show you how to complete it. You will be asked to record your food, drink, and activity over two weekdays and a weekend. You will be asked to bring this diary with you to visit 2.

Receive Study Food: You will receive 2-4 days of study food in a cooler. You will be asked not to eat anything not included in the study diet. The study diet is meant to replicate the standard American diet. The diet is not meant to make you gain or lose weight, but keep you at a consistent weight. The diet will provide a variety of options with fruits and vegetables and it is specifically tailored to meet your caloric needs.

Return Leftover Study Food: Depending on how much food is sent home with you on visit #2, the study team will collect your leftovers and provide more study food for you to eat for another 2-4 days. In total, you will be provided with about 7-8 days worth of meals, which will be provided over a couple of drop-offs and pick-ups. The study team will arrange for you to pick up your food at either the REACH lab or the KUMC Clinical Research Center.

Blood Draw: You will be asked to fast (not have anything to eat or drink) for at least 10 hours before visit 2. A sample of your blood (about 5 teaspoons) will be drawn from a vein in your arm for study testing.

Stool sample: You will be given kits at visits 1 and 2 containing all the materials needed to collect samples of your stool, and instructed how to obtain samples. Approximately 1-2 days before your arrival for visit #2, you will use one stool collection kit to gather a sample of stool and will bring the collected sample to visit #2. Approximately 1-2 days before your arrival for visit #5, you will use the second stool collection kit to gather another stool sample and will bring the collected sample to visit #5.

Pregnancy test: If you are a woman who could become pregnant a urine pregnancy test will be performed before the DEXA scan. If the result is positive you will not undergo the DEXA scan and may be removed from the study.

DEXA Scan: This test measures your body composition (fat, muscle mass, and bone density). You will be asked to wear clothing without any metal in it and remove all jewelry or other metal on your body. You will be asked to lie still on a table while the scanning arm of the machine passes over you.

Glucose Tolerance Test: This test will be done at Visit 5 and you will be asked to not eat or drink anything for at least 10 hours before this test. A small tube like an IV will be placed in your arm, and a sample of your blood (about 5 teaspoons) will be collected for

a baseline measure. You will then be asked to drink a concentrated sugary liquid (this liquid contains the same amount of sugar as 2 ½ cans of soda). For the next 2 hours, you will be asked to lie still on a bed while we will collect a small blood sample (1-2 tablespoons) every 15 minutes. You will be able to read or watch a video on a computer or tablet during this time.

RISKS

The risks associated with the maximal exercise test include: increased blood pressure, and heart arrhythmias (abnormal heart beat). There is a very small risk of a heart attack during the exercise test. Trained technicians supervise the maximal exercise test and if you have chest pain, shortness of breath, are unable to continue exercising, or have changes in your blood pressure or heart rhythm the test will be stopped. Although the heart rate monitor poses no risk, occasionally a person is allergic to the adhesive patches and may develop skin irritation.

Drawing blood may cause fainting, and some discomfort, soreness, pain, and/or bruising at the site on your arm where the blood was taken. Rarely an infection occurs at the blood-drawing site. There is also a risk of a blood clot forming in your vein and a risk of developing anemia. There is also a risk that the blood draw site will bleed more than expected. A nurse or trained technician will use sterile technique to draw the blood sample or to place the catheter. There is also the chance that your blood sugar levels may get low. If this occurs we will have you consume a sugary drink to increase your blood sugar back to normal levels.

The DEXA scan may make you slightly uncomfortable because you have to hold very still. You will be exposed to radiation in this study from x-rays as part of the DEXA scan. This radiation exposure is not needed for your medical care. You are exposed to radiation every day. This radiation comes from the sun and the earth. It is called background radiation. The amount of radiation you receive in this study is about the same amount that you receive in less than one day from background radiation. The risk from this radiation exposure is very low. If you have any concerns about radiation exposure please ask the investigator

There is also risk that the questionnaires that you fill out will contain sensitive topics that may cause you embarrassment or stress. These questionnaires will be kept private. However, you have the right to not answer any questions that make you feel uncomfortable.

There may be other risks of the study that are not yet known.

NEW FINDINGS STATEMENT

You will be told about anything new that might change your decision to be in this study. You may be asked to sign a new consent form if this occurs.

