Summer 8-8-2017

# Branched Chain Amino Acids: Causal or Predictive of Type 2 Diabetes

Jency George

ABSTRACT

Branched Chain Amino Acids: Causal or Predictive of Type 2 Diabetes

By

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6/13/2017

Branched Chain Amino Acids (BCAA) have been associated with insulin resistance and type 2 diabetes. However, there is not concrete understanding on whether BCAA is playing a causal factor or a predictive factor for type 2 diabetes. The aim of this review is to understand how Branched Chain Amino Acids act as a predictive biomarker of type 2 diabetes (T2D) or causal factor in insulin resistance. Two mechanisms have been proposed, but not enough studies have been done to prove which is true. There is still a lot of unknowns in BCAA mechanisms and how it affects metabolic disorders. Future study recommendations have been made to further understand BCAA pertaining to T2D and other metabolic disorders. After reviewing current studies, there is not enough evidence to confirm whether BCAAs are causative or predictive of T2D.

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Causal or Predictive
Of
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B.A., Augusta University

(List other degrees awarded in the same format)

A Capstone Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the
Requirements for the Degree

MASTER OF PUBLIC HEALTH

ATLANTA, GEORGIA
30303

APPROVAL PAGE

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Acknowledgments

Special thank you to Vijay Sekaran, Ph.D.

Author's Statement Page

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TABLE OF CONTENTS

List of Tables

**Chapter 1:** Background

*1.1 Branched Chain Amino Acids*

Branched Chain Amino Acids (BCAAs), specifically Leucine, Isoleucine, and Valine, are essential amino acids that are required for normal functioning. They are the building blocks for proteins, and therefore, are important in tissue expansion and regeneration as well as having metabolic functions. Recently, BCAAs have been used in performance enhancements for exercise. BCAAs also increase the secretion of insulin (1,2). An increase in circulating BCAAs has been associated with insulin resistance and type 2 diabetes (1,2). BCAAs are potentially a biomarker, rather than a causative factor of insulin resistance because insulin resistance increases the appearance rate of BCAAs and is linked to the reduced expression of mitochondrial BCAA catabolic enzymes (1,3). The aim of this review is to understand how Branched Chain Amino Acids act as a predictive biomarker of type 2 diabetes (T2D) or causal factor in insulin resistance.

It is known that the build-up of BCAA by-products can affect insulin sensitivity, but do the by-products blunt other metabolic pathways and potentially cause other metabolic disorders? Acylcarnitines are the connecting factor between BCAA and insulin resistance. Acetyl-CoA and propionyl-CoA, which are important components of the TCA cycle, which also creates by-products like C3-acylcarnitine and C5-acylcarnitines (4). C3 and C5 have been found to be involved in insulin administration. Acylcarnitines have only been studied regarding insulin resistance, and not many studies have determined whether they play a role in other metabolic disorders. It has been suggested that fatty acid oxidation, which yields energy, maybe be a

factor that leads to the accumulation of acylcarnitines via the TCA cycle (4). The metabolic by-products create more controversy regarding whether BCAAs are a causal factor with respect to T2D and insulin resistance.

*1.2 Diabetes*

Diabetes is a metabolic disorder with multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism that result from defects in insulin secretion, activity or both. Diabetes is the most common endocrine problem worldwide, with the incidence rate growing at an alarming rate and causing it to be the seventh leading cause of death. Roughly 90% of individuals with diabetes in the United States have type 2 diabetes. Many complications coincide with diabetes, such as high blood pressure, cardiovascular disease, stroke, and blindness (5). The CDC (6) states that 29.1 million people currently have diabetes across all age groups, including 21.0 million individuals who have been diagnosed with diabetes and 8.1 million people are undiagnosed. The National Health and Nutrition Examination Survey (NHANES) has permitted the ability to gather percentages of diabetic people in the United States. By collecting both fasting glucose and hemoglobin A1C levels, the data from the survey allow estimates of the percentage of undiagnosed diabetics and prediabetic individuals. In a 2014 report by the CDC, American Indians/Alaskan Natives had the highest age-adjusted percentage of people 20 years or older with diagnosed diabetes at 15.9%, while Non-Hispanic blacks were the second highest with 13.2%. Obesity has been observed as a contributing factor to diabetes. Approximately 85.2% of overweight adults older than 20 have been previously diagnosed with diabetes, and 54.8% of obese adults are diabetic (5, 6).

Individuals with type 2 diabetes manage their glucose levels by following a healthy meal plan, participating in the exercise along with weight loss, as well as taking medications. Insulin is commonly used to control glucose levels in those who have type 2 diabetes (5,6). Insulin is a hormone that is secreted when circulating glucose levels rise from the digestion of carbohydrates. Insulin triggers the absorption of glucose from the blood stream into cells. Individuals become insulin-resistant when tissues absorbing glucose do not properly respond to secreted insulin, and thus, blood glucose levels become abnormally high (7). Insulin resistance is a physiological condition in which target organs fail to respond to the insulin effectively. Issues in insulin response impair the use of these target organs leading to the development of type 2 diabetes (8). Insulin resistance that is not controlled by individuals that have diabetes mellitus can results in muscle protein degradation, though this can be improved upon treatment with insulin.

