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The Association of Exercise Training Modalities with Circulating Branched Chain Amino Acid and Ketone Body levels in Patients with Type 2 Diabetes

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ABSTRACT

Background: Elevated levels of circulating branched-chain amino acids (BCAA) and ketone bodies are recognized as biomarkers for cardiovascular disease (CVD) and other pathological conditions in type-2 diabetes mellitus (T2DM). Aerobic exercise interventions have been shown to decrease the levels of these markers, suggesting improved metabolic status and reduced risk of CVD. However, the efficacy of resistance training and concurrent programs in reducing BCAA and ketone body levels has not been well researched.

Methods: The current study was performed as a secondary analysis of the HART-D trial, a 9-month randomized, controlled exercise-training trial of 262 participants with T2DM. Participants were randomized to one of four groups: non-exercise control, aerobic training (AT), resistance training (RT), or a combined aerobic and resistance training (ATRT). The effects of the 9-month intervention on BCAAs (leucine, valine, and isoleucine) and ketone bodies (β-hydroxy-butyrate, BHB; acetoacetate, AcAc; and acetone) were quantified by nuclear magnetic resonance spectroscopy (NMR) at LabCorp (Morrisville, NC). Generalized linear models were used to examine effects of exercise training between groups with adjustments for age, sex, race, change in fat mass, glucose, and medication status and baseline trait value. Pearson correlation analysis was used to examine associations of the changes in BCAA and ketone levels with changes in concomitant cardiometabolic biomarkers.

Results: The ATRT group increased total BCAA and leucine levels compared to the AT group, and increased isoleucine compared to all other groups (all $p<0.05$). RT decreased BHB levels ($p<0.05$) compared to the AT group only. Across all exercise groups combined, changes in total ketone bodies ($r=0.2$), BHB ($r=0.21$), and Acetone ($r=0.17$) were weakly correlated with changes in HbA1c levels. Changes in total BCAAs ($r=0.30$) and valine ($r=0.36$) were moderately correlated with changes in fasting glucose levels, while isoleucine was weakly correlated with glucose ($r=0.2$) (all $p<0.05$).

Conclusions: Our results show that the ATRT group increased isoleucine levels compared to the control group in diabetics, the mechanism of which is unclear. Exercise induced changes in BCAA and ketone body levels are weakly to moderately related to some concomitant cardiometabolic biomarkers such as fasting glucose and HbA1c levels. Further research is needed to examine the association of exercise training on circulating BCAA and ketone body levels in diabetics.

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CHAPTER 1

INTRODUCTION

The branched-chain amino acids (BCAA); leucine, valine, and isoleucine are essential amino acids that distinguish themselves from the other amino acids due to very limited hepatic catabolism[1]. The majority of their metabolism resides in skeletal muscle. As such they play an important role in regulating muscle protein synthesis, and contributing to energy production via the tricarboxylic acid (TCA) cycle[2]. BCAAs are metabolized through two main processes. The first, a reversible transamination catalyzed by branched-chain aminotransferase (BCAT), produces branched chain α-ketoacids (BCKA) and glutamate. The second, is an irreversible oxidative decarboxylation of the BCKAs catalyzed by the branched chain α-ketoacid dehydrogenase (BCKDH) complex[3]. The complex is regulated by both covalent and allosteric mechanisms. Phosphorylation of its E1 component by BCKDH kinase downregulates activity, while mitochondrial protein phosphatase 2C (PP2Cm) dephosphorylates the complex, upregulating its activity[1]. BCKAs, specifically α-ketoisocaproate, can bind the BCKDH kinase and allosterically inhibit the phosphorylation of the complex, suggesting that deficiencies in BCAT activity could downregulate BCKDH activity[4]. A deficiency in the BCKDH complex can reduce its contribution to energy production and lead to a buildup of circulating BCAAs in the blood.

While the relationship is not completely understood, there appears to be an association between elevated levels of circulating BCAAs and T2DM, and related pathologies, such as cardiovascular disease (CVD)[5-7]. Improvements in insulin resistance levels, as measured by the homeostatic model assessment of insulin resistance (HOMA-IR), after weight loss have been found to have a greater correlation with decreases in BCAA levels (r=0.50) than the amount of weight lost (r=0.24) during an exercise intervention[5]. Elevated BCAA levels have also been identified as strong predictors of the development of T2DM as they have been shown to increase long before the onset of the condition[6]. Increased baseline levels of BCAAs more than doubled the risk of developing T2DM over a six-year period in men (OR 2.09: 95%CI 1.38-3.17)[6]. Tobias et al[8] discovered a positive relationship between BCAA levels and coronary cardiovascular events in women both with and without T2DM, although the relationship was stronger in the diabetic population (Relative Risk 1.2: CI 1.08-1.32). This association has also been reproduced in other prospective cohort trials[7,9]. While there hasn't been a determined causal link between BCAAs and CVD, impairment of BCAA catabolic pathways in the heart was associated with elevated superoxide production, oxidative injury, and mitochondrial permeability transition pore opening in heart failure[10,11].

Since BCAAs can be metabolized in skeletal muscle, unlike other amino acids, they can contribute to energy production during exercise. Therefore exercise has a large regulatory effect on the metabolism of BCAAs[12]. Aerobic exercise acutely[13-15] and chronically[16] increases BCKDH activity and decreases

expression of the BCKDH kinase protein. Due to enhanced oxidative capacity[17] and increased protein turnover[18] in skeletal muscle from resistance training it is thought that regular resistance training would be beneficial in regulating BCAA metabolism, however no actual studies have been performed to date. One study looking at a concurrent exercise training program has been conducted which showed a decrease in isoleucine and valine levels, however leucine levels did not significantly decrease[19].

Ketone bodies are molecules produced by the liver as a result of fatty acid oxidation under conditions of low glucose availability[20]. The β-oxidation of fatty acids results in acetyl-CoA, which can be converted via multiple steps of enzyme-catalyzed reactions into one of three ketone bodies: acetoacetate (AcAc), 3-β-hydroxybutyrate (BHB), or the least abundant, acetone. They are mainly thought of as an alternate energy source, but can also be important mediators of cell signaling, drivers of protein post-translational modification, and modulators of inflammation and oxidative stress[21]. The rate of production of ketones is controlled by acetyl-CoA carboxylase and mitochondrial HMG-CoA synthase[22], and the rate of clearance is regulated by succinyl-CoA-3-oxoacid CoA transferase (SCOT)[23] and monocarboxylate transporters (MCT1)[24]

Due to the role of insulin in regulating ketone production and metabolism, ketones are a metabolite with strong implications in T2DM[20]. Diabetic ketoacidosis is a serious condition that arises in diabetics, when ketone production becomes too deregulated[25]. In a study of over 9,000 men; levels of ketone bodies (AcAc & BHB) were significantly increased ($p<0.01$) in those with

diabetes compared to those with normal glucose control[26]. AcAc levels were increased by elevated in diabetics by 64% (95% CI, 16% to 109%), and BHB levels were increased by 99% (95% CI, 6% to 186%) compared with the reference group. After a five year follow up of over 4,000 of these men, elevated AcAc levels were associated with increased risk of incident T2DM in those with impaired fasting glucose (OR 1.49: 95%CI 1.12-1.99)[26]. Lower levels of adipocyte RNA expression of key enzymes in ketolysis, such as SCOT, were also found in those with diabetes and glucose tolerance issues[26]. Altered substrate utilization in myocardial metabolism is known to play a causative role in the development of CVD in those with diabetes[27,28]. A 2001 study[29] found that ketone body utilization in patients with heart failure is altered in a tissue specific manner. Skeletal muscle has a significantly lower uptake of ketones in heart failure patients than in healthy controls. Therefore, elevated levels of ketone bodies are often observed in patients with heart failure[29].

Due to the role of ketones as a fuel source under certain conditions, exercise can affect plasma ketone levels acutely and alter them chronically through both ketogenic inhibiting and ketolytic enhancing mechanisms. Since the rate of ketogenesis is increased as the ratio of glucagon to insulin increases, chronically, exercise works to inhibit the excess rates of ketogenesis by increasing insulin sensitivity in type 2 diabetics[30,31]. While specific long term training induced changes in expression of ketolytic enzymes has not yet been described in humans, changes are observed in ketone body metabolism during and after exercise in trained and untrained individuals, such as the attenuation of

post-exercise rises in ketone bodies[32]. However, rodent models have shown increased expression of ketolytic enzymes such as SCOT[33] and MCT1[24] from aerobic training programs in an intensity dependent manner.