BENEFITS

You may not directly benefit from participating in this study. One possible benefit from

participating in this study is that you will be provided with your aerobic fitness level, your height, and your weight. We will give you this information along with how you rank with individuals of your age.

It is hoped that this study may inform biomedical scientists about new ways by which aerobic fitness impacts health through differences in bile acid metabolism and microbiota. Ultimately, these findings could benefit society as a whole.

ALTERNATIVES

Participation in this study is voluntary. Deciding not to participate will have no effect on the care or services you receive at the University of Kansas Medical Center.

COSTS

Costs as a result of your participation will include the costs of transportation to and from KUMC and the Clinical Research Center. The study will pay for all the procedures and food in this study.

PAYMENT TO SUBJECTS

Participation in the screening exercise test will not be compensated. However, subjects who are eligible to participate and complete the first stool collection will be compensated $40.00 and those who complete the entire study through visit 5 will be compensated another $80.00 for their time and effort.

You will be given a ClinCard, which works like a debit card. After a study visit, payment will be added onto your card by computer. The money will be available within 1 business day. You can use the ClinCard at an ATM or at a store. No one at KUMC will know where you spent the money. You will be given one card during the study. If your card is lost or stolen, please call (866) 952-3795.

The KUMC Research Institute will be given your name, address, social security number, and the title of this study to allow them to write checks for your study payments. Study payments are taxable income. A Form 1099 will be sent to you and the Internal Revenue Service if your payments are $600 or more in a calendar year.

This study includes providing specimens to the researcher. The specimens will belong to the University of Kansas Medical Center. There are no plans for you to profit from new products that are developed from research on your specimens.

IN THE EVENT OF INJURY

If you have a serious side effect or other problem during this study, you should immediately contact Dr. John Thyfault at 913-588-1790. If it is after 5:00 p.m., a holiday or a weekend, you should call Dr. Thyfault's cell at 573-268-2131. A member of the research team will decide what type of treatment, if any, is best for you at that time.

If you have a bodily injury as a result of participating in this study, treatment will be provided for you at the usual charge. Treatment may include first aid, emergency care

and follow-up care, as needed. Claims will be submitted to your health insurance policy, your government program, or other third party, but you will be billed for the costs that are not covered by the insurance. You do not give up any legal rights by signing this form.

INSTITUTIONAL DISCLAIMER STATEMENT

If you think you have been harmed as a result of participating in research at the University of Kansas Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Under certain conditions, Kansas state law or the Kansas Tort Claims Act may allow for payment to persons who are injured in research at KUMC.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

The researchers will protect your information, as required by law. Absolute confidentiality cannot be guaranteed because persons outside the study team may need to look at your study records. The researchers may publish the results of the study. If they do, they will only discuss group results. Your name will not be used in any publication or presentation about the study.

Your health information is protected by a federal privacy law called HIPAA. By signing this consent form, you are giving permission for KUMC to use and share your health information. If you decide not to sign the form, you cannot be in the study.

The researchers will only use and share information that is needed for the study. To do the study, they will collect health information from the study activities. You may be identified by information such as name, address, phone, and date of birth. However, this information will be kept secure. Your health information will be used at KU Medical Center by Dr. John Thyfault and the members of his research team and the KUMC Human Subjects Committee and other committees and offices that review and monitor research studies. Study records might be reviewed by government officials who oversee research, if a regulatory review takes place.

Blood and stool samples will be sent to a research facility in Arkansas for analysis. All study information that is sent outside KU Medical Center will have your name and other identifying characteristics removed, so that your identity will not be known. Because identifiers will be removed, your health information will not be re-disclosed by outside persons or groups and will not lose its federal privacy protection.

Your permission to use and share your health information will not expire unless you cancel it.

QUESTIONS

Before you sign this form, Dr. John Thyfault or other members of the study team should answer all your questions. You can talk to the researchers if you have any more questions, suggestions, concerns or complaints after signing this form. If you have any

questions about your rights as a research subject, or if you want to talk with someone who is not involved in the study, you may call the Human Subjects Committee at (913) 588-1240. You may also write the Human Subjects Committee at Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

You may stop being in the study at any time. Your decision to stop will not prevent you from getting treatment or services at KUMC. The entire study may be discontinued for any reason without your consent by the investigator conducting the study.