*1.3 Insulin Signaling Pathway*

Many factors coincide with obesity and play a role in the development of type 2 diabetes (7). Adipokines are peptide hormones or cytokines that are produced and secreted by adipocytes, which play a part in the development of insulin resistance, and have the capacity to store excess lipids that become saturated during obesity, leading to the abnormal redistribution of lipids to organs and tissues. Adipose tissue exhibits insulin resistance through the cytokine-related molecule leptin. Further studies have also revealed that adipose cells produce adiponectin, retinol-binding protein-4, resistin, and proinflammatory cytokines. Leptin and adiponectin decrease triglyceride synthesis, which stimulates β-oxidation and increases insulin action in skeletal muscle and the liver. Inflammatory mediators may also lead to insulin

resistance (7). Insulin resistance is partly caused by changes in hormone and cytokine production by the liver, adipose tissue and immune cells in response to the chronic exposure to lipids (7, 8).

When insulin binds to the insulin receptor, it leads to autophosphorylation of the insulin receptor and promotes the binding of scaffold proteins, including insulin receptor substrate proteins, Src-homology-2- containing protein and the c-Cbl proto-oncogene (7). Phosphorylation of the scaffolding proteins engages various pathways in insulin signaling. The insulin receptor substrate 1 is the key mediator of insulin-stimulated glucose uptake and activation of anabolic pathways in muscle and adipose tissue. This insulin activity promotes glucose uptake and storage but can be attenuated by other regulators (7,8,9). Pathways initiation can be reversed by the protein Tyr phosphatase-1B (PTB1B) and insulin receptor Tyr kinase activity (7).

There are possible fundamental changes in metabolism that occur in response to over-nutrition. For example, lipid-derived metabolites accumulate outside the adipose depots in response to a high-fat diet (7,9,10). The accumulation of the metabolites leads to metabolic overload that causes a potential loss of insulin sensitivity. In the liver, chronic increases in malonyl coenzyme A levels promote *de novo* fatty synthesis and inhibit carnitine palmitoyltransferas-1 (CPT1) activity. Long chain-CoAs are diverted away from mitochondrial oxidation towards biosynthetic enzymes that produce triglycerides and signaling intermediates. Over-nutrition imposes an anabolic burden on the endoplasmic reticulum, which causes protein misfolding and activation of inositol-requiring kinase-1 (IRE1). These adverse events converge

on stress-induced Ser kinases that impede insulin-mediated suppression of gluconeogenesis while allowing lipid synthesis and restricting β-oxidation (7).

In skeletal muscle, over-nutrition stimulates fatty acid influx, and PPARα/δ-mediated activation of target genes promote β -oxidation without a coordinated increase in TCA cycle flux. This results in incomplete fat oxidation metabolic by-products, such as acyl carnitines, which are BCAA-derived, and ROS, which accumulates in the mitochondria. These by-products might activate Ser kinases that impede insulin signaling and glucose transporter-4 (GLUT4) translocation (7,10). Exercise has been shown to reduce lipid stress by increasing TCA cycle flux by coupling the ligand-induced PPARα/δ activity with PPARyco-activator-1α (PGC1α)-mediated remodeling of downstream metabolic pathways (7).

Obesity can also lead to insulin resistance (1,5,7,9); however, not all obesity-related insulin resistance progresses to type 2 diabetes. β-cell failure involves a decrease in β-cell mass and deterioration of glucose-stimulated insulin secretion (GSIS) (7). Insulin secretion from islet cells is stimulated by glucose metabolism, which leads to an increase in the ATP: ADP ratio and closure of the ATP-sensitive $K^+$ channel-dependent mechanism. This activates voltage-gated $Ca^{2+}$ channels, which creates an influx of $Ca^{2+}$ and stimulates insulin granule exocytosis. The failure of β-cells in type 2 diabetes could be caused by mitochondrial metabolic overload, endoplasmic reticulum stress and the deposition of harmful amyloid fibrils (7,9). As stated before, over-nutrition induces an increase in the lipid supply, resulting in the induction β-oxidation enzymes such as CPT1 (7,9,10). This increases acetyl-CoA levels, allosteric activation of pyruvate carboxylase and upregulation of pyruvate cycling, which ultimately blunts GSIS. Pyruvate cycling involves the entry of pyruvate or other substrates into the tricarboxylic acid

cycle to generate excess intermediates that exit the mitochondria and engage in different cytosolic pathways that eventually lead back to pyruvate. This increases the demand for insulin biosynthesis, which results in increased ER stress and protein misfolding (7,11).