In summary, circulating BCAAs and ketone bodies are heavily implicated with the development of T2DM and mediate some of the comorbidities and disease states that are synonymous with the condition, especially CVD. While some research has been published with promising results regarding the efficacy of regular exercise to manage these biomarkers, there is limited information on different training modalities or the pathways mediating these effects. In the original analyses performed on the HART-D cohort, their main outcome trait (HbA1c levels) was decreased compared to the control only in the combination training group (-0.34%: 95% CI, -0.64% to -0.3%)[34]. Therefore, it is of interest to research the effect of different training modalities on the levels of circulating BCAAs and ketone bodies to further develop our understanding and management of these biomarkers of complications in T2DM. We hypothesize that all modalities of exercise will elicit significant changes in circulating BCAAs and ketone bodies. We will test these hypotheses with the following aims:

Aim 1: Determine the association of different modalities of 9-month exercise training plan on circulating BCAA and ketone levels in type 2 diabetics from the HART-D study.

a. Determine the association of an aerobic training program on circulating BCAA and ketone levels

b. Determine the association of a resistance training program on circulating BCAA and ketone levels

c. Determine the association of a combined aerobic and resistance training program on circulating BCAA and ketone levels

We hypothesize that all exercise training modalities will significantly decrease circulating BCAA and ketone levels and ATRT will produce significantly greater decreases than AT and RT.

Aim 2: Determine the association of exercise induced changes in circulating BCAA and ketones with changes in concomitant cardiometabolic biomarkers (body fat %, lean mass, HbA1c, fasting glucose, fasting insulin, C-reactive protein, and Vo_2peak) in type 2 diabetics from the HART-D study.

We hypothesize that exercise induced changes in circulating BCAA and ketone levels will be associated with changes in concomitant cardiometabolic biomarkers with no significant differences between groups.

CHAPTER 2

BCAAS AND KETONE BODIES IN DIABETES

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by apoptosis and dysfunction of pancreatic β cells due to a decrease in insulin receptor sensitivity referred to as insulin resistance[35]. Chronic insulin resistance causes an upregulation of glucose transporter 2 (GLUT2) channels which leads to an increase in cytosolic calcium levels[35, 36]. These increased calcium levels cause the subsequent β cell apoptosis due to calcium activated intracellular cysteine protease calpain-2[37], and increased reactive oxygen species (ROS)[38, 39]. Calcium stimulates ROS through both, increased mitochondrial ROS metabolism[38] and the NADPH oxidase dependent generation of ROS due to activation of protein kinase C (PKC)[39]. The pancreatic β cells are believed to have increased exposure to ROS due to aging[40], chronic hyperglycemia[41], and elevated intracellular fatty acids[42], which has led to the notion that advanced age, poor diet, and sedentary lifestyle have a deleterious effect on β cell function and play a role in the development of T2DM[40-42].

Through large systematic reviews and meta-analyses, a plethora of risk factors have been identified and evaluated for their efficacy in predicting T2DM outcomes. Factors pertaining to diet and lifestyle habits, psychosocial factors, medical history, and blood biomarkers have been strongly linked to T2DM[43].

Obesity is the strongest risk factor known, with metabolically unhealthy obesity being associated with a 10-fold increase for the development of T2DM[43]. Lifestyle factors that promote obesity, such as increased sedentary time [risk ratio (RR) 1.9: 95% Confidence Interval (CI) 1.66-2.19], smoking (RR 1.4: CI 1.33-1.44), and increased sugar-sweetened beverage (RR 1.3: CI 1.21-1.41) and processed meat consumption (RR 1.4: CI 1.25-1.49) also therefore increase the risk of developing T2DM[43]. Biomarkers including C-reactive protein (RR 1.26: CI 1.16-1.37), alanine aminotransferase (RR 1.85: CI 1.57-2.18), and gamma-glutamyl transferase (RR 1.92: CI 1.66-2.21) are all positively associated with T2DM risk, while increased Vitamin D levels (RR 0.62: CI 0.54-0.70) are a negative risk factor[43].

Along with the transparent metabolic pathology of T2DM, there are a number of complications and comorbidities associated with the disease. Common complications of T2DM include hypertension, dyslipidemia, decreased glomerular filtration rate, and peripheral vascular disease[44]. In addition to the physical comorbidities that present themselves, an increased risk for mild cognitive impairment and depression is associated with T2DM[45]. Rates of clinically relevant depression among those afflicted with T2DM has been shown to be about 31%[46] which is much higher than the rates in the general population.

Diabetes and its' complications are having a drastically increasing societal and economic impact on the United States. Over the 20 year period from 1990 to 2010, the total number of adults diagnosed with diabetes tripled, while the incidence rate over that time period doubled[47], with type 2 being the vastly more

prevalent diagnosis[48]. Based on data from the 2016 National Health Interview Survey, there are 21 million adults in the United States currently diagnosed with T2DM[48], which equates to 8.58% of the population. Along with the myriad of health complications and comorbidities associated with it, adults diagnosed with diabetes have a 50% higher all-cause mortality rate than those without the diagnosis[49]. On top of the individual burden brought upon those diagnosed with diabetes, the burden on the healthcare system is enormous. In 2017, the cost of diabetes to the US healthcare system was $237 billion, more than double the $116 billion it cost in 2007[50]. This total accounts for roughly a quarter of all healthcare dollars spent, and equals out to an average cost of $16,572 per annum for each individual with the condition[50].

Cardiovascular disease (CVD) is the leading cause of death in the United States, attributing to approximately one third of total deaths in 2016[51]. The risk for developing CVD increases in diabetics one to three times for women and two to five times for men. Diabetics have 1.7 times the mortality risk from CVD[52]. Additionally, on average, atherosclerotic cardiovascular disease manifests itself 14.6 years earlier in those with T2DM than the non-diabetic population[53]. There are many cellular and molecular pathophysiologic factors that elucidate the increased incidence and severity of cardiovascular disease in the diabetic population. The impairment of insulin signaling, hyperinsulinemia, and hyperglycemia presented by type-2 diabetics contributes to numerous issues such as elevated free fatty acids, protein kinase-C activation, mitochondrial dysfunction, oxidative stress, and advanced glycogen end-product, which causes

endothelial dysfunction and inflammation[52]. This leads to an increase in the formation of foam cells in the vulnerable subendothelial layers of vasculature. These foam cells release inflammatory mediators such as tumor necrosis factor-α, leading to stenosis and necrosis of the vessel[52].

Exercise has long been identified as a key factor in the prevention and management of T2DM. The most recent American Diabetes Association guidelines recommend at least 150 minutes per week of moderate to vigorous aerobic exercise and 2-3 days per week of resistance training[54]. The efficacy of aerobic exercise in the management and prevention of diabetes has been well studied with positive results. Lifestyle interventions in the form of dietary energy restriction and 150-175 minutes per week of aerobic exercise have shown a 40-70% reduction in the risk of developing T2DM in subjects with impaired glucose tolerance[54]. Aerobic exercise has been shown to improve many markers associated with dysfunction in diabetics such as glycosylated hemoglobin levels, regulation of lipid and lipoprotein metabolism[55], insulin resistance, fasting plasma glucose, fasting insulin, and systolic blood pressure[56]. More recently it has been acknowledged that resistance training is also a viable method of exercise to combat diabetes related complications. Along with the obvious beneficial physiological adaptations caused by resistance training, such as increased lean body mass, strength, and bone mineral density, it also has been shown to reduce HbA1c and blood pressure, and increase insulin sensitivity in type 2 diabetics[30]. There is an inverse association between skeletal muscle index, the ratio of estimated total skeletal muscle mass as a ratio of total body weight, and

developing insulin resistance. From the lowest quartile to the second lowest quartile the RR is 0.72 (CI 0.63-0.83) and from the lowest to highest quartile the RR is 0.59 (CI 0.48-0.72)[31]. This suggests resistance training may be important and beneficial in the diabetic population. There is evidence that a concurrent (resistance and aerobic training) program produces greater results than either modality by itself. Concurrent programs have been shown to produce greater improvements in body composition and performance characteristics, such as lean body mass, fat mass, strength, and VO_2max, as well as HbA1c levels[34], and pro-inflammatory biomarkers such as interleukin-6 and tumor necrosis factor-α[57].