You have the right to cancel your permission for researchers to use your health information. If you want to cancel your permission, please write to John Thyfault. The mailing address is John Thyfault, Dept. of Molecular and Integrative Physiology, MS3043, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. If you cancel permission to use your health information, you will be withdrawn from the study. The research team will stop collecting any additional information about you. The research team may use and share information that was gathered before they received your cancellation.

CONSENT

Dr. John Thyfault or the research team has given you information about this research study. They have explained what will be done and how long it will take. They explained any inconvenience, discomfort or risks that may be experienced during this study.

By signing this form, you say that you freely and voluntarily consent to participate in this research study. You have read the information and had your questions answered.
You will be given a signed copy of the consent form to keep for your records.

_____
Print Participant's Name

_____ _____ _____
Signature of Participant Time Date

_____
Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

OPTIONAL SAMPLE STORAGE AND FUTURE USE

Purpose:

The researchers would like to save your left over blood samples and study information for research in the future. By studying these samples, researchers hope to learn more about the relationship between fitness and metabolism.

What is involved?
No additional blood will be drawn for this optional sample storage study.
In order to do the research with your sample, researchers may need to know some of your information. This helps researchers answer questions about diseases. The information that will be given to the researcher may include age, sex, gender, fitness, and physical activity patterns. This future use may include investigations of biomarkers. Biomarkers are substances in blood or tissue that may reflect information on metabolism and overall health.

How will information about me be kept private?
Samples will be stored in a locked freezer in Dr. Thyfault's laboratory at KUMC. Data will be stored in a format that is de-identified and does not include any information that can link the information to you. Your samples will be labeled with a random number code and a date indicating when they were collected but will not be marked with your name. Samples and data will be saved indefinitely. Once you have given us permission, you will not be able to cancel that permission because there is no way to identify which samples belong to you. The information about the uses and disclosures of your health information for the main study also applies to this future research. Reports about research done with your samples will not be given to you or your doctor. These reports will not be put into your medical record. The research will not have any effect on future medical care. If results are published, your name and other personal information will not be given.

What are possible risks?
The blood storage for future use will apply to extra samples that remain after the main study analyses are complete and there is no additional physical risk. The main risk of this optional research is possible loss of privacy and confidentiality. We will take reasonable precaution to reduce this risk.

Research methods are rapidly changing. In the future, researchers may develop methods that allow your samples to be linked back to you
The choice to share your samples and information is completely voluntary. You can decide not to have your samples used and still participate in the main study. Please mark your choice "Yes" or "No" below. If you have any questions you can talk to the investigator or the study team.

You give permission that your blood samples/data may be stored and used for future research.

☐ YES ☐ NO

_____
Print Participant's Name

_____ _____ _____
Signature of Participant Time Date

_____
Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

Appendix C: Health History Questionnaire

University of Kansas Medical Center – Thyfault Lab

Health History and Medical Questionnaire

Subject ID #: _____ Height:_____ Weight:_____

Name:_____ Date:_____Age: _____

Address:_____ Sex: Male Female

Telephone: _____ Email:_____

Personal Physician's Name:_____

Address & Telephone:_____

Emergency Contact:

Name:_____

Relationship: _____ Phone #:_____

Ethnicity: (Select one or more of the following)

- ⁄ ***Hispanic or Latino*** - A person of Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin," can be used in addition to "Hispanic or Latino."
- ⁄ ***Non-Hispanic or Latino***

Race: What race do you consider yourself to be? Select one or more of the following:

- ⁄ **American Indian or Alaska Native.** A person having origins in any of the original peoples of North, South, or Central America, and who maintains a tribal affiliation or community attachment.
- ⁄ **Asian.** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in the previous data collection strategies.)
- ⁄ **Black or African American.** A person having either origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or "African American."
- ⁄ **Native Hawaiian or Pacific Islander.** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific islands.
- ⁄ **White.** A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Health History:

Have you ever had:	High Blood Pressure **Y / N**	Low Blood Pressure **Y / N**
Heart Disease **Y / N**	Irregular Heart Beat **Y / N**	Diabetes **Y / N**
Heart Murmurs **Y / N**	Chest Pain **Y / N**	Thyroid Disease **Y / N**
Lung Disease **Y / N**	Arthritis **Y / N**	High Blood Cholesterol **Y / N**