*1.4 BCAA Metabolic Pathway and Mechanisms*

The BCAA metabolic pathway crosses with the mechanism for insulin resistance. The first step in BCAA metabolism takes place in the peripheral tissue, except the liver, and is catalyzed by the mitochondrial isoform of branched-chain-amino-acid transaminase (BCAT(m)), which is encoded by the BCAT2 gene. The deletion of the BCAT2 gene prevents BCAA metabolites from forming in peripheral tissues. When BCAT2 is present, BCAAs are converted into branched-chain α-ketoacids via the removal of the amino groups. Next, the α-ketoacids are decarboxylated by branched-chain α-ketoacid dehydrogenase (BCKD). Finally, the BCAA metabolites are catabolized by a series of enzyme reactions to their final products, acetyl-CoA, and succinyl-CoA, which enter the TCA cycle (2,3,11,12).

There are two hypothesized mechanisms that play a role in insulin resistance and type 2 diabetes. The first mechanism is activated by elevated BCAA levels resulting in insulin resistance in the phosphorylation of insulin receptor substrate 1. mTORC1 is a serine/threonine kinase that regulates important cellular and developmental processes (2,3,12,13). mTORC1 promotes insulin resistance through serine phosphorylation of insulin receptor substrates (IRS)-1 and IRS-2, which increases inflammation and the demand for insulin due to the impaired insulin activity. The rate of protein degradation in muscle can be inhibited by insulin and insulin-like growth factor 1 (2,3,12,13). Though there is evidence that supports this mechanism, it is still a

controversial mechanism because exercise and BCAA supplementation have been shown to improve metabolism, even when mTORC1 is activated (2,3).

The second proposed mechanism is known as BCAA dysmetabolism. Impairments in BCAA metabolism can induce the accumulation of toxic intermediates that impair cellular function(s) (3). This model suggests that the accumulation of toxic intermediates, such as increases in acylcarnitines and acetyl-CoA, promotes β-cell mitochondrial dysfunction and eventually leads to β-cell apoptosis as well as type 2 diabetes, which is observed with elevated BCAA levels (3,14). Altered gene expression can be caused by either mutations or epigenetic regulation. Tiffen et al. identified BCKDH4, a gene that encodes the regulatory subunit in BCKDC. BCKDC activity is impeded by long-chain fatty acids and their metabolites by directly affecting the redox state or the concentration of acetyl-CoA or indirectly by inhibiting enzyme carbonylation (2,3).

**Chapter 2**: What we know about Insulin Resistance and BCAAs

Elevated BCAA levels have been shown to improve metabolic parameters, including body composition, glycaemia levels, and satiety. For many years, BCAAs have frequently been used by body builders as performance-enhancing supplements (2,11). With insulin, BCAA functions as an anabolic signal to alter the growth of energy-consuming tissues. Oral supplementation of leucine has been shown to alter protein synthesis in rats; however, when given a carbohydrate meal, there were no changes in protein synthesis. The supplementation prompted an increase in insulin, suggesting that leucine is a direct nutrient signal to induce protein synthesis (2,3,11).

Acute exercise has also been shown to have positive effects on metabolism by activating the BCKD complex in skeletal muscle, whereas under a regular diet, there was no increase in enzyme activity (11). Both BCAA supplementation and BCAA-rich diets support the dietary recommendation for building proteins and have been shown to increase BCAA levels (2,3,11).

Though BCAAs have been shown to promote positive effects, there is still controversy regarding whether elevated BCAA levels can lead to insulin resistance and type 2 diabetes (T2D). Individuals with obesity and T2D are often characterized as having increased BCAA levels. For example, the study by Fiehn et al. supports the association between a BCAA signature and insulin resistance when leucine and valine were increased in African American women with T2D. Moreover, longitudinal studies have shown that BCAA levels in the blood can predict the likelihood of developing insulin resistance or T2D (2). Table 1 provides a summary of current studies on BCAA, obesity, and T2D.

*2.1 Current Studies*

Newgard et al. discuss the potential cause-effect relationships between BCAAs and metabolic diseases, as there are strong associations between BCAAs and metabolites that play a role in T2D. When there is an influx of BCAAs, there is a simultaneous increase in BCAA catabolism, which enhances the production of propionyl-CoA and succinyl-CoA. This results in possible contributions to the incomplete oxidation of fatty acids. Another possibility that is that a high-fat diet could cause the substrates involved in BCAA metabolism to overflow the TCA cycle; this would contribute to the accumulation of incompletely oxidized fatty acid and BCAA oxidation intermediates, which could contribute to insulin resistance. Newgard continues to discuss that BCAA levels and the increase in insulin resistance are not completely understood;

however, there are several possibilities to explain what might cause their correlation. The first possibility is through increased protein consumption, but studies that were previously discussed suggest that the relationship between BCAAs with insulin resistance and the risk of T2D are not influenced by protein consumption. A second possibility is genetic variations in the expression of BCAA enzyme genes that control protein synthesis. Lastly, they hypothesized that BCAAs synergize with hyperlipidemia to contribute to developing insulin resistance. Readily available glucose and lipid substrates in obesity may decrease the need for amino acid catabolism in adipose tissues; however, the mechanism of the increased supply of substrates, which causes BCAA catabolism enzyme down-regulation, is not well understood (8).