A factor that has more recently been identified as having a strong association with metabolic disease and CVD is the circulating level of branched-chain amino acids (BCAA) and their metabolites[58]. Metabolic profiling shows that changes in circulating levels of BCAAs have an inverse association with insulin sensitivity (r=-0.38), and are a potential predictor of CVD risk[59]. The BCAAs; leucine, valine, and isoleucine are essential amino acids that play an important role in activating the anabolic signaling molecule, mammalian target of rapamyacin complex 1 (MTOR1C), regulating muscle protein synthesis, and energy production[2]. BCAAs are broken down through two main processes, the first catalyzed by branched-chain aminotransferase (BCAT), and the second catalyzed by the branched chain α-ketoacid dehydrogenase complex (BCKDH)[3]. In the first process, BCAT catalyzes the transamination of BCAAs, a substitution reaction that replaces the amine functional group of the BCAA with a ketone group to form branched-chain α-keto-acids(BCKA) and glutamate. In the BCKDH

complex the BCKA products then undergo oxidative decarboxylation to produce acyl-CoA derivatives which are subsequently converted through several downstream reactions into acetyl-CoA and succinyl-CoA which enter the tricarboxylic acid (TCA) cycle to be involved in energy production[60]. A deficiency in the BCKDH complex can reduce its contribution to energy production and lead to a buildup of BCAAs in the blood. Adipose specific over-expression of GLUT-4 creates a concerted decrease in multiple BCAA catabolic enzymes in adipose tissue, resulting in increased levels of circulating BCAAs[61]. Deficiencies in the BCKDH complex can often be caused by overexpression of BCKDH kinase, an inhibitor of the complex, and a decrease in expression of mitochondrial BCAT, which catalyzes the initial transamination of BCAAs to produce BCKA for oxidative decarboxylation via the BCKDH complex[62].

While the relationship is not completely understood, there appears to be an association between T2DM and elevated levels of circulating BCAAs. Improvements in insulin resistance levels, as measured by the homeostatic model assessment of insulin resistance, after weight loss have been found to have a greater correlation with decreases in BCAA levels (r=0.50) than the amount of weight lost (r=0.24) during an exercise intervention[59]. Elevated BCAA levels have also been shown to be strong predictors of the development of T2DM as they have been shown to increase long before the onset of the condition[5]. Increased baseline levels of BCAAs more than doubled the risk of developing T2DM over a six year period in men (OR 2.09: CI 1.38-3.17)[5]. Increased levels of BCAAs, BCKAs, and medium and long-chain acylcarnitines, by-products of

mitochondrial catabolism of BCAAs, all distinguish between obese people who have features of insulin resistance versus those who do not[3].

There are several hypotheses as to how BCAA levels might affect insulin resistance especially in those with T2DM. There are some genetic factors, as genetic variants in the protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1K, are associated with T2DM. The PPM1K gene is responsible for encoding the mitochondrial protein phosphatase 2C (PP2Cm) which activates the BCKDH complex through dephosphorylation[6]. There are also theories involving the MTORC1 pathway. Activation of the MTORC1 pathway involves insulin and glucose, as well as crucially requiring BCAAs for signaling of translocation to the lysosome, so an overload of BCAA could play a role in developing insulin resistance[63] with MTOR being a central signal in cross-talk between BCAAs and insulin[64]. There is also some evidence to suggest that inhibition of sodium-glucose cotransporter-2 increases BCAA metabolism and therefore the sodium-glucose cotransporter-2 may play a role in BCAA levels in T2DM as expression of these proteins is increased in diabetic nephropathy[65].

While the relationship between BCAA's and T2DM is relatively well documented, the association of BCAAs with CVD is more contentious. Tobias et al[8] discovered a positive relationship between BCAA levels and coronary cardiovascular events in women both with and without T2DM, although the relationship was stronger in the diabetic population (RR 1.2: CI 1.08-1.32). This association has been reproduced in other prospective cohort trials[7, 9], however some studies have found that after adjusting for cofounding variables, BCAA

levels are not a significant predictor of cardiovascular events[66]. While there hasn't been a determined causal link between BCAAs and CVD, a recent study found that impairment of BCAA catabolic pathways in the heart was associated with elevated superoxide production and oxidative injury[10], and were regulated by Kruppel-like factor 15 in which deficiencies have been shown to be linked to CVD[67].

As BCAAs, unlike other amino acids, can be metabolized in skeletal muscle, they can contribute to energy production during exercise, and therefore exercise has a large regulatory effect on the metabolism of BCAAs[12]. Aerobic exercise acutely increases BCAA metabolism by activating the BCKDH complex[13-15] and decreasing BCKDH Kinase activity[15]. Chronic repeated bouts of aerobic exercise decrease BCKDH Kinase protein expression in skeletal muscle, thereby increasing activation of the BCDKH complex[16]. Exercise intolerance may develop in severe cases of deficiencies in BCAA metabolism, as exhibited in studies with BCAT knockout mice[68].

There is very little information to date on the impact of resistance training on BCAA metabolism. Due to the increased oxidative capacity in skeletal muscle from enhanced mitochondrial function[17] and stimulation of muscle protein turnover[18], it would be assumed to have a beneficial effect. The effects of a combined program of aerobic and resistance training on BCAA levels have been studied only once. In this study, leucine levels did not change with training, however, isoleucine and valine levels did decrease[19]. Due to the lack of studies regarding resistance and combination training, there is a need for further

research to increase our understanding of the efficacy of different modes of exercise as a treatment for lowering or maintaining circulating BCAA levels. It is also not known how exercise induced changes in BCAAs correlate with changes in some other important concomitant cardiometabolic biomarkers related to T2DM, such as HbA1c, C-reactive protein, and lipid panel. There is no accepted working model as to the exact physiological mechanisms underlying enhanced BCAA metabolism from exercise, and therefore the biological plausibility needs to be further examined.

Ketone bodies are molecules produced by the liver as a result of fatty acid oxidation under conditions of low glucose availability[20]. They are mainly thought of as an alternate energy source, but can also be important mediators of cell signaling, drivers of protein post-translational modification, and modulators of inflammation and oxidative stress[21]. Normal ketone levels are below 0.6mmol/L, however issues with hormonal balance or enzyme malfunctions in ketolysis can lead to a build-up of unused ketone bodies in the blood, which causes a drop in pH and acidosis. The β-oxidation of fatty acids results in acetyl-CoA, which can be converted via multiple steps of enzyme-catalyzed reactions into one of three ketone bodies: acetoacetate (AcAc), 3-β-hydroxybutyrate (3HB), or the least abundant, acetone.

The rate of production of ketones is controlled by three hormones: hormone-sensitive lipase, acetyl-CoA carboxylase, and mitochondrial HMG-CoA synthase. The activity of these hormones is determined by the ratio of circulating levels of insulin and glucagon[20]. Ratios favoring insulin act to inhibit ketogenesis,

while when glucagon is favored ketone production is stimulated[22]. Glucagon signals for the body to raise the concentration of glucose in the bloodstream whereas insulin lowers the concentration, signaling for the uptake of glucose into tissues to be used for energy production[22]. When the levels of glucagon are higher than insulin it promotes fatty acid oxidation in order to produce acetyl-CoA for energy production through the TCA cycle. If glucagon levels are raised too much, due to issues such as insulin resistance, acetyl-CoA production via fatty acid oxidation may increase beyond the body's need for it as an energy substrate and will instead be converted to ketone bodies to be stored instead of entering the TCA cycle[69]. Insulin also promotes peripheral ketone body clearance, thus reduced insulin levels will cause increased plasma ketone levels due to both enhanced ketogenesis and diminished ketolysis[70].

Succinyl-CoA-3-oxoacid CoA transferase (SCOT) is an enzyme derived from the OXCT1 gene that catalyzes the transfer of CoA between carboxylic acid groups[23]. SCOT catalyzes the first and rate determining step of ketolysis by transferring a CoA group from succinyl-CoA to acetoacetate to form acetoacetyl-CoA, which is further broken down into two acetyl-CoA groups to enter the TCA cycle for energy production[71]. Deficiencies in SCOT interfere with the ability to utilize ketones as an energy source, as the process of ketolysis cannot be initiated in order to produce Acetyl-CoA groups for the TCA cycle. As a result they build up in the blood and can cause recurrent episodes of ketoacidosis[23]. Monocarboxylate transporters (MCT1) are responsible for the transport of ketones through the cell membrane. Loss of function or decreased expression in

MCT1 can therefore reduce uptake in organs to cause elevated circulating ketone bodies[24].