Abnormal Electrocardiogram (ECG) **Y / N** Seizures **Y / N** Stroke **Y / N**

Anxiety (diagnosed) **Y / N** Depression (diagnosed) **Y / N** Recurrent Fatigue **Y / N**

Insomnia **Y / N** Asthma **Y / N** Tuberculosis **Y / N**

Chronic Infection **Y / N** Stomach/GI problems **Y / N** Hepatitis/Liver Disease **Y / N**

Bleeding Disorder **Y / N** Kidney/Urinary problems **Y / N** Cancer **Y / N**

Has a parent or sibling had any heart disorders prior to age 55? **Y / N**
Has a sudden death ever occurred in a parent or sibling? **Y / N**

Current Medications: include over the counter drugs

Name/Dosage/How often taken:

_____

_____

_____

_____

Allergies: _____

Have you ever taken medication for? Medication/Dosage/Date

Y / N High Blood Pressure: _____

Y / N Low Blood Pressure: _____

Y / N Heart Disease: _____

Y / N Diabetes: _____

Y / N Thyroid Disease: _____

Y / N Lung Disease: _____

Y / N Arthritis: _____

Smoking History:

Do you smoke: Cigarettes? **Y / N** Pipe/Cigar? **Y / N** Other? **Y / N**

If you quit, what month and year did you quit? _____/_____

Of packs smoked per day_____ For how many years _____

Alcohol Consumption History:

Do you currently drink alcohol? **Y / N**

If you drank alcohol previously, when did you stop? _____

If you ever did drink alcohol, what is (was) the volume consumed?

_____# of drinks per (circle one) day week month for _____ # of years

(**1 Drink** = 5oz glass of wine **OR** 12oz bottle of beer **OR** 1.5oz shot of distilled spirits)

Highest Education Level Achieved: if currently enrolled, highest degree received.

- ⁄ Some high school, no diploma
- ⁄ High school graduate, diploma or the equivalent (for example: GED)
- ⁄ Some college credit, no degree
- ⁄ Trade/technical/vocational training
- ⁄ Associate degree
- ⁄ Bachelor's degree
- ⁄ Master's degree
- ⁄ Professional degree
- ⁄ Doctoral degree

Exercise History:

Do you currently exercise aerobically? **Y / N** If yes, for how many years? _____

Duration: _____hours _____minutes Frequency: _____times per week

Types of exercise: _____

What time of the day do you usually exercise? (Circle one)

Morning Afternoon Evening

If you usually exercise in the **morning**, when do you eat breakfast? (Circle one)

Before Exercising After Exercising

Do you compete in endurance events? **Y / N** If yes, for how many years? _____

Frequency: _____times per year

What events? _____

Are you currently sedentary? **Y / N**

If yes, when did you last exercise? _____ Years ago

Duration: _____hours _____minutes Frequency: _____times per week

Types of exercise: _____

Medical Questionnaire:

1. Have you ever been advised by a physician to avoid exercise? **Y / N**
2. Do you ever have shortness of breath during or after exercise? **Y / N**
3. Have you ever experienced fainting or dizzy spells? **Y / N**
4. Have you ever experienced pain or discomfort in the chest? **Y / N**
5. Have you ever experienced swollen ankles (excluding sprains)? **Y / N**
6. Have you recently experienced heart palpitations (rapid heart beat) at rest? **Y / N**
7. Have you ever experienced claudication (unexplained lameness)? **Y / N**
8. Is there any other health condition that might limit your participation in exercise programs (e.g., bone or joint disorders, pregnancy, etc.)? **Y / N** If Yes, please explain:_____
9. Are you taking any medication not listed above? **Y / N** If Yes, please list:_____
10. Have you had a medical exam in the last 12 month? **Y / N** If Yes, please list date:_____

Signature:_____ Date:_____

University of Kansas Medical Center – Thyfault Lab

Follow-up Medical History Questions

11. Have you had any surgeries? Please list

12. Did you have any medical conditions for which you received treatment during the past year?

13. Do you take any vitamins or dietary supplements?
    Please list Name/Dosage/How often taken:

University of Kansas Medical Center – Thyfault Lab

Additional Medical Questionnaire
(For Women only)

1. Do you have menstrual cycle? Yes No
   If Yes, go to question #2
   If No, go to question #5

2. What is the frequency of your menstrual cycle? _____ times/year

3. How many days does each cycle last? _____ days.

4. When was your last menstrual period?

5. Are you taking birth control pills? Yes No

5a. If Yes, please record the brand name_____,
the amount_____.

