Food and Nutrition Sciences, 2025, 16(1), 137-152
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Isoleucine Enriched Branched-Chain Amino Acid Supplement Improves Glucose and Insulin Sensitivity in Healthy College-Age Males but Not in Females

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How to cite this paper: Dellogono, M., Clark, L., Garelnabi, M. and Wilson, T.A. (2025) Isoleucine Enriched Branched-Chain Amino Acid Supplement Improves Glucose and Insulin Sensitivity in Healthy College-Age Males but Not in Females. *Food and Nutrition Sciences*, **16**, 137-152.
https://doi.org/10.4236/fns.2025.161008

Received: December 19, 2024
Accepted: January 23, 2025
Published: January 26, 2025

Abstract

Athletes often use branched-chain amino acid (BCAAs) supplements with a ratio of 2:1:1 (leucine:isoleucine:valine) for their impact on muscle building. Research suggests that by altering the ratio, an improvement in glucose metabolism might be possible. The purpose of this study was to examine how isoleucine would influence glucose tolerance. We recruited healthy male (n = 13) and female (n = 5) participants who were asked to fast for 12 hours before coming to the laboratory. A fasting blood sample was collected, followed by the subjects consuming a breakfast containing 113 g carbohydrates, 8 g protein, 1.5 g fat, and BCAA powder in the 2:1:1 ratio (Control) or BCAA powder enriched with Isoleucine (2:6:1), both added to orange juice. The opposite meal was consumed on a second visit one week apart. Blood was collected at 30, 60, 90, and 120 minutes post-meal. No differences were observed between the Control and Isoleucine for changes in serum glucose or insulin response when examining all subjects together. However, when comparing between genders, males tended to have a significantly lower serum glucose response compared to females when consuming the Isoleucine, with no difference between the genders when consuming the Control. Also, males had significantly lower serum glucose responses when consuming the Isoleucine compared to when they consumed the Control, while females had significantly higher serum glucose responses when consuming the Isoleucine compared to when they consumed the Control. In general, males tended to have a lower serum insulin response than females when consuming both the Control and the Isoleucine. Our study indicates a significant difference in the way genders respond to BCAA supplementation, where isoleucine may improve glucose tolerance

and insulin response in males but not females.

Keywords

Isoleucine, Branched-Chain Amino Acids, Glucose Tolerance, Insulin

1. Introduction

The branched-chain amino acids (BCAAs): Leucine, Isoleucine, and Valine, have been used in both the sports nutrition industry and the clinical healthcare setting for many years. In athletes and exercising populations, BCAAs have been used to effectively stimulate muscle protein synthesis, reduce exercise-induced muscle damage, and prevent central fatigue [1]-[7]. Outside of sports nutrition, BCAAs have been examined for their impact on diseases, such as cirrhosis, insulin resistance, metabolic disease, and the immune system [8]-[14]. Current research shows mixed results and conclusions on how we should view BCAAs and their respect for health. Clinical studies looking at metabolic and cardiovascular disease (CVD) have predominately been conducted as epidemiological studies. These studies have identified a negative relationship between elevated plasma BCAA levels with impaired insulin sensitivity and cardiovascular events [12] [15]. Contrary to the epidemiological work, however, the work done *in vivo* on liver health has shown significant improvements in outcomes [10] [16]-[18]. The mixed conclusions of BCAA research suggest that further research is needed to understand how they influence health.

One possibility for the mixed results is that most studies involving BCAAs do not state the exact ratio of BCAAs being used. When the ratio has been identified, it is most frequently a 2:1:1 (leucine:isoleucine:valine) ratio. This ratio is the most commonly found in natural food such as chicken and turkey as well as nutritional supplements. Although 2:1:1 is the most frequently encountered, we have observed that each amino acid has a unique biological action. In studies examining the amino acids for muscular development and recovery, it is evident that leucine is the primary amino acid responsible for muscle protein synthesis [19] [20]. While current research has predominately focused on leucine alone or the BCAAs supplemented in a 2:1:1 ratio, unique physiological effects have been observed when isoleucine is taken alone as a supplement or as a bolus in addition to diet. Multiple *in vitro* and rodent studies have demonstrated that isoleucine alone does not increase muscle protein synthesis significantly; however, it does seem to significantly reduce blood glucose concentrations [21]-[24]. Very few studies have examined the physiological response to isoleucine supplementation in humans. In studies, there has been a significant decrease in the blood glucose area under the curve when isoleucine is consumed with a bolus of glucose [25]-[27]. While in these previous studies, the intervention was predominantly to supply additional amounts of pure isoleucine to greatly increase the amount normally consumed

compared to leucine intake; the current study wanted to increase the amount of isoleucine through a BCAA supplemental dose that would be similar to these studies at two to three times more isoleucine than leucine, thus a ratio of 2:6:1 was used.