Due to the role of insulin in regulating ketone production and metabolism, ketones are a metabolite with strong implications in T2DM[20]. Diabetic ketoacidosis is a serious condition that arises in diabetics, when ketone production becomes too deregulated. It is characterized by blood glucose levels of more than 13.9mmol/L, serum ketone levels of more than 3.0 mmol/L and arterial pH of less than 7.3[25]. In a study of over 9,000 men; levels of ketone bodies (AcAc & BHB) were significantly increased ($p<0.01$) in those with diabetes compared to those with normal glucose control[26]. AcAc levels were increased by elevated in diabetics by 64% (95% CI, 16% to 109%), and BHB levels were increased by 99% (95% CI, 6% to 186%) compared with the reference group. After a five year follow up of over 4,000 of these men, elevated AcAc levels were associated with increased risk of incident T2DM in those with impaired fasting glucose (OR 1.49: 95%CI 1.12-1.99)[26]. Lower levels of adipocyte RNA expression of key enzymes in ketolysis, such as SCOT, were also found in those with diabetes and glucose tolerance issues[26].

Dysregulation of ketone levels in diabetics is a multi-factorial issue that stems from several deficiencies and pathologies caused by the condition within both the endocrine system and enzymatic proteins. The effects of T2DM on insulin resistance and β-cell function cause disparity in the glucagon/insulin ratio, which promotes ketogenesis and the diminished insulin secretion can also cause decreases in ketolysis[70]. Increased activity of free radicals of nitrogen and

oxygen species in diabetics can lead to non-enzymatic nitration of tyrosine residues of SCOT, which attenuate its function, as has been reported in diabetic mice models[72]. MCT1 expression is also lowered due to decreases in lactate production as a result of insulin resistance[73] as well as muscle inactivity[74] due to sedentary lifestyle.

Altered substrate utilization in myocardial metabolism is known to play a causative role in the development of CVD in those with diabetes[27, 28]. Ketone metabolism is one substrate associated with cardiovascular events. One characteristic of CVD, such as cardiomyopathy, is decreased utilization of fatty acid oxidation for energy production in the cardiac muscle tissue, leading to an increase in glucose utilization in the heart[28]. Increased glucose utilization results in increased rates of gluconeogenesis and therefore acetyl-CoA produced by fatty acid oxidation is broken down into ketone bodies rather than reacting with oxaloacetate to enter the TCA cycle[20]. A 2011 study[29] found that ketone body utilization in patients with heart failure is altered in a tissue specific manner. Skeletal muscle has a significantly lower uptake of ketones in heart failure patients than in healthy controls. Therefore, elevated levels of ketone bodies are often observed in patients with heart failure[29]. Further highlighting the importance of ketones in CVD, patients with recessive mutations of the OXCT1 gene that encodes the SCOT protein often present with dilated cardiomyopathy due to defects in ketone body metabolism[75].

Due to the role of ketones as a fuel source under certain conditions, exercise can affect plasma ketone levels acutely and alter them chronically

through both ketogenic inhibiting and ketolytic enhancing mechanisms. Since the rate of ketogenesis is increased as the ratio of glucagon to insulin increases, chronically, exercise works to inhibit the excess rates of ketogenesis by increasing insulin sensitivity in type 2 diabetics[30, 31]. Acute exercise promotes the ketolytic pathways to increase ketone body clearance. The relationship between plasma ketone concentrations and skeletal muscle oxidation of ketones is curvilinear with saturation kinetics. The contribution of ketones to skeletal muscle ATP production increases with elevating concentrations until it reaches saturation levels at approximately 1-2mmol/L and then declines as the concentrations increase further[76, 77].

While specific long term training induced changes in expression of ketolytic enzymes has not yet been described in humans, changes are observed in ketone body metabolism during and after exercise in trained and untrained individuals, such as the attenuation of post-exercise rises in ketone bodies[32]. However, these enzymatic pathways have been studied more in rodent models and suggest that intense aerobic exercise training results in increased expression of ketolytic enzymes SCOT, BDH, and ACAT[33]. It has been well established that MCT1 expression increases with training in an intensity dependent manner, to increase cells uptake of ketones[24, 78]. While research is lacking on the effects of resistance training on mechanisms of ketone body clearance, the resulting increased skeletal muscle mass would have a positive effect on insulin sensitivity[31]. This would decrease ketogenic activity via better regulation of the glucagon/insulin ratio of glucagon and inhibiting ketogenesis

promoting hormones[20]. Resistance training, like aerobic training, has also been shown to increase MCT1 expression in certain trials[79].

While positive effects of aerobic training on ketone metabolism have been documented, there are large gaps in current knowledge regarding the effects of resistance training and concurrent training on ketone metabolism. Therefore, further research is required to determine the potential benefits of exercise programs for ketone body metabolism. It is also not known how exercise induced changes in ketones correlate with changes in some other important concomitant cardiometabolic biomarkers related to T2DM, such as HbA1c, C-reactive protein, and lipid panel. There is no accepted working model as to the exact physiological mechanisms underlying enhanced ketone metabolism from exercise, and therefore the biological plausibility needs to be examined.

Exercise increases BCAA catabolism by increasing BCKDH activity while reducing BCKDH kinase activity. One proposed mechanism behind this effect is exercise-induced increases in adiponectin expression[80, 81] and the subsequent activation of downstream substrates. Adiponectin is a protein hormone produced in adipose tissue that has roles in regulating glucose levels, fatty acid breakdown[81] and has more recently been shown to be linked to BCAA catabolism[82]. One mechanism of action for adiponectin is activation of 5’ adenosine monophosphate-activated protein kinase (AMPK)[83]. Adiponectin, specifically through its downstream substrate AMPK works to activate PP2Cm. This causes an overall shift towards dephosphorylation of BCKDH[82].

Dephosphorylated BCKDH is free to catalyze the decarboxylation of BCKA[60, 82] (Figure 2.1).

Both aerobic training and resistance training have well established mechanisms behind physiological adaptions which improve insulin sensitivity to decrease ketone levels[56, 30, 31]. Ketogenesis becomes inhibited due to decreased release of ketogenic hormones; hormone-sensitive lipase, acetyl-CoA carboxylase, and mitochondrial HMG-CoA synthase[55]. However, the role of exercise on lowering ketones via mechanisms controlling ketolytic enzymes is less clear. MCT1, a plasma membrane transporter, catalyzes the proton-linked transport of monocarboxylates which includes not only ketones, but pyruvate and lactate, which are accumulated during exercise and transported in the cell to go through the TCA cycle to produce ATP[78, 84]. Lactate accumulation increases with increasing intensity of exercise[85], which causes an increase in MCT1 activity[78, 86]. MCT1 communicates with basigin (CD147), a cell surface glycoprotein, which facilitates MCT1 turnover. As the activation of these transporters increases during exercise, CD147 causes an acute increase in degradation of MCT1 through activation of matrix metalloproteinase-2/9 as well as directly increasing MCT1 transcription[87]. Increased CD147 activity results in upregulation and increased expression of MCT1 in heart and skeletal muscle which increases ketone body uptake into the cells for utilization in the TCA cycle (Figure 2.2).