6. Are you on estrogen therapy? Yes No

7. If Yes, please list the name (s), and frequency.

Name	Frequency (i.e. 1 per day)

Additional comments:

Appendix D: Participant 3-Day Food Record

NAME/ID #: _____	Reviewed: _____ Entered: _____ Checked: _____

Fitness and Metabolism Study

INSTRUCTIONS:

- Using the forms provided, please write down **ALL** of the foods you eat and beverages you drink on the following 3 consecutive days:

DAY 1 _____

DAY 2 _____

DAY 3 _____

Note: 3 consecutive days must include 2 weekdays and 1 weekend day. For example, Thursday/Friday/Saturday or Sunday/Monday/Tuesday

- Write down **ALL** of the foods you eat and beverages you drink each day, including soda, water, coffee, and even small bites of food and snacks between meals.

- Write down **AS MANY DETAILS AS YOU CAN** about the foods and beverages you ate in the appropriate columns:
  - ✓ **MEAL:** What would you call the meal or snack – breakfast, snack, lunch, brunch, dinner, etc.?
  - ✓ **TIME:** When did you eat or drink each food or beverage?
  - ✓ **LOCATION:** Where did you eat or drink each food or beverage?
  - ✓ **FOOD/BEVERAGE:** What did you eat or drink?
  - ✓ **AMOUNT:** How much of each food and beverage did you consume?
  - ✓ **OTHER DETAILS:** What is the brand name? What else does it say on the label – fat free, low fat, regular, etc.? How was the food prepared? Did you add anything to the food before eating it?

- Please list one item per line and write legibly. ***SEE EXAMPLE ON BACK.***

Example:

MEAL	TIME	LOCATION	FOOD/BEVERAGE	AMOUNT	OTHER DETAILS
Breakfast	6:00am	Home	2% Chocolate Milk	1 cup	Purchased ready to drink; 2% reduced-fat
			Banana	1 medium	Fresh, 7 inches long
			Blueberry bagel	1 whole	Thomas brand, (3.5" diameter)
			Peanut butter	3 Tbsp.	All-natural, Smucker's, Creamy
Snack	9:00am	School	Protein Bar	1 bar–2.75 oz	Pure Protein Chocolate Peanut Butter bar
			Water	16.9 fl. oz.	bottled
Lunch	11:30am	School cafe	Chicken Nuggets	5 each	
			Barbecue Sauce	1 packet	Heinz
			Cheddar Sun Chips	1.5 oz bag	
			Cinnamon Applesauce	1 container	
			Skim Chocolate Milk	1 carton	
			Powdered Sugar Mini Donuts	1 package (6 ea)	
Snack	4:00pm	School	Gatorade, lemon-lime	24 fl oz bottle	
			Snickers candy bar	1 regular size	
Dinner	6:30pm	Home	Cold Cut Combo Sub (Subway)	12" sub	On Italian bread, with American cheese, lettuce, tomato, regular mayonnaise
			Nacho Cheese Doritos	1.5 oz bag	
			Coca-Cola, with ice	40 oz cup	
Water			Water, without ice	36 fl oz	Throughout the day

Please list all vitamin, mineral, and herbal supplements you took today.

Type/Brand of Supplement	Reason for Taking	Amount Taken (dosage)	Frequency of Dose (times/day)
Example: *Flintstones Complete Multivitamin Chewable*	*General Health*	*1 Tablet*	*Once per day*
Example: *Nature made Vitamin D3 (2000IU)*	*Low vitamin D – prescribed by doctor*	*1 softgel (2000 IU)*	*Once per day*

- **Would you consider your intake of foods and beverages today to be typical of most days or was it considerably more or less? Explain why if not typical:** Typical of school day
- **Would you consider this food record to be complete and match what you really ate this day or are there any missing meals, snacks or beverages? Explain why if not complete and accurate:** All foods and beverages recorded

NAME _____ **DAY 1** _____

MEAL	TIME	LOCATION	FOOD/BEVERAGE	AMOUNT	OTHER DETAILS

Please list all vitamin, mineral, and herbal supplements you took today.