Lackey et al. conducted a study on the white adipose tissue (WAT), which is considered a player in BCAA metabolism in their study. They tested whether BCKD expression is reduced in obese WAT and regulated by metabolic signals. The results found that BCKD expression was reduced 35-50% in obesity models and that BCKD component transcripts were significantly lower in subcutaneous adipocytes compared to lean Pima Indians. Perturbated metabolic signals in WAT, which are observed in insulin resistance and T2D, could put stress on WAT BCAA utilization. Though there was a reduced expression of the BCKD complex and other BCAA catabolism factors in the obese and insulin-resistant individuals, there is not enough evidence to equate to these factors as promoting lower BCAA utilization and catabolism in adipose tissue. However, Lackey et al. conclude that obesity is less important than WAT metabolic regulators in BCAA catabolism (15).

Excess BCAAs can promote the development of β-cell dysfunction. Leucine has been found to be a principle component associated with insulin resistance by causing a mutation that leads to hyperinsulinism. Excess BCAA levels cause a constant secretory pressure on b-cells that can contribute to b-cell dysfunction (17,18). Newgard et al. conducted a metabolic profiling study in 2009 in obese vs. lean humans to discover whether there is a correlation between increased BCAA catabolism and insulin resistance. The results showed that insulin resistance is induced by high-fat BCAA feeding and associated with chronic mTOR and IRS1 phosphorylation and the accumulation of multiple acylcarnitines. In contrast, insulin resistance was reversed by the mTOR inhibitor rapamycin. Therefore, they concluded that BCAAs are associated with the development of obesity and associated insulin resistance in individuals with poor diet patterns that include high-fat consumption (9).

An interventional study tested whether manipulation of dietary BCAA levels alters fasting BCAA levels independent of other factors. In this study, five healthy males were given a low BCAA diet for four days followed by a high BCAA diet for four days after a two week period. The study found that fasting valine was significantly lower ($p=0.02$) and the fasting isoleucine and leucine were lower in the low BCAA diet compared with the high BCAA diet. The study found that short-term manipulation of BCAA intake leads to a modest change in fasting BCAA levels (16).

Lotta et al. studied the genetic predisposition of BCAAs and the risk of T2D in a Mendelian randomization analysis; this study examined whether the association is causal by studying human genetics and metabolomic pathways in muscle biopsies. The study found that participants that genetically predisposition to insulin resistance was associated with higher

plasma BCAA levels, which potentially mediate the insulin resistance mechanism. The link between insulin resistance and higher BCAA levels is possibly mediated by BCKD activation; this has also been suggested by other studies (Ref). Other studies have stated that BCAAs are not a causal factor for insulin resistance, rather a biomarker that can lead to insulin resistance or T2D (17).

Obesity and T2D are drastically increasing in children in the United States, with nearly one-third of children being either overweight or obese. SEARCH for diabetes in youth is a national multicenter study that has aimed to understand more about diabetes among children and young adults in the United States. The study began in 2000 and is following children until 2020, with funding from the CDC and NIH. To date, they have found that there are 1.82 cases of diabetes per 1000 youth, with an average of 56 months of diabetes. Hispanic and Asian Pacific Islanders have the highest prevalence of diabetes compared to other races (5,18,19). There have been internet-delivered interventions that help families receive education about treatment and prevention options to reduce the risk of obesity and diabetes. With regards to BCAAs, most of the research conducted is performed on middle-aged populations and not in younger adult populations (18,19).

Another interventional study suggests that there are predictors for insulin resistance and suggestions for treatment. Shah et al. conducted a study in 2011 in the United States to identify biomarkers that predict changes in insulin resistance in regards weight loss. The study population was the weight loss management (WLM) clinical trial that aimed at determining the effects of two different weight loss interventions compared to a control group. Phase 1

included 1,685 participants who had behavioral interventions for six months, followed by the collection of fasting blood samples. The participants who lost over 4 kg were randomized into phase 2 of the study, which only included 500 participants. BCAA levels were measured via mass spectrometry, and insulin resistance was calculated from HOMA-IR. The study found a mean weight loss of 8.67±4.28 kg, with a mean HOMA-IR difference of -0.80±1.73 in phase 2. After performing principal component analysis, BCAAs and associated catabolites were shown to correlate with IR at baseline ($r = 0.50$, $p<0.001$) and be independently associated with the differences in the HOMA-IR at the 6-month follow-up ($r=0.28$, $p<0.0001$). Weight loss itself only moderately correlated with the differences in HOMA-IR, which suggests that BCAA catabolites are predictors of changes in HOMA-IR. The study concluded that BCAA metabolism is associated with IR in non-diabetic obese individuals and can predict improvements in IR with moderate weight loss independent of the amount of weight lost (20).