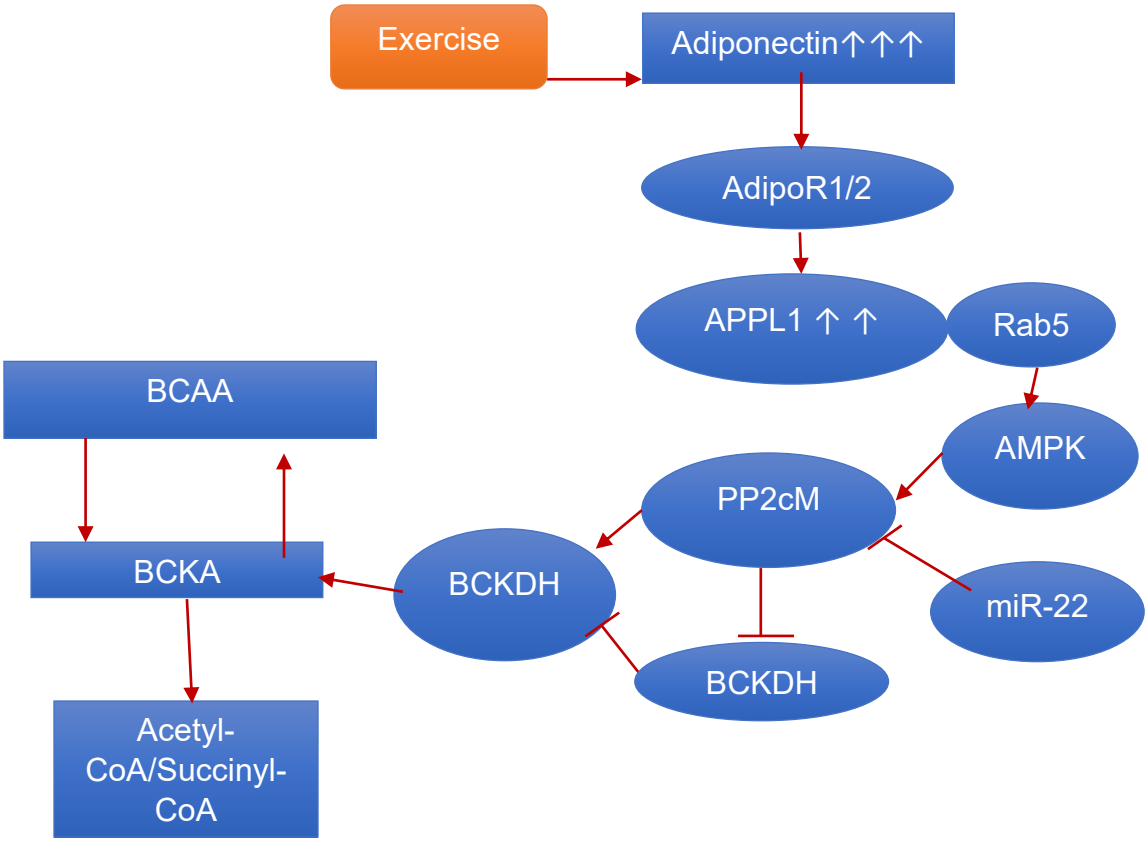

Figure 2.1 Hypothetical working model of the effect of exercise on BCAA metabolism.

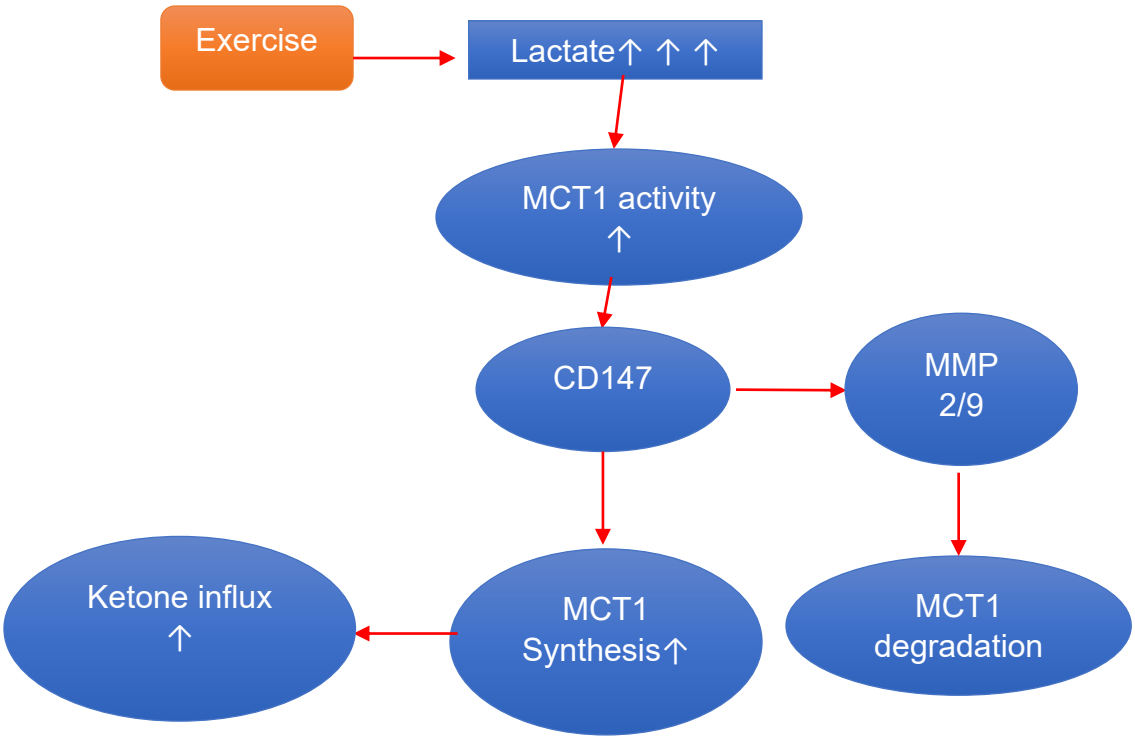

Figure 2.2 Hypothetical working model of the effect of exercise on ketone body metabolism.

CHAPTER 3

METHODOLOGY

Study Design and Participants:

The current study will be performed as a secondary analysis of the Health Benefits of Aerobic and Resistance Training in individuals with Type 2 Diabetes (HART-D) trial. The full design and methodology of the HART-D trial has been published previously[56]. Briefly, HART-D was a 9-month randomized, controlled exercise-training trial comparing the effects of different modalities of exercise training on HbA1c levels in sedentary participants with T2DM. A total of 262 participants were recruited from the greater Baton Rouge, Louisiana area. They were then randomized to one of four groups; a non-exercise control group, an aerobic training group (AT), a resistance training group (RT), or a combination of aerobic and resistance training group (ATRT). Exclusion criteria for the study included a BMI >48 kg/m^2, age <30 or >75 years, blood pressure ≥160/100 mmHg, fasting triglyceride levels ≥500 mg/dL, use of insulin pump, urine protein levels >100 mg/dL, history of stroke, and advanced neuropathy or retinopathy or any serious medical condition that prevented adherence to the study protocol or the ability to exercise safely. For the current study, participants (n=180) who completed greater than 70% of their prescribed exercise program and had complete data were included in the per-protocol analysis. consent was obtained from all participants prior to screening. All training sessions were performed

under staff supervision in an exercise training laboratory at Pennington Biomedical Research Center, Baton Rouge, Louisiana.

Exercise Interventions:

Participants in the AT group (n=44) exercised 3–5 days/week at an intensity of 50–80% of their VO_2 peak fitness for a total dose of 12 kcal/kg/week (KKW), which is estimated to be equivalent to the 150 minutes of physical activity per week recommended by the federal activity guidelines[88]. The caloric dose was adjusted on a weekly basis based on changes in body weight. American College of Sports Medicine equations were used to estimate caloric expenditure rates and, therefore, the time required per session[89].

The RT group (n=49) exercised 3 days/week, with each session consisting of two sets of four upper-body exercises (chest press, lateral pull-down, military press, and seated row), three sets of three lower-body exercises (leg press, leg extension, and hamstring curl), and two sets of abdominal and back exercises. Each set consisted of 10–12 repetitions. The prescribed weight was increased when the participant was able to complete 12 repetitions of a final set of each exercise on two consecutive sessions.

The ATRT group (n=54) had the same guidelines for performing their aerobic training but had a lower weekly dose of 10KKW. The resistance training for the ATRT group required two sessions per week, with each session consisting of one set of each of the aforementioned nine exercises. They also used a progressive increase in weight when 12 repetitions could be performed on

an exercise, as described above. The training regimen for the combination training group was consistent with federal physical activity guidelines[88] and ensured equal time commitment among all exercise groups. The non-exercise control group (n=33) was offered weekly stretching and relaxation classes and was asked to maintain their baseline activity levels during the 9-month study period.

BCAA and Ketone body measurement:

After a 10 hour fast, blood samples were obtained at baseline. Post-training blood sampling was performed 24-48 hours after completion of the final exercise session and after a 10 hour fast. Plasma ketone and BCAA levels were quantified by nuclear magnetic resonance spectroscopy (NMR) at LabCorp (Morrisville, NC) using an optimized version of NMR LipoProfile algorithm (LP4)[90]. The methyl groups of the 3 branched-chain amino acids (valine, leucine, isoleucine) give rise to characteristic signals in the ^{1}H NMR spectrum that enable their accurate quantification as validated by comparison with LC/MS/MS values[91]. The three ketone bodies (3-β-hydroxybutyrate, acetoacetate, acetone) give rise to resolved NMR signals that serve as the basis of their quantification[91].

Demographic and Cardiometabolic phenotypes measurement:

Weight was measured on a GSE 450 electronic scale (GSE Scale Systems, Novi, Michigan) and height was measured using a standard stadiometer. Lean mass and fat mass were measured by Dual x-ray

absorptiometry scans using the QDR 4500A whole-body scanner (Hologic Inc, Bedford, Massachusetts).