The primary goal of this study was to examine how supplementing a meal with isoleucine-enriched BCAAs in a 2:6:1 ratio influences the glucose response to that meal. By increasing the amount of isoleucine in the BCAA ratio, we hypothesize that we could see a significant improvement in glucose tolerance and that this study would begin to fill the gaps in the current literature with regard to BCAA ratios. By identifying a ratio of BCAAs that improves glucose metabolism, we hope to provide a safe resource for blood glucose management through nutritional supplementation.

2. Materials and Methods

2.1. Study Participants

We recruited a group of college students (men, n = 13; women, n = 5) through flyers, oral presentations in classrooms, and on-campus by study personnel. Participants were screened and excluded from the study if they were experiencing conditions that required physician care, had medically implanted devices, were currently taking oral contraceptives, were pregnant, or had a BMI outside of 18.5 - 29.9 (kg/m^2). The female subjects were asked when their last menstrual cycle period ended, and all were initiated and completed the study within 10 - 20 days following this date. We explained the purpose and design of the study, as well as all known risks associated with the study procedures, to each subject. Each subject signed an informed consent form, and the Institutional Review Board approved all study procedures at the University of Massachusetts Lowell.

2.2. Experimental Design

Following the completion of the informed consent, we collected anthropometric data for each subject. This included height, weight, and body composition. Body composition was measured via A Tanita® bioelectrical impedance scale (Tanita Corporation, Tokyo, Japan) was used to measure body composition.

Each subject was studied on two occasions, separated by 2 - 7 days. On both occasions, subjects were asked to refrain from strenuous activity and alcohol for the 24 hours before the study. Subjects were also asked to fast for at least 12 hours, only consuming water during that time frame. Upon arrival at the facility, subjects were asked: "Did you eat or drink anything other than plain water in the past 12 hours?". Upon confirmation, we collected a fasting blood sample via the median cubital vein.

Following the fasting blood draw, subjects were asked to consume a meal containing 16 fl. Oz. of Tropicana® Orange Juice (220 calories; 52 g carbohydrates, 44 g sugar; 4 g protein; 0 g fat), which contained the BCAAs, Wonder® Classic White Bread (140 calories; 29 g carbohydrates, 5 g sugar; 4 g protein; 1.5 g fat), and 4

tablespoons of Welch's® Natural Grape or Strawberry Jelly (120 calories; 32 g carbohydrates, 32 g sugar; 0 g fat; 0 g protein). Total meal consumption was 480 calories, 113 g carbohydrates (81 g sugar, 2 g fiber), 8 g protein, 1.5 g fat. Subjects were asked to consume the meal within 10 minutes.

During one of the two days, unflavored BCAA powder (Bodybuilding.com, Boise, Idaho) in a ratio of 2:1:1 (2.5 g leucine, 1.25 g isoleucine, 1.25 g valine) was added to the orange juice and blended (Control). On the other day, the same unflavored BCAA powder was used and enriched with an additional unflavored isoleucine powder (Infinite Lab, Orlando, Florida) (Isoleucine). The enriched isoleucine and BCAA powder were added to the orange juice and blended. This gave the BCAAs a ratio of 2:6:1 (2.5 g leucine, 7. 5g isoleucine, 1.25 g valine). We did not inform subjects of the ratio of BCAAs they were consuming.

2.3. Serum Glucose and Insulin Analyses

Following consumption of the meal, we collected a venous blood sample via the median cubital vein of the arm at 30, 60, 90, and 120 minutes post-meal consumption. During the 120-minute collection period, subjects were asked to remain seated and relaxed.

We centrifuged the blood samples at 1500 × *g* 4˚C for 20 minutes; serum was collected and stored at −80˚C until analyses. The analysis of serum glucose was completed with the Medica EasyRA Clinical Chemistry Analyzer (Medica Corporation, Bedford, MA) using a hexokinase enzyme reaction glucose assay kit (Medica Corporation, Bedford, MA). Serum insulin was measured using the Insulin ELISA, a sandwich-type immunoassay (ALPCO, Salem, NH).

2.4. Statistical Analysis

Statistical evaluations were performed using SPSS for Windows 19.0 software (SPSS, INC., Chicago, IL). The data was analyzed using Repeated Measures Two Way ANOVA to examine differences between time points within the subjects and at the same time points between subjects. A Student's *t*-test was used to analyze the area under the curve (AUC) for the glucose and insulin response to the meal and between male and female subjects. All values are expressed as means ± SD, and statistical significance was set at a $p \leq 0.05$.