Lips et al. observed an association between obesity and T2D with increased BCAA levels. Their study was conducted in the Netherlands in 2013 to observe whether plasma BCAA levels are reduced in obese women independent of weight loss or the presence of T2D. The study included 30 obese non-diabetic participants, 32 obese T2D participants, and 12 lean female subjects. The obese women underwent restrictive procedures such as gastric binding or the restrictive/bypass via Roux-en-Y-Gastric Bypass surgery. Fasting blood samples were drawn four weeks before and three weeks and three months after the intervention. This interventional study found that plasma BCAA levels were higher in the T2D patients compared with the normal non-diabetic and lean subjects. There were no specific amino acids that were affected by weight loss through the gastric bypass in the normal glucose tolerant (NGT) subjects

However, NGT subjects that had the RYGB surgery displayed a decrease in amino acids (Leucine $P=2.3x10^{-5}$, Valine $P= 3.1x\ 10^{-7}$) in a mixed-model analysis. BCAA levels were also correlated with insulin sensitivity and glucose tolerance. The study concluded that there is a significant decrease in BCAAs 3 weeks after the procedure. However, after three months, the bypass procedure reduced BCAA levels in the obese subjects independent of weight-loss or T2D. This study does not justify whether the surgeries are what caused the decrease in circulating BCAA levels. Calorie restriction has a similar effect on insulin sensitivity and glucose tolerance without affecting BCAA levels, though a reduction in BCAA levels is not necessary for improvements in obesity-associated insulin resistance (21).

The study by Qin et al. was conducted within the International Study of Macro/Micronutrients and Blood Pressure and examined various cross-sectional relationships between dietary BCAA intake and body weight status/obesity in China, Japan, the United Kingdom and the United States. By involving many different countries, inferences pertaining to lifestyle, ethnicity and cultural backgrounds can be shown to play a distinct role in BCAA metabolism and obesity. The study included 4,429 diabetes-free overweight and obese participants with the objective being to determine whether there was an association between dietary BCAA intake and obesity. The levels of BCAAs and other nutrients were determined from urine tests. Participants' diets were observed through 4 multi-pass 24 hour recalls, and the measurements of dietary BCAAs were derived from country-specific food tables. After performing ANOVA and chi-square tests to compare the data across 4 BCAA quartiles, the authors found that the mean BCAA intake was 2.6± 0.6% energy, which is equivalent to the total protein intake. The BCAA intake was observed to be significantly lower in the Chinese

population compared to the populations from the other countries. The multivariable-adjusted OR for the association between BCAA intake and the overweight quartiles with the 1st quartile being a reference category are as follows: (1) 2nd quartile - 0.97 (0.80-1.17), (2) 3rd quartile - 0.91 (0.75-1,11), and (3) 4th quartile - 0.70 (0.57-0.86). The BCAA intake was inverse with associated with the prevalence of obesity (P-trend = 0.03), suggesting that a higher BCAA dietary intake may reduce the prevalence of overweight/obese status among healthy individuals from East Asian and Western countries (22).

Li et al. did a study by specifically observing young adults. This study was conducted in China in 2014 and observed the association between the ratio of BCAA intake and the risk of obesity among young people in an Internet-based cross-sectional study. There was a total of 948 randomly selected participants who were asked to finish an internet-based questionnaire that asked about their diet. Ninety of the participants were asked to come in and fasting blood samples were taken to measure glucose levels. The dietary BCAA ratios in the obese participants were significantly lower than the non-obese participants. There was a negative correlation between the ratio of dietary BCAAs and BMI ($r= -0.197$, $p<0.001$) or waist circumference ($r = -0.187$, $p<0.001$). After stratification by gender, the significance of the dietary BCAA ratio for overweight/obesity existed for the 3rd and 4th quartiles (before stratification by gender OR, 0.508[95% CI 0.265-0.972] and 0.389[95%CI 0.193-0.783] respectively). The study found that the dietary BCAA ratio was inversely associated with BMI. There are possible biases that arise with an internet study. Because studies have shown that obesity is correlated with an increase in BCAAs in the blood, it is possible that the participants

did not honestly answer the questions about their daily lives and could have answered with ideals of how they want to be perceived (23).

Another study also looking at pathways related to insulin resistance suggested that insulin resistance is associated with alterations in skeletal muscle BCAA metabolism. BCAA measurements performed on skeletal muscle biopsies via gene expression and metabolomics analyses found that perturbed BCAA and fatty acid oxidation are found in insulin-resistant humans. The results showed two genes, MUT and ALDH6A1, were down-regulated in multiple steps in the pathway to insulin resistance. This study concluded that impaired BCAA catabolism might contribute to the development of insulin resistance by perturbing BCAA and fatty acid oxidation, suggesting that treatments targeting BCAA metabolism dysfunction could be promising for individuals with T2D (24).

The Chen et al. study was conducted in China from 2005 to 2015 and predicted the potential to develop T2D in a Chinese population in a secondary analysis study. The study population was gathered from a longitudinal cohort study and a cross-sectional study and included 429 Chinese participants in various stages of developing diabetes. In this study, BCAA levels were measured via mass spectrometry. The 213 subjects with the risk of developing diabetes were followed for ten years as a longitudinal study and compared to 216 subjects in a cross-sectional study that encompassed metabolically healthy or unhealthy status. The authors discovered that five amino acids (leucine, isoleucine, valine, phenylalanine, and tyrosine) that were differentially expressed in the healthy lean, overweight/obesity and diabetes individuals, when tested using an AA and metabolic levels heat map, are predictive of the risk of future

diabetes. The longitudinal study showed that individuals who became diabetic during the 10-year follow-up period had significantly elevated AA levels compared to baseline levels, which suggests that elevated AA levels are predictive of diabetes. Valine was shown to have increased by 251% during the development of diabetes in the longitudinal study. In the cross-sectional study, the AA levels progressively increased in the overweight/obese and diabetic subjects. The study concluded that there was a close correlation between BCAA levels, insulin resistance and the development of diabetes and that BCAA levels can predict the development of diabetes (25).