Diabetes status and duration was confirmed by medical history review. Diabetes medication type and dosage were assessed by detailed questionnaire with visual confirmation of prescription bottles. Participants were categorized as either increased, decreased, or no change in diabetes medications based on baseline and follow-up medication dosages. Race/ethnicity was obtained through written self-report.

HbA1c was assessed from a finger prick sample run on an automated glycosylated hemoglobin analyzer (DCA2000+, Bayer, Dublin, Ireland). Fasted blood samples from baseline and post-intervention clinic visits were used to measure glucose and insulin levels. Glucose levels were analyzed on a DXC 600 Pro (Beckman Coulter Inc, Brea, California). Insulin was measured using an immunoassay on the Siemens 2000 (Siemens, Deerfield, Illinois).

Exercise testing to determine VO_2peak was conducted on a treadmill (Trackmaster 425, Carefusion, Newton, Kansas), with respiratory gases sampled using a True Max 2400 Metabolic Measurement Cart (Parvomedics, Salt Lake City, Utah).

For all continuous variables, change in response to the exercise training program (Δ) was calculated by subtracting the baseline value from the post-training value.

Statistical Analysis:

Primary outcome analyses used the per-protocol principle and included only participants who completed greater than 70% of their prescribed exercise program (n=180).

Within-group exercise induced changes in levels of circulating BCAA and ketones were analyzed using a paired t-test. Exercise induced changes in levels of circulating BCAA (total, leucine, isoleucine, valine) and ketones (total, AcAc, BHB, acetone) were analyzed between intervention groups using generalized linear regression models adjusting for age, race, sex, Δfat mass, Δglucose, cholesterol and blood pressure medications, diabetes medication changes, and baseline trait value. If the main effect of intervention group showed a p-value <0.05 in the model, post-hoc analyses determined between-group differences in adjusted least squared means values across all pairwise comparisons (Aim 1).

As an exploratory analysis for aim 1, changes in total circulating BCAA levels were analyzed within intervention groups using unadjusted generalized linear regression models stratified by baseline total circulating BCAA levels. Participants were stratified as less than/equal to or greater than the proposed risk cut-off threshold of 450 μmol/L.

Pearson Correlation analysis was used to determine the association between exercise induced changes in circulating BCAA (total, leucine, isoleucine, valine) and ketone (total, AcAc, BHB, acetone) levels (independent variable) and changes in concomitant cardiometabolic biomarkers (HbA1c, fasting glucose,

fasting insulin, CRP, lean mass, and body fat %) (dependent variable). Due to the small sample sizes of the exercise intervention groups, all exercise groups were combined for correlation analyses (Aim 2).

All statistical analyses were performed using SAS version 9.4 (Cary, NC). $P<0.05$ was considered significant for all analyses.

CHAPTER 4

RESULTS

Baseline characteristics, and average changes from baseline to post-intervention, for participants by group including age, BMI, and other cardiometabolic risk factors are shown in Table 4.1. No significant differences were found between groups at baseline. Within exercise groups, ATRT experienced an increase of 27.0 μmol/L for total BCAAs, 8.10 μmol/L in leucine, and 6.69 μmol/L in isoleucine (all $p<0.05$) while no significant changes were found in the AT or RT groups (Table 4.2). Between groups, the adjusted exercise induced changes in total BCAA and leucine levels in the ATRT group were significantly increased compared to the control and AT groups, while changes in isoleucine levels in ATRT were significantly increased compared to other groups (Table 4.3).

Within exercise groups, RT exhibited a -33.0 μmol/L decrease in BHB ($p<0.05$) and was the only change in ketone bodies from the exercise program (Table 4.4). The only significantly different change between groups in ketone bodies was that the RT group had significantly larger decreases in BHB compared with the AT group. The decrease in the RT group was not significantly larger compared to the control group though (table 4.5).

There was a large amount of heterogeneity in intraindividual responses to the exercise program throughout all groups. There were large ranges in levels of all traits at baseline and the changes in response to exercise varied within groups. The SE of calculations for within-group changes in the exercise groups ranged from 19.2–21.5 μmol/L in total BCAA levels (Table 4.2) and 23.4-26.3 μmol/L in total ketone body levels (Table 4.4).

Exercise induced changes in the outcome traits in all exercise training groups combined were ($p<0.05$) correlated with changes in a few concomitant cardiometabolic biomarkers (Table 4.6). Exercise induced changes isoleucine were weakly, negatively correlated with the duration since diagnosis of T2DM and weakly, positively correlated with fasting blood glucose levels. Changes in total BCAA and valine levels were also moderately positively correlated with changes in glucose. Changes in leucine levels were weakly negatively correlated with change in VO_2 peak. Total ketone body, BHB, and Acetone levels were all weakly positively correlated with changes in HbA1c (Table 4.6).

When stratified by baseline total circulating BCAA levels, participants across all exercise groups above the threshold at baseline (n=47) experienced average decreases of 6.7 μmol/L, compared to 19.91 μmol/L increases experienced across all exercise groups in participants below the threshold (n=100) ($p<0.05$). Within exercise groups, those in the AT group above the threshold experienced an average decrease of 28.73 μmol/L compared to 3.90 μmol/L increases experienced by those above the threshold in the AT group (n=33) ($p<0.05$) (figure 4.1).

Table 4.1. Participant baseline characteristics.

Variable	Timepoint	Control, n=33	AT, n=44	RT, n=49	ATRT, n=54
Age		58.6 (1.3)	52.7 (1.1)	56.9 (1.0)	55.4 (1.0)
BMI (kg/m^2)	Baseline	34.8 (1.0)	34.7 (0.7)	34.1 (0.6)	35.8 (0.7)
	Change	0.2 (0.3)	-0.2 (0.2)	-0.2 (0.2)	-0.5 (0.2)
Body fat (%)	Baseline	38.8 (1.2)	37.0 (1.2)	37.6 (1.1)	38.1 (0.9)
	Change	0.2 (0.3)	-0.1 (0.3)	-1.2 (0.3)[a]	-1.1 (0.2)[a]
Fat mass (kg)	Baseline	37.9 (2.1)	34.7 (1.4)	37.2 (1.4)	38.2 (1.6)
	Change	0.2 (0.6)	-0.3 (0.4)	-1.5 (0.4)[a]	-1.7 (0.4)[a]
Lean mass (kg)	Baseline	56.0 (2.1)	56.5 (1.7)	58.7 (1.5)	58.6 (1.7)
	Change	0.1 (0.4)	-0.4 (0.3)	0.8 (0.3)[a]	0.0 (0.3)
VO_2peak (mL/kg/min)	Baseline	19.7 (0.7)	21.2 (0.8)	20.4 (0.7)	18.9 (0.5)
	Change	-0.3 (2.4)	0.5 (2.0)	0.3 (2.1)	1.3 (2.6)[a]
SBP (mmHg)	Baseline	127.1 (2.2)	124.5 (1.5)	124.2 (1.5)	129.4 (1.5)
	Change	1.9 (2.3)	-0.8 (2.0)	-0.9 (2.0)	-4.2 (1.9)[a]
DBP (mmHg)	Baseline	76.4 (1.3)	75.8 (1.1)	75.1 (1.0)	75.3 (18.9)
	Change	-3.8 (1.4)[a]	-0.2 (1.4)	-0.1 (1.3)	-0.2 (1.2)
Insulin (pmol/L)	Baseline	17.7 (2.3)	18.5 (2.0)	20.3 (1.7)	16.9 (6.3)
	Change	-1.0 (2.2)	-1.7 (1.2)	-4.5 (1.7)[a]	-0.8 (1.1)
HbA1c (%)	Baseline	7.9 (1.3)	7.6 (1.0)	7.6 (0.9)	7.6 (1.0)
	Change	0.1 (0.2)	-0.1 (0.2)	-0.2 (0.1)	-0.3 (0.1)[a]
Glucose (mg/dL)	Baseline	158.4 (6.4)	146.4 (3.6)	153.8 (4.6)	148.8 (4.1)
	Change	4.6 (8.8)	11.2 (6.5)	0.9 (5.9)	2.9 (4.1)

*All values expressed as means (standard error).
[a]$p<0.05$ for difference between post-training and baseline value from paired t-test

Table 4.2. Within-group changes in BCAA traits.