3. Results

3.1. Subject Characteristics

A total of 18 subjects (13 males; 5 females) completed the study (Table 1). The mean (SD) age of the subjects was 23 years (± 3.3), body mass index (BMI) was 24.6 kg/m^2 (± 2.2), and body weight was 74.4 kg (± 10.38). Lean body mass was 60.3 kg (± 11.4), and the percentage of body fat was 19.2% (± 8.2%). Age and BMI did not differ between males and females. However, male participants had significantly less body fat (males, 15.5%; females, 29.0%) and significantly more lean body mass (males, 65.9 kg; females, 44.5 kg) than females ($p \leq 0.001$).

Table 1. Subject anthropometrics.

Variable	n = 18	Males (n = 13)	Females (n = 5)	
	Mean (SD)	Mean (SD)	Mean (SD)	$p \leq$ [a]
Age (years)	23.1 (3.23)	24.0 (3.39)	20.8 (1.79)	0.065
Weight (kg)	74.4 (10.38)	78.1 (8.68)	64.7 (8.32)	0.009
Height (cm)	173.7 (10.59)	177.6 (7.19)	163.6 (11.90)	0.007
BMI (kg/m2)	24.6 (2.21)	24.7 (1.90)	24.1 (3.12)	0.620
Body Fat %	19.2% (8%)	15.5% (5.45%)	29.0% (5.93%)	<0.001
Lean Body Mass (kg)	60.3 (11.14)	65.9 (6.92)	44.5 (3.40)	<0.001

BMI = Body Mass Index; [a]Two (independent) sample *t*-tests on sex.

3.2. Isoleucine versus Control

There were no statistical differences at any time point between the Isoleucine and Control for serum glucose concentrations following the meal (Table 2). From baseline to 30 minutes post-meal, serum glucose rose 18.5% in response to the Control and only 14.3% in response to the Isoleucine. In both groups, the serum glucose returned to a below fasting value within 60 minutes after the feeding. At 60 minutes the Control resulted in a −3.8% decrease in serum glucose from baseline, while the Isoleucine decreased serum glucose −10.6% from baseline values. At 90 and 120 minutes, both the Control and Isoleucine were similar. The area under the curve (AUC) for the Isoleucine also showed a slight reduction compared to Control but failed to achieve statistical significance (Table 2).

Table 2. Serum glucose and insulin responses to control and isoleucine.

Glucose (mg/dL)	Control (n = 18)	Isoleucine (n = 18)	$p \leq$ [a]
Baseline	90.11 (10.43)	91.50 (4.83)	0.87
30 minutes	106.72 (28.84)	103.83 (27.52)	0.79
60 minutes	86.28 (20.56)	81.22 (19.95)	0.16
90 minutes	87.44 (19.42)	85.62 (18.79)	0.61
120 minutes	86.94 (12.92)	86.61 (13.80)	0.92
AUC	11086.67 (2091.03)	10729.17 (1928.75)	0.59
Insulin (pmol/L)			
Baseline	49.31 (21.60)	48.10 (20.46)	0.77
30 minutes	466.05 (227.39)	523.15 (287.16)	0.32
60 minutes	282.09 (122.91)	226.89 (118.20)	0.05
90 minutes	201.64 (101.08)	223.96 (160.49)	0.39
120 minutes	145.91 (87.95)	154.23 (134.57)	0.74
AUC	32126.98 (11985.20)	34078.17 (17615.46)	0.69

Values are Mean (SD).

Serum insulin followed a similar response to serum glucose between the Isoleucine

and Control. However, a significant decrease in serum insulin was observed at 60 minutes with the Isoleucine compared to Control (−19.5%; $p \leq 0.04$) (Table 2). The 60-minute time point was the only time in which the Isoleucine serum insulin response was less than that of the Control. At 30 minutes post-meal, serum insulin elevation translated to a 1033% increase with the Isoleucine and a 951% increase with the Control. At 60 minutes, the percent change from baseline was 505% with the Control and 425% with Isoleucine. At 90 minutes, the change from baseline was 338% for the Control and 375% with Isoleucine. At 120 minutes post-meal, the insulin response remained slightly elevated for both the Control (199%) and Isoleucine (220%). Ultimately, the serum insulin AUC response was very similar and did not statistically differ between the Isoleucine and Control (Table 2).