Zheng et al. conducted a meta-analysis study in 2016 that included three prospective cohort studies conducted between 1980 and 2012. The cohort studies included were the Nurses' Health Study (NHS), NHS II and the Health Professionals Follow-up Study. The main objective was to determine how BCAA dietary intake affects the risk of T2D. There were 16,097 incidents of T2D during the 32 years of follow-up. After adjustments for established risk factors, higher total BCAA intake was associated with an increased risk of T2D. All of the tests for linear trends across increasing BCAA quintiles were shown to be significant ($p<0.005$). Leucine, Isoleucine and Valine intake showed a 13% [HR (95% CI) 1.13 (1.05-1.22)], 8%, [1.08 (0.99-1.18)] and 15% [1.15 (1.03-1.29)] higher risk of T2D compared to the lowest AA intake, respectively. Stratification for diabetes risk factors, such as obesity, did not modify the associations between BCAAs and diabetes. The analysis concluded that high BCAA consumption is associated with an increased risk of T2D; moreover, these results were similar to many other studies (26).

Lee et al. conducted a secondary analysis using data from the Insulin Resistance Atherosclerotic Study, a cohort study conducted in the United States with recruitment between 1992 and 1994 and specific analysis conducted in 2015 (9). The study recruited 1,625 participants and only included 685 non-diabetic participants of Caucasian, African American, and Hispanic descent. Each participant had their plasma BCAA sum measured by mass spectrometry. Additionally, insulin sensitivity (Si), acute insulin response (AIR), and metabolic clearance rate of insulin (MCRI), which are factors that describe insulin clearance and glucose effectiveness, were also determined. Insulin sensitivity was measured using the Frequently Sampled Intravenous Glucose Tolerance methodology. The study examined associations between BCAAs and these indices of insulin resistance. After adjusting for potential confounders, the study found an inverse relationship between plasma BCAA levels and insulin sensitivity and MCRI (b = -0.0012 [95% CI -0.0018, -0.0059], P< 0.001 for Si; b= -0.0013 [95% CI -0.0018, -0l.00082] P<0.001 for MCRI) and a positive association with fasting insulin (b=0.0015 [95% CI 0.0008, 0.0023] P<0.001 for fasting insulin). Being of Caucasian or Hispanic descent was found to be significantly associated with Si. The adjusted odds ratio for plasma BCAAs was 1.67 [95% CI 1.21, 2.29, P=0.02], though there was no significance in African Americans (8). These data suggested that race is a potential factor in BCAA levels, though other studies have proven otherwise (8). The results from this cohort study suggest that increased BCAA levels lead to lower insulin sensitivity, which subsequently promotes insulin resistance and T2D (27).

McCormick et al. observed a younger population in their secondary analysis study. The study was conducted in the United States from 2007 to 2009 to understand whether pediatric obesity is associated with an increase in future insulin resistance. The participants are chosen

from a completed prospective cohort for 18 months. Sixty-nine healthy participants were selected from a cross-sectional study. BCAA levels were measured through mass spectrometry, and BMI and insulin resistance were measured from blood samples. Increases in BCAAs were significantly associated with BMI (rho =0.27, P=0.03) in the cross-sectional cohort. The goal of the study was to observe whether obesity is associated with future insulin resistance in children and adolescents. Obese children had higher BCAA concentrations (p=0.008), though there was not a significant association between BCAA concentrations and baseline insulin resistance. The study concluded that obesity in children is associated with elevated BCAA concentrations and that both of these characteristics are independently associated with future insulin resistance (28).

Considering that BCAA levels are suggestive of predicting insulin resistance, Wurtz et al. conducted a study in young adults. This study was conducted in urban and rural areas in Finland. The first survey was given in 1980 and included children and adolescents between the ages of 3 and 18. Participants attended a follow-up in 2001, which was used as a baseline and ended in 2007. There was a total of 1,809 participants that were eligible for this prospective cohort study. Insulin resistance was calculated by HOMA-IR after fasting plasma insulin levels were determined from drawn blood, and BCAA levels were quantified by NMR. The 6-year follow-up found that the sum of the BCAA and Aromatic amino acid (AAA) concentrations were associated with insulin resistance in men (odds ratio 2.09[95%CI 1.38,3.17]; P=0.0005). There were 168 individuals that were classified as insulin-resistant at follow-up. After a 6-year follow-up, insulin resistance was calculated by the homeostasis model assessment (HOMA) at baseline and concluded that isoleucine, leucine, valine, phenylalanine, and tyrosine were with insulin

resistance for men. In contrast, only valine, leucine, and phenylalanine were predictors for IR in women. BCAAs and AAAs are markers for the development of insulin resistance in young, non-diabetic adults. They also find that BCAAs and AAAs predict the risk of T2D that is partially mediated through insulin resistance (29).