Type/Brand of Supplement	Reason for Taking	Amount Taken (dosage)	Frequency of Dose (times/day)

- **Would you consider your intake of foods and beverages today to be typical of most days or was it considerably more or less? Explain why if not typical: _____**
- **Would you consider this food record to be complete and match what you really ate this day or are there any missing meals, snacks or beverages? Explain why if not complete and accurate: _____**

NAME _____ **DAY 2** _____

MEAL	TIME	LOCATION	FOOD/BEVERAGE	AMOUNT	OTHER DETAILS

Please list all vitamin, mineral, and herbal supplements you took today.

Type/Brand of Supplement	Reason for Taking	Amount Taken (dosage)	Frequency of Dose (times/day)

- **Would you consider your intake of foods and beverages today to be typical of most days or was it considerably more or less? Explain why if not typical: _____**
- **Would you consider this food record to be complete and match what you really ate this day or are there any missing meals, snacks or beverages? Explain why if not complete and accurate: _____**

NAME _____ **DAY 3** _____

MEAL	TIME	LOCATION	FOOD/BEVERAGE	AMOUNT	OTHER DETAILS

Please list all vitamin, mineral, and herbal supplements you took today.

Type/Brand of Supplement	Reason for Taking	Amount Taken (dosage)	Frequency of Dose (times/day)

- **Would you consider your intake of foods and beverages today to be typical of most days or was it considerably more or less? Explain why if not typical: _____**
- **Would you consider this food record to be complete and match what you really ate this day or are there any missing meals, snacks or beverages? Explain why if not complete and accurate: _____**

Appendix E: Participant Stool Collection Instructions

Bile Acid Study Stool Collection Instructions

Please avoid touching the scoop or sample to decrease contamination of the sample.

1. **Prepare your Materials**

   Make sure you have everything you need. You should have:

   - Screw-top fecal container with spoon
   - Stool collection device
   - Plastic specimen bag (says “Biohazard”)
   - Silver insulated envelope
   - Ice pack

2. **Get the Specimen**

   Wash your hands and urinate before you start. Getting urine mixed in with the poop contaminates the sample. You can use gloves if you’d like, but you shouldn’t have to touch any stool.

   - Peel the adhesive off of the two tabs of the stool collection device and tape it to the toilet.
   - Poop into the tissue paper part of the liner.
   - Using the spoon attached to the lid of the specimen container, scoop at least 2 spoonfuls of poop into the container. Try not to get any on the outside of the container.
   - Screw the lid on tight.

3. **Pack it Up**

   - Tear the tissue paper part of the liner away from the cardboard and flush it down the toilet with the remaining stool.
   - Remove the cardboard section from the toilet and throw it in the garbage.
   - Wash your hands.
   - Double check that the lid is screwed on tight, and place the sealed container inside the plastic specimen bag.
   - Fill out the paper slip with your name and date and time of collection. Place the paper form into the pouch on the outside of the plastic bag.
   - Place the plastic bag into the silver insulated envelop along with the ice pack and place it in your freezer.

4. **Give it Back**

   - Bring the sample to your next scheduled visit and we will get it from you there.

Appendix F: Diet Instructions and Recording Sheet

Diet Instructions

1.) Over the next 7-8 days, you will be asked to consume only the foods provided and/or prescribed to you.
    a. The meals provided are designed to meet your total daily caloric needs.
    b. All foods will be provided to you in a pack-out cooler.

2.) Please use the attached check-off log. This log indicates which foods to consume throughout the day. There is also a section to report how much you ate/drank a particular food and to document what time of day this occurred.
    a. You may consume the assigned food for a particular day at anytime as long as they are consumed only during that day.

3.) You are required to consume ALL foods in their entirety. Do not throw away the uneaten food. If you cannot eat all the food provided, please place the uneaten food back in the refrigerator (as you will return this to us). Also, do not eat or drink anything else besides the items provided to you.
    a. Allowable outside beverages include: water, diet soda, black coffee, unsweetened tea.
    b. Please document the beverages you consume in the provided sections.