3.3. Gender Differences for Serum Glucose

With the Control, we observed no significant differences in serum glucose response between the males and females at any time point (Figure 1(A); Table 3). However, with the Isoleucine, males responded with lower serum glucose levels at every time point, with the 90-minute time point being significantly lower compared

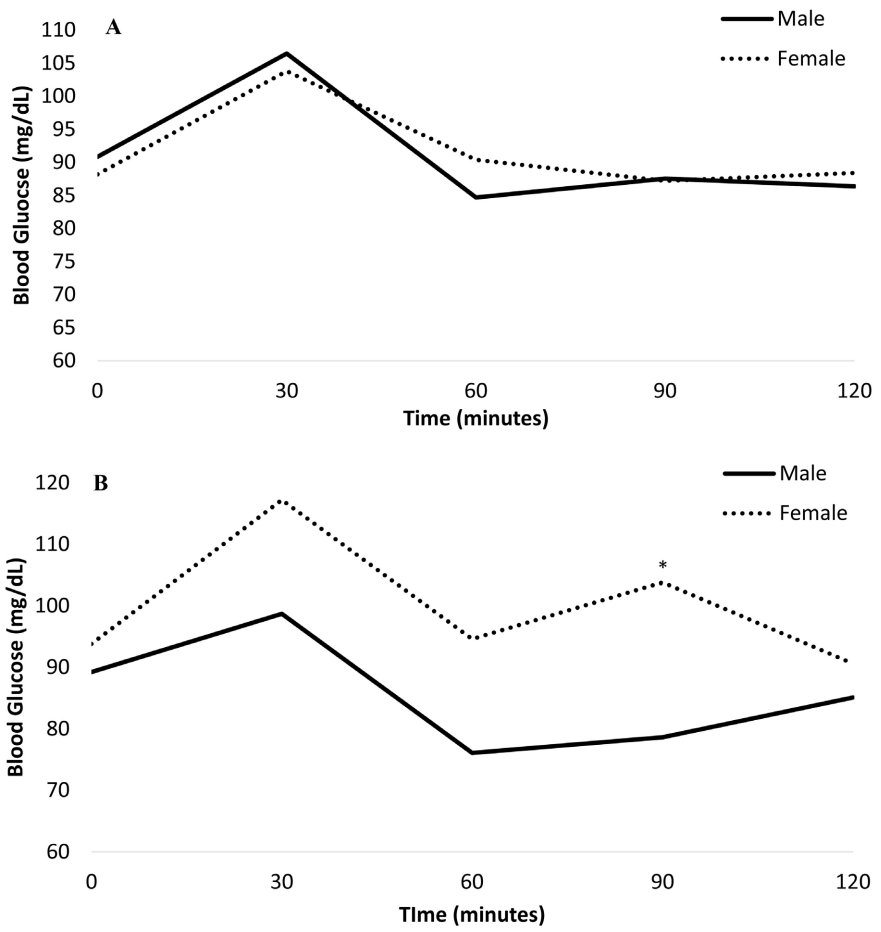

Figure 1. Gender differences in serum glucose response to control (A) and isoleucine (B). *Significantly different at $p \leq 0.01$.

Table 3. Gender differences in serum glucose (mg/dL) response to control and isoleucine.

	Control		Isoleucine	
	Male (n = 13)	Female (n = 5)	Male (n = 13)	Female (n = 5)
Baseline	90.85 (11.72)	88.20 (6.61)	89.23 (4.55)	93.80 (4.27)
30 minutes	106.46 (32.10)	103.80^{+} (20.90)	98.69 (29.81)	117.70^{+} (15.64)
60 minutes	84.69^{+} (23.16)	90.40 (12.54)	76.08^{+} (18.70)	94.60 (18.26)
90 minutes	87.54^{+} (22.39)	87.20^{+} (9.96)	78.62$^{+,*}$ (15.95)	103.80$^{+,*}$ (12.87)
120 minutes	86.38 (14.48)	88.40 (8.76)	85.08 (13.39)	90.60 (15.63)
AUC	11019.23 (2384.94)	11262.00 (1210.66)	10216.15 (1923.48)	12063.00^{+} (1277.44)

Values are Mean (SD). $^{+}$Significantly different at $p \leq 0.05$ between treatments within gender. *Significantly different at $p \leq 0.05$ between genders within the treatment.