**Chapter 3**: What is Unknown about BCAA

All of the studies discussed here were focused on whether BCAA metabolism is causal for insulin resistance or T2D. However, to date, it is not well understood whether adipose tissues alter BCAA metabolism during insulin resistance. There is evidence that BCAA catabolism is altered in obesity, which increases BCAA-derived acyl carnitines and therefore promotes obesity and insulin resistance. However, the question remains, are changes in BCAAs causal or just a biomarker that can potentially predict the development of T2D (1,12).

There are many pathways and mechanisms that have been hypothesized, but there have not been any concrete conclusions on which mechanism is correct. Currently, there are no preventative methods that specifically target BCAAs, though exercise and having a healthy diet has been shown to decrease BCAA levels (1,2). Body-builders and people that enjoy working out take BCAA supplements and have positive effects; however, there is no clarification on whether these supplements can lead to insulin resistance. Because this is a biological issue, identifying a preventative method could potentially be difficult. So far, all of the studies in this review have examined the effects of elevated BCAA levels; however, it is not determined whether the elevated BCAA levels are high enough to be toxic to the body. Additionally, there are not recommendations for defining what are safe BCAA levels in the body. Therefore, more

studies should be conducted on whether BCAA levels can be high enough to promote toxicity and negative effects.

## **Chapter 4**: Recommendations and Conclusions

### *4.1 Recommendations*

Because there is still controversy regarding whether BCAA metabolism is a causal or predictive factor for T2D and insulin resistance, understanding what would be considered toxic levels of BCAAs and BCAA metabolites would be beneficial for future studies. A long-term longitudinal study on healthy exercise enthusiasts who take BCAA supplement and test whether this supplementation leads to insulin resistance could examine whether increased BCAAs have lasting effects. This study would require the investigators to follow the participants and perform routine blood and glucose tolerance tests over a long period to examine BCAA levels and insulin sensitivity over time. A baseline muscle biopsy and follow-up biopsy would be beneficial to test gene expression to further understand the contributions of the two BCAA mechanisms proposed earlier. Having a better understanding of these mechanisms would also be beneficial regarding how to treat individuals that are insulin-resistant, especially if BCAAs are determined to be a causal factor for the development of T2D.

Another proposed study could focus on BCAA and BCAA by-product levels and how they affect the development of metabolic disorders. Compared to the studies performed in this review, this study would be more biochemically focused, but would also benefit from epidemiological analyses of the tested patients for determining correlations between specific genes and the risk/development of obesity, insulin resistance, and/or T2D. This study could

examine varying BCAA and BCAA by-product levels on the development metabolic disorder by combining next-generation sequencing approaches and proteomics to look at the levels of various genes and proteins, respectively, in a variety of patients with different ages, exercise habits, diets, and body types as well as having different ethnic origins. Then, the generated data be annotated with Gene Ontology (GO) or the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to determine the types of genes and/or pathways that are enriched/affected with changes in BCAA levels. This analysis could identify substantial correlations between BCAAs and their by-products with relevant cellular pathways; risk factors for obesity, insulin resistance, and T2D; and a variety of potential metabolic disorders, while also suggesting methods/mechanisms that could prevent disease development and promote healthy lifestyles.

*4.2 Conclusions*

In conclusion, although there are numerous studies that have shown associations between branched-chain amino acids and obesity, insulin resistance, and type 2 diabetes, it remains unclear whether BCAA levels are a causative factor that promotes these conditions. Several mechanisms have been postulated regarding the roles that BCAAs play in the metabolic pathways that lead to diabetes. However, most of these mechanisms have yet to be confirmed, suggesting that more in-depth research is necessary. Regardless, several studies support that idea that increased BCAA levels have the potential to be a biomarker for the loss of insulin effectiveness.

Table 1. Summary of Current Studies on BCAA

| Author | Type of Study | Population studied | Sample Size | Outcome Measures | Conclusions |
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| **Newgard et al.[19,20]** | Interventional<br><br>Experimental | Wistar Rats<br><br>Adults that are "healthy" | Rats given a high fat diet, high fat and BCAA supplement or standard chow<br><br>73 obese and 67 lean subjects | Rats with HF/BCAA rats were equally resistant as HF rats<br><br>Wilcoxon rank sum test and PCA found BCAA positively correlated with HOMA r=0.58 | High fat consumption and poor diet, BCAA contributes to development of obesity-associated insulin resistance |
| **Lackey et al.[8]** | Experimental | Obese rats with white adipose tissue | Fa/fa rats, db/db mice, diet induced obese mice | BCKD expression was reduced 35-50% in obesity models and that BCKD component transcripts were significantly lower in subcutaneous adipocytes compared to lean Pima Indians. | Obesity is less important than WAT metabolic regulators in BCAA catabolism |
| **Cavallaro et al.[3]** | Interventional | Healthy males | 5 healthy males | Fasting BCAA were found to be significantly lower in valine (p=0.02) | Short term dietary manipulation of BCAA intake led to modest changes in |