Intervention Group	N	Baseline Mean	Baseline SD	Follow-up Mean	Follow-up SD	Within-group changes Mean*	Within-group changes SE
Total BCAA (µmol/L)							
Control	33	402.5	66.4	397.9	67.5	-13.8	21.6
AT	44	407.5	68.5	403.3	67.8	-15.8	21.5
RT	49	419.3	86.4	426.8	80.3	3.2	21.1
ATRT	54	421.3	69.6	448.9[a]	103.4	27.0	19.2
Valine (µmol/L)							
Control	33	224.7	40.2	222.8	35.1	-3.5	11.2
AT	44	226.0	33.4	227.1	33.9	-5.1	11.1
RT	49	235.3	42.4	236.1	42.1	1.2	10.9
ATRT	54	238.1	36.2	248.1	51.6	11.1	9.9
Leucine (µmol/L)							
Control	33	120.2	21.1	122.6	26.1	-5.1	8.9
AT	44	123.4	28.5	118.2	30.7	-9.4	8.8
RT	49	122.4	35.2	129.7	31.2	1.7	8.7
ATRT	54	123.1	28.0	133.9[a]	37.2	8.1	7.9
Isoleucine (µmol/L)							
Control	33	57.7	14.7	55.3	14.1	-4.3	5.0
AT	44	58.2	15.5	58.0	15.7	-2.5	4.9
RT	49	61.5	17.1	61.1	16.5	-1.0	4.9
ATRT	54	60.0	14.4	66.9[a]	22.6	6.7	4.4

*Values adjusted for age, sex, race, change in fat mass and glucose, cholesterol and blood pressure medication status, change in diabetes medication, and baseline trait value.

[a]$p<0.05$ for difference between post-training and baseline value from paired t-test

Table 4.3. Between-groups comparison in BCAA traits.

Intervention Group	N	Between-group: comparison vs Control group changes Mean*	95% CI	pairwise p-value
Total BCAA (µmol/L)				
AT	44	-1.9	(-31.2 to 27.3)	0.90
RT	49	17.0	(-11.1 to 45.1)	0.24
ATRT	54	40.8[a]	(12.2 to 69.4)	0.005
Valine (µmol/L)				
AT	44	-1.6	(-16.7 to 13.6)	0.84
RT	49	4.7	(-9.8 to 19.3)	0.53
ATRT	54	14.6	(-0.26 to 29.5)	0.054
Leucine (µmol/L)				
AT	44	-4.4	(-16.5 to 7.7)	0.48
RT	49	6.7	(-5.0 to 18.4)	0.26
ATRT	54	13.1[a]	(1.2 to 25.0)	0.03
Isoleucine (µmol/L)				
AT	44	1.8	(-4.9 to 8.5)	0.60
RT	49	3.3	(-3.2 to 9.8)	0.32
ATRT	54	11.0[b]	(4.4 to 17.5)	0.001

*Values adjusted for age, sex, race, change in fat mass and glucose, cholesterol and blood pressure medication status, change in diabetes medication, and baseline trait value

[a]$p<0.05$ for difference compared to AT group, [b]$p<0.05$ for difference compared to all other groups

Table 4.4. Within-group changes in ketone bodies traits.

Intervention Group	N	Baseline Mean	Baseline SD	Follow-up Mean	Follow-up SD	Within-group changes Mean*	Within-group changes SE
Total Ketone Bodies (μmol/L)							
Control	33	156.1	51.1	181.3	64.3	-15.7	26.4
AT	44	164.9	65.3	189.4	116.5	-8.3	26.3
RT	49	190.2	95.7	170.2	59.8	-49.3	25.8
ATRT	54	185.7	69.4	172.8	76.8	-30.5	23.4
Betahydroxybutyrate (BHB) (μmol/L)							
Control	33	91.4	30.5	103.1	37.1	-15.4	16.4
AT	44	97.3	41.7	113.4	76.4	-3.4	16.3
RT	49	106.5	59.8	96.5[a]	37.8	-33.0	16.0
ATRT	54	105.4	42.5	99.8	43.3	-20.5	14.5
Acetoacetate (AcAc) (μmol/L)							
Control	33	41.1	18.2	47.9	22.5	-0.6	8.2
AT	44	42.5	19.1	48.0	29.8	-2.7	8.2
RT	49	53.3	27.3	45.9	21.4	-10.8	8.0
ATRT	54	49.8	21.3	47.1	29.8	-4.7	7.3
Acetone (μmol/L)							
Control	33	23.6	10.7	30.3	15.4	0.1	4.5
AT	44	25.1	13.2	28.0	15.9	-2.5	4.4
RT	49	30.4	15.7	27.8	10.7	-5.3	4.4
ATRT	54	30.4	13.9	25.9	12.3	-5.0	4.0

*Values adjusted for age, sex, race, change in fat mass and glucose, cholesterol and blood pressure medication status, change in diabetes medication, and baseline trait value.

[a]$p<0.05$ difference between post-training and baseline value from paired t-test

Table 4.5. Between-group changes in ketone bodies traits.

Intervention Group	N	Between-group: comparison vs Control group changes Mean *	95% CI	pairwise p-value
Total Ketone Bodies (µmol/L)				
AT	44	7.4	(-28.4 to 43.2)	0.68
RT	49	-33.6	(-68.3 to 1.2)	0.06
ATRT	54	-14.8	(-50.1 to 20.6)	0.410
Betahydroxybutyrate (BHB) (µmol/L)				
AT	44	12.0	(-10.2 to 34.3)	0.29
RT	49	-17.5[a]	(-39.0 to 4.0)	0.11
ATRT	54	-5.1	(-26.9 to 16.8)	0.65
Acetoacetate (AcAc) (µmol/L)				
AT	44	-2.1	(-13.3 to 9.0)	0.71
RT	49	-10.2	(-21.1 to 0.6)	0.06
ATRT	54	-4.2	(-15.1 to 6.8)	0.46
Acetone (µmol/L)				
AT	44	-2.6	(-8.7 to 3.4)	0.39
RT	49	-5.4	(-11.3 to 0.5)	0.07
ATRT	54	-5.1	(-11.1 to 0.9)	0.09

*Values adjusted for age, sex, race, change in fat mass and glucose, cholesterol and blood pressure medication status, change in diabetes medication, and baseline trait value.

[a]$p<0.05$ for difference compared to AT group.

Table 4.6. Correlation between BCAA and ketone body traits and concomitant cardiometabolic biomarkers

	T2DD	bf%	lean mass	CRP	HbA1c	insulin	glucose	VO_2 peak
BCAA	NS	NS	NS	NS	NS	NS	**0.30**	NS
Val	NS	NS	NS	NS	NS	NS	**0.36**	NS
Leu	NS	NS	NS	NS	NS	NS	NS	**-0.18**
Ileu	**-0.17**	NS	NS	NS	NS	NS	**0.20**	NS
KetBod	NS	NS	NS	NS	**0.20**	NS	NS	NS
BHB	NS	NS	NS	NS	**0.21**	NS	NS	NS
AcAc	NS	**0.17**	NS	NS	NS	NS	NS	NS
Acetone	NS	NS	NS	NS	**0.17**	NS	NS	NS

Values in bold indicate significant correlations. All correlations listed were significant at $p<0.05$. NS, not significant ($p>0.05$). T2DD: duration since T2DM diagnosis, bf%: body fat %, CRP: C-reactive protein.