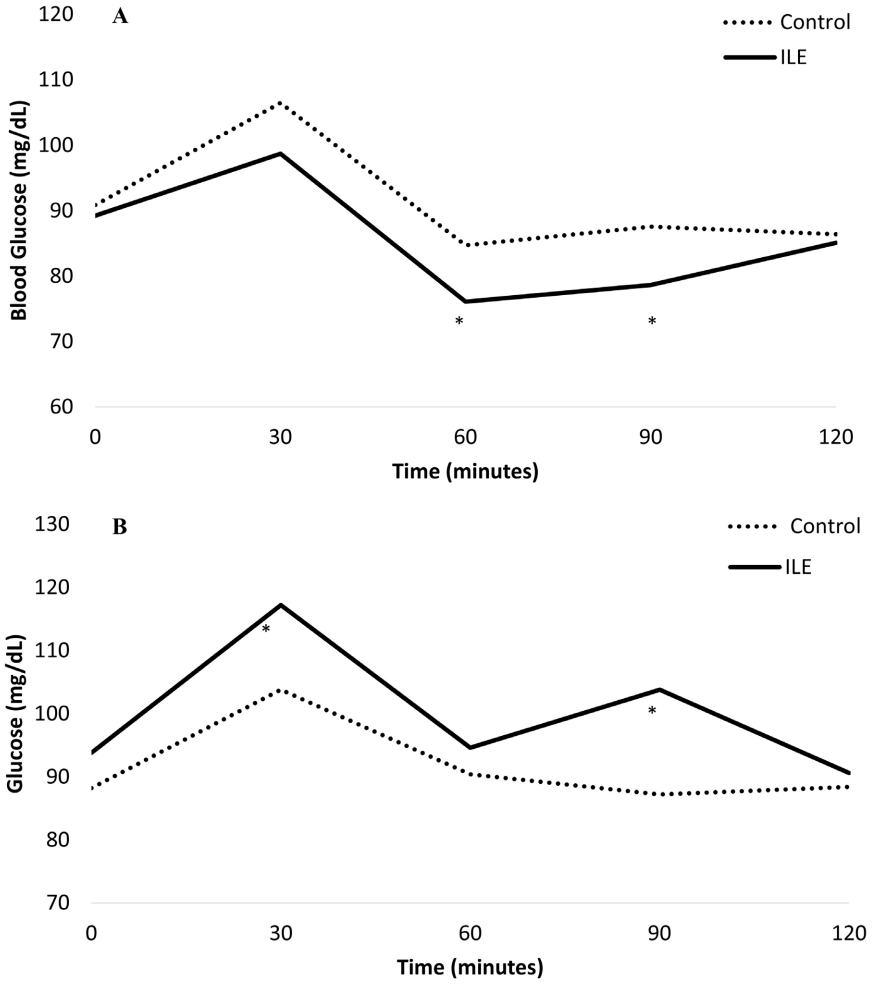

Figure 2. Serum glucose responses in male (n = 13) (A) and female (n = 5) (B) subjects between the control and isoleucine. *Significantly different at $p \leq 0.05$.

to females (32%; $p \leq 0.01$) (Figure 1(B); Table 3). This variation in serum glucose levels at 90 minutes translated to a −12% decrease from baseline in males ($p \leq 0.009$) and a 10% increase from baseline in females with the Isoleucine. Serum

glucose AUC was not found to have a significant difference between males and females within the Control or Isoleucine (Table 3).

When examining males alone, we demonstrated a trend for a decreased serum glucose response at every time point with Isoleucine when compared to Control (Table 3). A significant decrease in serum glucose response was seen at the 60-minute (−10%, $p \leq 0.04$) and 90-minute (−10%, $p \leq 0.01$) time points post-meal (Figure 2(A)). A significant difference was not observed in the serum glucose AUC when comparing Isoleucine with Control within males (Table 3).

When examining females alone, they responded to the Isoleucine with increased serum glucose response at every time point when compared to the Control (Table 3). This increase in serum glucose response was statistically significant at 30 minutes (13%, $p \leq 0.03$) and 90 minutes (19%, $p \leq 0.01$) when compared to the Control (Figure 2(B)). We also observed a significant increase in serum glucose AUC with ILE compared to Control within females (7%; $p \leq 0.003$) (Table 4).

Table 4. Gender differences in serum insulin (pmol/L) response to control and isoleucine.