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| | | | | | fasting levels of BCAAs |
| **Lotta et al.**[13] | Case Study | Individuals identified with 5 genomic regions where genetic variants were associated with BCAA levels | 1,992 incident cases of T2D and 4,319 non-cases | Meta-analysis found that stronger associations between higher level of each BCAA and higher risk of T2D | Large-scale human genetic and metabolic study is consistent with a causal role of BCAA metabolism of T2D |
| **Shah et al.**[25] | Interventional | Adults participating in the Weight Loss Maintenance trial | 1053 WLM participants for Phase 1 and 500 participants in Phase 2 | PCA and mixed models BCAA correlated with baseline HOMA-IR ($r=0.50$, $p<0.0001$) and independently with change HOMA-IR ($p<0.0001$) | BCAA and related analytes predicts improvements in HOMA-IR independent of weight lost |
| **Lips et al.**[12] | Interventional | Obese women with and without T2D | 30 obese normal glucose tolerant, 32 obese subjects with T2D and 12 lean female subjects | PCA revealed a correlation between BCAA and insulin sensitivity and glucose tolerance. | There is an association between the deregulation of BCAA and insulin resistance. Bypass RYGB surgery, is independent of weight-loss or presence of T2D, reduces BCAA levels |

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| | | | | | in obese subjects |
| **Qin et al.[23]** | Cross Sectional | Adults in the International Study of Macro/Micronutrients and Blood Pressure in China, Japan, UK and US | 4429 middle-aged men and women between the ages 40-59 years | Multivariable OR (95% CI) of dietary BCAA intake $2^{nd}$ -$4^{th}$ quartile 0.97 (0.80-1.17), 0.91 (0.75-1.11) and 0.70 (0.57-0.86). BCAA intake and obesity are inversely associated (P-trend = 0.03) | Higher dietary BCAA intake is associated with lower prevalence of overweight status/obesity among health middle-aged adults from East Asia and Western countries. |
| **Li et al.[11]** | Cross-sectional | Young Northern Chinese Adults between the ages 18-40 that completed the internet study, non-diabetic, and not on a diet in the past 6 months | 948 randomly recruited participants and 90 subjects recruited to explore a mechanism | Multivariable OR (95% CI) of dietary BCAA ratio with obesity of $3^{rd}$ and $4^{th}$ quartile 0.351 (0.145-0.845) and 0.376 (0.161-0.876) all with $p<0.05$. | Higher ratio of dietary BCAA is inversely associated with prevalence of obesity and status of inflammation in young northern Chinese adults |
| **Lerin et al.[10]** | Cross sectional | Humans with normal glucose tolerance and Type 2 diabetes | 41 humans with Normal Glucose Tolerance and 11 with T2D | Perturbed BCAA metabolism and fatty acid oxidation in muscle from insulin resistant humans | Impaired muscle BCAA catabolism may contribute to the development of IR |

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| **Chen et al.[4]** | Cross-sectional and Longitudinal cohort study | Chinese adults at various stages of diabetes development | 429 Chinese participants 213 with the risk of developing diabetes in the longitudinal study and 216 healthy individuals in the cross-sectional study | Logistic regression model with 5 AAs with risk of diabetes p-values ≤0.001 | The 5 AAs are predictive of the risk of future diabetes as a marker for diabetes not causal. |
| **Zheng et al.[30]** | Prospective cohort | Men and women at risk for T2D living in the US | 16,097 men and women incident T2D events during up to 32-year follow-up | Meta-analysis of all cohorts with hazard ratios for leucine 1.13 (1.07-1.19), isoleucine 1.13 (1.07-1.19), and valine 1.11 (1.05-1.17) P-trend <0.001 | High consumption of BCAAs is associated with an increased risk of T2D |
| **Lee et al.[9]** | Secondary analysis Cohort | Adults without diabetes in the Insulin Resistance Atherosclerosis Study in 4 clinical location in the US | 685 men and women. 290 Caucasians, 165 African American, and 230 Hispanics. | Regression models found elevated plasma BCAA were inversely associated with Si and positively with fasting insulin. Si (B= -0.0012 (-0.0018—0.00059) fasting | Plasma BCAA are associated with incident diabetes and underlying metabolic abnormalities. |

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| **McCormack et al.**[17] | Cross-sectional and Longitudinal Cohort | Children and adolescents in Boston that are pre- or early pubertal. | 69 healthy subjects between 8-18 years old and a subset of 17 participants for a longitudinal cohort | Using a spearman correlation r=0.27, p=0.03 find that there is a correlation between BCAA and BMI in the cross-sectional study and longitudinal cohort. | Elevated concentrations of circulating BCAA are significantly associated with obesity in children and adolescents and may predict insulin resistance. |
| **Wurtz et al.**[27] | Prospective Cohort | Young adults in the Cardiovascular Risk in young Finns Study | 1680 non-diabetic young adults | Linear regression models found that BCAA is associated with insulin resistance OR 2.09 (CI=1.38-3.17) | BCAA are markers of the development of IR in young nonglycemic adults. |

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