4.) At the end of the 7-8 day period, please return all wrappers and containers (containing any food not consumed).

5.) Eat ONLY the food or drink provided to you by staff.

If you have any questions please call or text Adrianna at 913-634-0857.

Aerobic Capacity and Bile Acid Study: Intake Record

Day #1

ID #: Date:

Breakfast		**Time:**
Honey bunches of oats cereal	How much of this did you eat? 0% 25% 50% 75% 100%	
1% Milk	How much of this did you eat? 0% 25% 50% 75% 100%	
Banana	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Turkey sandwich w/cheese, lettuce, tomato, mustard	How much of this did you eat? 0% 25% 50% 75% 100%	
Lay's potato chips	How much of this did you eat? 0% 25% 50% 75% 100%	
Chocolate chip cookies	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Spaghetti & meat sauce	How much of this did you eat? 0% 25% 50% 75% 100%	
Salad w/Italian dressing	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
Mixed Nuts	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek Yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #2

ID #: Date:

Breakfast		**Time:**
Apples and Cinnamon Instant Oatmeal	How much of this did you eat? 0% 25% 50% 75% 100%	
1% milk	How much of this did you eat? 0% 25% 50% 75% 100%	
Blueberry Muffin	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Peanut Butter and Jelly Sandwich	How much of this did you eat? 0% 25% 50% 75% 100%	
Pretzels crisps	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek Yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Beef Burrito w/lettuce, cheese, sour cream	How much of this did you eat? 0% 25% 50% 75% 100%	
Chips & salsa	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
String cheese	How much of this did you eat? 0% 25% 50% 75% 100%	
Apple	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #3

ID #: Date:

Breakfast		**Time:**
Bagel	How much of this did you eat? 0% 25% 50% 75% 100%	
Cream Cheese	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek Yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Pulled BBQ chicken sandwich	How much of this did you eat? 0% 25% 50% 75% 100%	
Celery w/ranch	How much of this did you eat? 0% 25% 50% 75% 100%	
Lay's Potato chips	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Chicken Stir-fry	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
Peanut, almond and dark chocolate protein bar	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #4

ID #: Date:

Breakfast		**Time:**
Bacon, egg and cheese biscuit	How much of this did you eat? 0% 25% 50% 75% 100%	
Carnation breakfast milk chocolate drink	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Creamy chicken and pasta	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Mandarin orange fruit cup	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Chicken and Seasoned vegetables	How much of this did you eat? 0% 25% 50% 75% 100%	
Rice	How much of this did you eat? 0% 25% 50% 75% 100%	
Ice cream bar	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
Trail Mix	How much of this did you eat? 0% 25% 50% 75% 100%	
String cheese	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #5

ID #: Date:

Breakfast		**Time:**
Honey bunches of oats cereal	How much of this did you eat? 0% 25% 50% 75% 100%	
1% Milk	How much of this did you eat? 0% 25% 50% 75% 100%	
Banana	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Turkey sandwich w/cheese, lettuce, tomato, mustard	How much of this did you eat? 0% 25% 50% 75% 100%	
Lay's potato chips	How much of this did you eat? 0% 25% 50% 75% 100%	
Chocolate chip cookies	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Spaghetti & meat sauce	How much of this did you eat? 0% 25% 50% 75% 100%	
Salad w/Italian dressing	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
Mixed Nuts	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek Yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #6

ID #:

Date:

Breakfast		**Time:**
Apples and Cinnamon Instant Oatmeal	How much of this did you eat? 0% 25% 50% 75% 100%	
1% milk	How much of this did you eat? 0% 25% 50% 75% 100%	
Blueberry Muffin	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Peanut Butter and Jelly Sandwich	How much of this did you eat? 0% 25% 50% 75% 100%	
Pretzels crisps	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek Yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Beef Burritos w/lettuce, cheese, sour cream	How much of this did you eat? 0% 25% 50% 75% 100%	
Chips & salsa	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
String cheese	How much of this did you eat? 0% 25% 50% 75% 100%	
Apple	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #7

ID #: Date:

Breakfast		**Time:**
Bagel	How much of this did you eat? 0% 25% 50% 75% 100%	
Cream Cheese	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek Yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Pulled BBQ chicken sandwich	How much of this did you eat? 0% 25% 50% 75% 100%	
Celery w/ranch	How much of this did you eat? 0% 25% 50% 75% 100%	
Lay's Potato chips	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Chicken Stir-fry	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
Peanut, almond and dark chocolate protein bar	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Aerobic Capacity and Bile Acid Study: Intake Record

Day #8

ID #: Date:

Breakfast		**Time:**
Bacon, egg and cheese biscuit	How much of this did you eat? 0% 25% 50% 75% 100%	
Carnation breakfast milk chocolate drink	How much of this did you eat? 0% 25% 50% 75% 100%	
Lunch		**Time:**
Creamy chicken and pasta	How much of this did you eat? 0% 25% 50% 75% 100%	
Greek yogurt	How much of this did you eat? 0% 25% 50% 75% 100%	
Mandarin orange fruit cup	How much of this did you eat? 0% 25% 50% 75% 100%	
Dinner		**Time:**
Chicken and Seasoned vegetables	How much of this did you eat? 0% 25% 50% 75% 100%	
Rice	How much of this did you eat? 0% 25% 50% 75% 100%	
Ice cream bar	How much of this did you eat? 0% 25% 50% 75% 100%	
Snacks		**Time:**
Trail Mix	How much of this did you eat? 0% 25% 50% 75% 100%	
String cheese	How much of this did you eat? 0% 25% 50% 75% 100%	
Additional Beverages:	**Amount:**	**Time:**

Appendix G: Study Recruitment Flyer

HEALTHY WOMEN NEEDED FOR FITNESS AND NUTRIENT METABOLISM STUDY

(Endurance athletes AND non-regular exercisers)

Researchers think there may be a relationship between fitness level and how people absorb nutrients.

Participation in this study will involve:

- VO_{2max} treadmill test
- Body measurements - (Weight, height, body composition)
- Eating a 7-8 day meal plan that is provided
- Blood draw and oral glucose tolerance test
- Stool collection
- Keeping a 3-day food diary and activity record
- Completing questionnaires
- 4-5 visits ranging from 15min-2.5hour
  (1-KU Med Center campus/3-4-fairway campus)

Participants who qualify and complete this study will be paid for their time.

Qualifications for Participation

- Female – ages 18-35
- Good health – no chronic diseases, not currently taking any medications
- Non-smoker

For more information about the study or to see if you might qualify as a participant:

Call: Adrianna Maurer, RD
(913) 634-0857 at KUMED
Or E-mail:
a126m463@kumc.edu

Appendix H: Bile Acid Names

aMCA	alpha-Muricholic Acid
bMCA	beta-Muricholic Acid
CA	Cholic Acid
CDCA	Chenodeoxycholic acid
CDCA-G	Chenodeoxycholic Acid-3-b-D-Glucuronide
CDCA-S	Chenodeoxycholic Acid-3-Sulfate
DCA	Deoxycholic acid
GCA	Glycocholic acid
GCDCA	Glycochenodeoxycholic Acid
GCDCA-S	Glycochenodeoxycholic Acid-3-sulfate
GDCA	Glycodeoxycholic Acid
GHCA	Glycohyocholic Acid
GHDCA	Glycohyodeoxycholic Acid
GLCA	Glycolithocholic Acid
GUDCA	Glycoursodeoxycholic Acid
HCA	Hyocholic acid
HDCA	Hyodeoxycholic acid
LCA	Lithocholic Acid
TaMCA	Tauro-alpha-Muricholic Acid
TbMCA	Tauro-beta-Muricholic Acid
TCA	Taurocholic acid
TCDCA	Taurochenodeoxycholic Acid
TDCA	Taurodeoxycholic acid
THCA	Taurohyocholic Acid
THDCA	Taurohyodeoxycholic Acid
TLCA	Taurolithocholic Acid
TLCA-S	Taurolithocholic Acid-3-Sulfate
TUDCA	Tauroursodeoxycholic Acid
TwMCA	Tauro-omega-Muricholic Acid
UDCA	Ursodeoxycholic acid
UDCA-S	Ursodeoxycholic Acid-3-Sulfate
wMCA	omega-Muricholic Acid