	Control		Isoleucine	
	Male (n = 13)	Female (n = 5)	Male (n = 13)	Female (n = 5)
Baseline	44.88 (21.06)	60.85 (20.54)	40.93* (14.43)	66.76* (23.51)
30 minutes	409.83 (217.96)	612.22 (217.96)	432.34* (230.44)	759.25* (307.71)
60 minutes	231.97* (99.55)	412.42* (99.55)	186.03* (101.30)	333.14* (95.19)
90 minutes	179.76^{+} (100.79)	258.53^{+} (85.67)	147.75$^{+,*}$ (74.73)	422.12$^{+,*}$ (157.31)
120 minutes	123.73 (85.43)	203.57 (72.27)	94.18* (59.80)	310.35* (155.07)
AUC	28436.10 (11511.40)	42451.46 (5878.49)	28001.87* (15080.23)	51091.80* (12892.69)

Values are Mean (SD). $^{+}$Significantly different at $p \leq 0.05$ between treatments. *Significantly different at $p \leq 0.05$ between genders.

3.4. Gender Differences for Serum Insulin

Females had slightly higher serum insulin levels at each time point with the Control, reaching statistical significance at 60 minutes when compared to males (78%; $p \leq 0.002$) (Figure 3(A); Table 4). When we added the Isoleucine to the meal, females had a significant increase in serum insulin response at every time point when compared to the male's serum insulin response to Isoleucine ($p \leq 0.05$) (Figure 3(B); Table 4). Both the Control and Isoleucine resulted in females having a significantly elevated serum insulin AUC compared to males on the Control (49%; $p \leq 0.02$) (Table 4) and on the Isoleucine (82%; $p \leq 0.01$) (Table 4).

When examining the males alone, they had a similar serum insulin response to both the Isoleucine and Control (Table 4). However, a significant decrease in serum insulin was seen at the 90-minute time point with the Isoleucine compared to Control in the males (−18%, $p \leq 0.04$) (Figure 4(A)). The serum insulin AUC was not significantly different between the Isoleucine and Control in the males (Table 4).

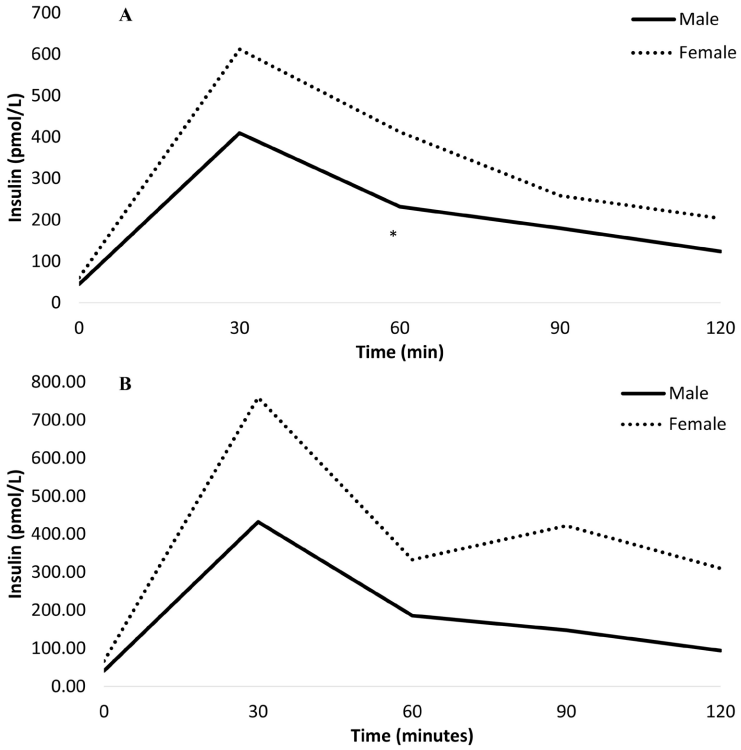

Figure 3. Gender differences in serum insulin response to control (A) and isoleucine (B). *Significantly different at $p \leq 0.002$.