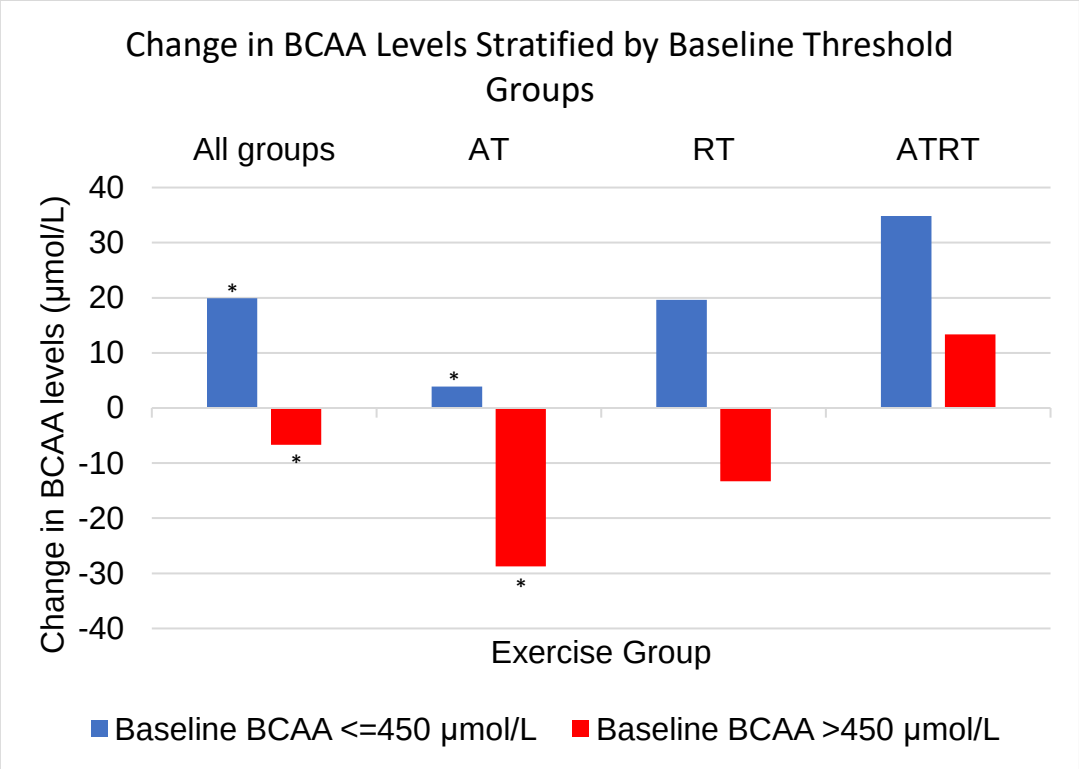

Figure 4.1. Change in BCAA levels stratified by baseline threshold groups.

CHAPTER 5

DISCUSSION

Our findings do not support our hypothesis that all exercise modalities would result in significant decreases in BCAA and ketone body levels, as no exercise groups showed a significant decrease in any outcome trait when compared with the control group. In fact, we found significant exercise induced increases in measures of isoleucine in the ATRT group compared to controls. Exercise induced changes over the nine-month period in some of the BCAA and ketone bodies also showed some weak and moderate correlations with concomitant cardiometabolic biomarkers such as bf%, glucose, HbA1c, and VO_2 peak. To our knowledge this is the first large scale randomized exercise control trial that has analyzed the changes in circulating BCAA and ketone body levels in response to different long-term exercise training modalities in type 2 diabetics.

While the existing body of literature suggests that aerobic training improves both BCAA and ketone body metabolism, this studies results did not find the aerobic, resistance, or combination training provided any significant benefits (i.e., decreased levels) for circulating BCAA or ketone body metabolism in those with T2DM. The RT group experienced a significantly larger decrease in BHB levels compared to the AT group, however this change was not different

than the control group. Moreover, the AT group experienced decreased levels compared to the AT and ATRT groups, but not different compared to the control.

The baseline levels of participants in this study for both circulating BCAA and Ketone body levels are consistent with the elevated levels expected for type 2 diabetics from previous research. The average total circulating ketone bodies within exercise groups in this study ranged from 156 μmol/L to 190 μmol/L compared to 182 μmol/L that was found to be the average level in a cohort of 373 subjects with T2DM in the Insulin Resistance Atherosclerosis Study (IRAS) cohort[91]. As expected, these levels are elevated compared to the average of non-diabetic subjects from the IRAS cohort, who had average circulating ketone body levels of 142 μmol/L[91]. Diabetics from the IRAS cohort had average levels of circulating BCAAs of 393 μmol/L, which was significantly elevated compared to the average of the non-diabetic cohorts (337 μmol/L)[5]. The average circulating levels of BCAAs in the HART-D cohort within groups range from 403 μmol/L to 421 μmol/ which is similar to the elevated levels that were found in the IRAS cohort.

While increased circulating BCAA levels are associated with increased risk for CVD and associated metabolic risk factors, there is some evidence that there may be a cut-off threshold that exists where increased BCAAs become a risk biomarker. Sun et al.[92] calculated this threshold concentration to be approximately 450 μmol/L in a longitudinal study of over 600 people. Results from a 2019 study support this proposed cut-off threshold. They found that for

males, compared to their reference group (Total BCAA concentration <361.9 μmol/L), only those in the upper quartile of their cohort (>448 μmol/L) were at a significantly increased risk of incident hypertension (Hazard Ratio 1.36: 95% CI 1.11-1.68) after an 8 year follow up[93]. Despite their diabetic status, the average BCAA concentration of participants in this study ranged was below that threshold at baseline. When stratified into those that started above (n=47) or below (n=100) this threshold from the exercise groups as an exploratory analysis, we did find that those above decreased compared to those below across all exercise groups. Those with baseline levels >450 μmol/L had an average change of -6.7 μmol/L and those with baseline levels >450 μmol/L had an average change of 19.9 μmol/L. When examined by exercise group, those above the baseline threshold in the AT group showed decreases in total BCAA levels compared to those above the threshold at baseline. The RT group decreased but not significantly different to the increases that were seen in those above the baseline, while the ATRT group still exhibited increases.

Despite a body of literature suggesting that aerobic exercise increases ketone body clearance acutely[76-78], there were no decreases shown from any exercise group. The RT group did experience decreases in BHB compared to AT. As muscle mass is a key regulator in the regulation of glucagon and insulin ratios[31], an increase in lean mass mediated by resistance training may be a potential mechanism behind RT experiencing larger decreases than AT.

Given the unhealthy metabolic status and age of the individual participants the possibility of exercise resistance also may have played a factor. A notable

quantity of non-responders to exercise in diabetic and obese individuals has been observed for several traits including glucose and insulin sensitivity measures[94]. Aging is also known to lead to anabolic resistance and non-response to exercise[95]. We found large heterogeneity in the exercise responses across all eight traits, regardless of exercise modality or adherence. This heterogeneity may be explained by a combination of numerous factors, such as genetic and epigenetic factors, differing clinical profiles of individuals (despite being similar at baseline), and other as of yet unknown factors.

Exercise may also just be a mediating factor for regulating concentrations of these metabolites. Individuals who are physically fit (higher VO2max) and have higher lean mass and lower fat mass have lower circulating BCAA levels compared to obese individuals[63]. The relationship between fitness and BCAA was minimally found in this study with a weak negative correlation between leucine concentration and VO_2 peak.

The relationship between ketone bodies and HbA1c levels is not well established. Different associations have been shown between HbA1c and AcAc and between HbA1c and BHB, between prediabetics and diabetics, and even between sexes and different races[96]. Zhang et al[96] found that increasing concentrations of HbA1c were associated with decreasing concentrations of acetoacetate in those with European background (regression coefficient in males=−0.13: 95% CI −0.24 to −0.004, females −0.17: 95% CI −0.30 to −0.05), but were associated with increasing concentrations of acetoacetate in African Surinamese men (0.09: 95% CI 0.02–0.17) as well as subjects with Ghanaian

background (males 0.13: 95% CI 0.05–0.20, females 0.08: 95% CI 0.01–0.154). Total ketone bodies, BHB, and acetone showed a weak positive correlation with HbA1c in this study, while AcAc showed no association, further suggesting that while circulating ketone body concentrations and HbA1c may be associated, the relationship is quite contentious. No mediating factor between the two biomarkers is known.

Increase in BCAAs, especially isoleucine, has been shown to correlate in increase with fasting glucose in both Caucasians (OR 1.021: 1.006-1.030), and African Americans (OR 1.021: 1.006-1.038) without impaired fasting glucose[96]. The correlation becomes even stronger in those with impaired fasting glucose, Caucasians (OR 1.026: 1.015-1.037) and African-Americans (OR 1.034: 1.019-1.050)[96]. Our results further support these findings although total circulating levels of total BCAAs and valine were found to have a stronger correlation than isoleucine.

Strengths of the HART-D study include that this is a large, randomized control trial using a diverse population in age, sex, ethnicity, medication use, and comorbidities leading to generalizable findings. All exercise sessions were tightly controlled and completed in a laboratory and were monitored by exercise training professionals. However, these ideal training conditions also represent a limitation in terms of dissemination. A food frequency questionnaire was administered at baseline and follow-up to assess changes in diet which limits the ability to identify changes in caloric intake.

Although the findings from this study do not fully support our hypothesis there is some evidence provided that different exercise modalities can have different impacts in management of circulating BCAA and ketone body levels in individuals with T2DM. Associations with other important cardiometabolic biomarkers in diabetes such as HbA1c and fasting blood glucose levels also further support the notion that ketone bodies and BCAAs are clinically significant metabolites in the treatment and management of T2DM. As this was the first large scale study looking at the association of different exercise modalities with circulating BCAA and ketone body levels in diabetics more research is needed to establish a better understanding of how exercise effects the concentrations of these metabolites and the mechanisms that mediate these changes.

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