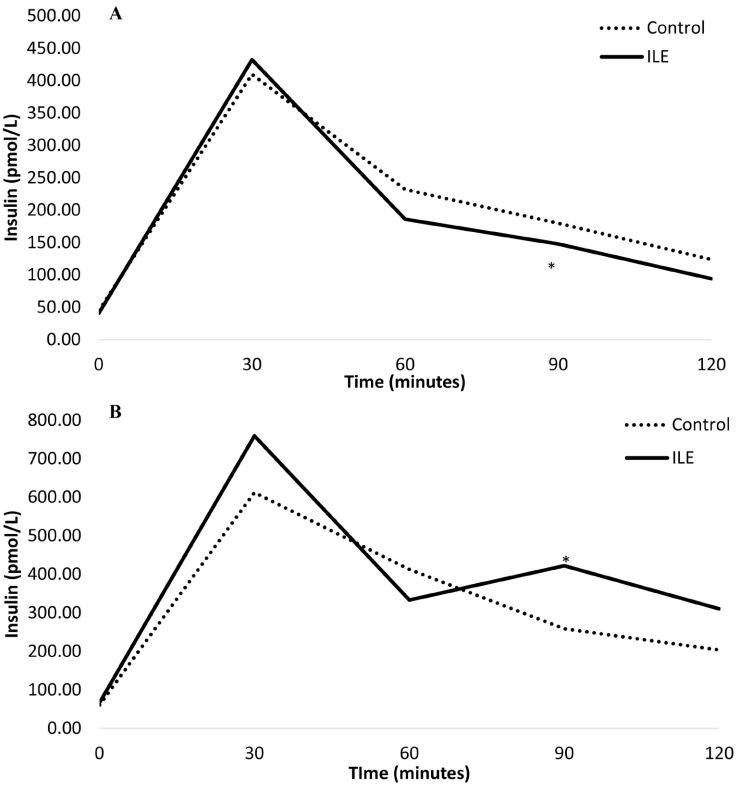

Figure 4. Serum insulin responses in male (n = 13) (A) and female (n = 5) (B) subjects between control and isoleucine. *Significantly different at $p \leq 0.05$.

When examining the females alone, serum insulin was significantly higher at the 90-minute time point following Isoleucine when compared to the 90-minute time point with Control (63%, $p \leq 0.01$) (**Figure 4(B)**). We did not find a significant difference between the ILE and Control AUC in the females (**Table 4**).

4. Discussion

BCAAs have been studied for a variety of applications, including liver disease, exercise performance and recovery, and metabolic disease [28]-[32]. A survey of the current nutritional supplement market reveals that BCAA supplements are most commonly used in a 2:1:1 ratio (leucine:isoleucine:valine). However, there is a gap in the literature examining how altering the ratio of branched-chain amino acids impacts their physiological effect. It has been demonstrated that leucine and isoleucine have unique metabolic effects, with leucine working to regulate muscle protein synthesis through mTOR regulation and isoleucine impacting glucose metabolism [19] [23] [24] [33]. Some epidemiological studies have also suggested that BCAAs are associated with an increased cardiometabolic risk [34]. To properly use BCAAs in nutritional therapy, we need to understand how BCAA ratios impact metabolic pathways. In this study, we measured the glucose and insulin response of healthy college-aged subjects to a meal with two different ratios of BCAAs. We compared the traditional BCAA mixture of 2:1:1 with an Isoleucine-rich BCAA mixture of 2:6:1. To our knowledge, this is the first study to specifically compare two ratios of BCAAs and examine how they alter glucose tolerance and insulin.

The Isoleucine and Control showed no significant changes in serum glucose response. We observed slightly lower serum glucose levels at 30, 60, and 90 minutes with Isoleucine; however, this decrease did not generate statistical significance. The trend we saw in a lower serum glucose response with Isoleucine was not matched with increased insulin, suggesting that the increased amino acid concentration did not stimulate the release of additional insulin. Serum insulin AUC did not significantly vary between the Control and the Isoleucine groups. We saw the expected spike in serum insulin following ingestion of the high carbohydrate meal, but serum insulin levels quickly began returning to baseline. With the Isoleucine, we saw that the rate of return was slightly quicker than the Control meal. These trends in a lower serum glucose and insulin response to isoleucine demonstrate that isoleucine may either positively influence and/or enhance insulin sensitivity in the subjects, which may help with reducing the need for insulin in controlling serum glucose levels in humans, especially those with diabetes.

Our results also indicate that there may be a significant difference in how males and females may utilize BCAA supplements when it comes to serum glucose and insulin responses following a meal. When we compared the male and female serum glucose response with the Control, we saw no differences between the genders, the curves were basically identical. However, when consuming the Isoleucine, we saw that males had consistently lower serum glucose response than the

females. Female subjects had serum glucose values that were, on average, 19 mg/dL higher than males at both 30 and 60 minutes and were significantly higher at 90 minutes with an average increase of 25 mg/dL. When examining the serum insulin response between genders, it appears that males, in general, showed a much lower serum insulin response across all time points whether consuming the Control or Isoleucine with the high carbohydrate meal, with males showing a significantly lower serum insulin AUC compared to females with the Isoleucine. Again, this significantly lower serum insulin response in males when taking the Isoleucine with a high carbohydrate meal may help with reducing the need for insulin in controlling serum glucose levels in males with diabetes.

Another interesting finding, when looking at the serum glucose and insulin responses within each gender, is that males decreased both their serum glucose and insulin responses with the Isoleucine compared to Control. Whereas, females showed the opposite effect and had a significant increase in serum glucose response with the Isoleucine compared to Control. This particular finding shows that for males, consuming an isoleucine-rich BCAA supplement may help to reduce their serum glucose response following the consumption of a carbohydrate-rich meal, but for females, the opposite effect may be true. For males, this may again help to have a positive effect when trying to control their serum glucose levels postprandial.

There is currently a lack of previously published data when comparing the gender differences examining similar outcomes to our study. Thalacker-Mercer *et al.* [35] used a hyperinsulinemic-euglycemic clamp in nondiabetic and type-2 diabetic individuals to examine the relationship between amino acids and insulin action. They reported that the leucine/isoleucine had the strongest negative association with glucose disposal rate. They also showed that the glucose disposal rate was greater in males than in females with leucine/isoleucine. Ultimately, it was concluded that leucine/isoleucine is associated with insulin resistance in non-obese and T2DM subjects [35]. Unfortunately, they did not separate leucine and isoleucine in their analysis but instead grouped them as one measurement. Contrary to their findings, our study suggests that glucose disposal was improved in the males and had minimal impact on insulin. This contradictory evidence further suggests that more research is needed on the individual amino acids and the ratios in which they are consumed.

Further looking at the gender differences, Kawaguchi *et al.* [36] found that BCAA supplementation at breakfast and dinner improved insulin resistance and β-cell function in male patients with chronic viral liver disease. Insulin resistance and β-cell function were evaluated using fasting glucose, and insulin was then quantified using the HOMA method. Similar to our findings with regard to gender differences, Kawaguchi *et al.* [36] observed significant improvements in glucose and insulin metabolism in males, but not in females. In their study, the BCAA supplement was given in the traditional ratio of 2:1:1 [36]. However, they state it was confounded with trace minerals, which they report as potentially impacting

the outcome.

Everman *et al.* [37] conducted a study on healthy college-aged students in which they used a hyperinsulinemic-euglycemic clamp to determine the impact of BCAA supplementation on insulin-mediated glucose turnover. They found that the short-term supplementation of BCAA did not cause a negative impact on insulin-mediated glucose disposal [37]. It should also be noted that while there was not a significant difference between the control group and the BCAA group, the glucose levels in the BCAA group decreased slightly during infusion, while the control group saw a slight increase in blood glucose. This trend continued during the hyperinsulinemic-euglycemic clamp, suggesting BCAAs may improve blood glucose. The infusion ratio of BCAAs was 1:1:(0.9), which should be noted as it strays away from the more commonly seen 2:1:1 ratio [37].

Although it was not part of the current study since we only examined the effect of taking a single isoleucine-rich BCAA once with a meal, there may be potentially some toxicity problems with taking higher amounts of isoleucine with each meal every day. Isoleucine supplementation is usually well tolerated, but some people may experience gastrointestinal disorders like nausea and diarrhea [38], none of which were experienced in our study, possibly due to the single dose used.

Overall, we found that males had a greater sensitivity and clearance of serum glucose than females. With Isoleucine, males demonstrated a trend toward improving their glucose clearance with a trend for decreased serum insulin. This may, in part, be due to their increased muscle mass, which we know to improve glucose clearance. However, when we look at females, independent of males, we see an opposite response.

The current data indicates there may be significant differences in how specific populations are affected by BCAA supplementation. A lack of emphasis has been placed on the ratio of amino acids given. In most cases, we see them supplemented in a 2:1:1 ratio, but few studies have compared various dosages. In our study, we saw that we could improve glucose removal in males by increasing the isoleucine, whereas females experienced the opposite effect. Although we observed several significant differences between the genders, our study is limited by a small female sample size (five female subjects versus 13 male subjects), and because of this, we focused our discussion on the possible positive benefits of isoleucine supplementation in males rather than the possible negative benefits in females and that future studies should expand upon the potential gender differences in the metabolism of individual BCAAs along with their long-term safety in humans. In this study, we demonstrated that a 2:6:1 ratio led to improvements in serum glucose response in males, but future studies should analyze other ratios to fully elucidate the BCAAs interaction with serum glucose.

Funding

This work was supported by an Internal Seed Grant from the University of Massachusetts Lowell (TAW).

Authors' Contributions

MD, CF, and TAW designed the study. MD and LC oversaw its conduct in the Animal Facility and completed the day-to-day care of the animals and the laboratory assays. CF, MG, and TAW advised on the statistical analysis. MD, LC, and TAW wrote the manuscript, and CF and MG provided their expert comment, as well as interpretation of the data, during the preparation of this manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Beth Halaby for her work and time on this project.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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