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RAPID COMMUNICATION

Leucine Supplementation: A Possible Anti-Inflammatory Strategy Evidences from a Pilot Study

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ABSTRACT

Background: Branched-Chain Amino Acids (BCAAs) are well-known nutrients able to promote therapeutic effects under proinflammatory conditions. We aimed to evaluate the effects of acute supplementation with a mixture of BCAAs or with leucine on serum cytokine pattern in human volunteers. Findings: In a cross-over, double-blind design, eight healthy men were randomly submitted to three experimental conditions: supplementation with BCAAs or LEU in comparison with isonitrogenous placebo (PLA). After an overnight fasting, participants ingested a single dose of the supplement and blood samples were collected to determine serum glucose and insulin concentrations, lipid profile, and interleukins (IL)-6, tumor necrosis factor alpha (TNF-α) and IL-10 concentrations. No significant differences were found on serum TNF-α level 30 minutes after supplements intake. However, serum IL-6 and IL-10 concentrations significantly decreased 60 minutes after LEU supplementation when compared to PLA intervention ($p < 0.05$). Conclusions: These preliminary results demonstrated that free leucine supplementation, but no within a mixture of BCAAs can influence serum cytokine pattern, in order to promote a more anti-inflammatory patter, in healthy humans.

Abbreviations: BCAAs: Branched-Chain Amino Acids; IL-6: Interleukin-6; IL-10: Interleukin-10; LEU: Leucine-Supplemented Group; PLA: Placebo-Supplemented Group; TNF-α: Tumor Necrosis Factor-Alpha

Background

Several animal studies have demonstrated that BCAAs, particularly leucine, exert significant effects on skeletal muscle protein such as stimulation of muscle protein synthesis (primarily through translation initiation [1], attenuation of muscle proteolysis [2], effects on glucose homeostasis through increased insulin secretion [3,4], and possibly interactions with innate immune system with resulting modified cytokines expression [5]. Therefore, given the physiological effects of these amino acids in animal models, the understanding of the physiological and metabolic responses modulated by the ingestion of individual and mixed BCAAs in human volunteers are of interest.

Inflammatory status associated with physiopathological situations such as cancer [6], sepsis [7], burning [8], trauma [9], and obesity [10] are often characterized by high levels of serum pro-inflammatory cytokines or Interleukins (IL). In this context, we hypothesize that BCAAs can indirectly interact with the innate immune system. Then, we tested the effects of BCAAs on both cytokine circulating concentrations and on insulin secretion in human volunteers.

Material and Methods

In a cross-over, double-blind design, eight adult, healthy, and sedentary men were randomly submitted to three experimental conditions: BCAAs, leucine (LEU), and placebo (PLA). Experimental sessions were conducted on different days (7-day washout period). Subjects' characteristics are presented in (Table 1).

Table 1: Subjects' characteristics and food intake of days prior experimental sessions.

	Mean	SE	Minimum	Maximum
Age (years)	24.4	1.2	21.0	30.0
Body weight (kg)	79.1	3.5	63.0	94.0
Height (cm)	179.5	2.5	170.0	188.0
BMI ($kg \cdot m^{-2}$)	24.6	1.0	18.5	27.4
Energy ($kcal \cdot d^{-1}$)	2554.0	394.4	1330.0	4399.0
Energy ($kcal \cdot kg^{-1} \cdot d^{-1}$)	31.7	3.9	18.5	49.8
Carbohydrate ($g \cdot d^{-1}$)	311.7	36.7	143.5	473.6
Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$)	3.9	0.4	2.0	5.0
Protein ($g \cdot d^{-1}$)	147.5	16.0	89.2	233.5
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.9	0.2	1.1	2.5
Nitrogen ($g \cdot d^{-1}$)	23.6	2.6	14.3	37.4
Nitrogen ($g \cdot kg^{-1} \cdot d^{-1}$)	0.30	0.02	0.17	0.39
Fat ($g \cdot d^{-1}$)	79.4	24.9	21.0	208.5
Fat ($g \cdot kg^{-1} \cdot d^{-1}$)	1.0	0.3	0.3	2.5

BMI: Body Mass Index; Data are presented as mean; standard error; minimum and maximum values for each variable. The values originate from 8 volunteers.

Participants were asked to avoid resistance exercise 24 h prior test days and were supplemented with one dose of BCAAs (2.4 g of leucine, 1.6 g of isoleucine, and 1.6 g of valine), LEU (2.4 g of leucine + 3.2 g of alanine), or alanine as PLA (5.6 g of alanine) into capsules (Ajinomoto®, Tokyo, Japan). Food intake was standardized at the day prior to the first experimental session and participants were asked to maintain it each day prior to the subsequent experimental sessions. After an overnight fast, participants ingested a single dose of the supplement and blood samples (15, 30, 60, 90, and 120 minutes) were collected to determine tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and IL-10 concentrations.

All of the procedures were approved by the local Ethics Committee (2009/02896-6). The results are expressed as mean ± SEM and were tested by analysis of variance (ANOVA) one-way (treatment) with a post hoc test with a Tukey adjustment for multiple comparison purposes. The significance level was set at $p < 0.05$.

Results and Discussion

Systemic TNF-α concentrations were evaluated at baseline and after 30 minutes of supplements intake in the 3 interventions. There was no significant difference among interventions at baseline (Figure 1A; PLA 5.6 ± 1.5 pg•mL-1, BCAAs 5.3 ± 0.5 pg•mL-1, and LEU 6.6 ± 0.7 pg•mL-1) and 30 minutes after supplements intake (Figure 1A; PLA 6.4 ± 1.2 pg•mL-1, BCAAs 5.9 ± 0.8 pg•mL-1, and LEU 5.0 ± 1.4 pg•mL-1). TNF-α tended to decrease 30 minutes after leucine supplementation (Figure 1B; LEU -1.6 ± 1.4 pg•mL-1), while tended to increase for the BCAAs and placebo groups (Figure 1B; BCAAs 0.6 ± 1.2 pg•mL-1 and PLA 0.9 ± 1.4 pg•mL-1). At baseline, serum IL-6 concentration was similar in all the interventions (Figure 1C; PLA 46.2 ± 1.3 pg•mL-1, BCAAs 47.2 ± 1.7 pg•mL-1, and LEU 45.5 ± 1.3 pg•mL-1) but was significantly decreased 60 minutes after LEU intake when compared to both PLA and BCAAs ingestions (Figure 1C; LEU 43.8 ± 1.0 pg•mL-1 versus PLA 48.5 ± 1.0 pg•mL-1 and BCAAs 48.3 ± 1.3 pg•mL-1; $p < 0.05$). It was also measured that 60 minutes after LEU intake, serum IL-6 levels significantly decreased (-

1.7 ± 0.3 pg•mL-1) when compared to the BCAAs and PLA ingestions (Figure 1D; $p < 0.05$).

After 60 minutes of PLA intake, serum IL-6 concentration increased (2.3 ± 0.4 pg•mL-1).

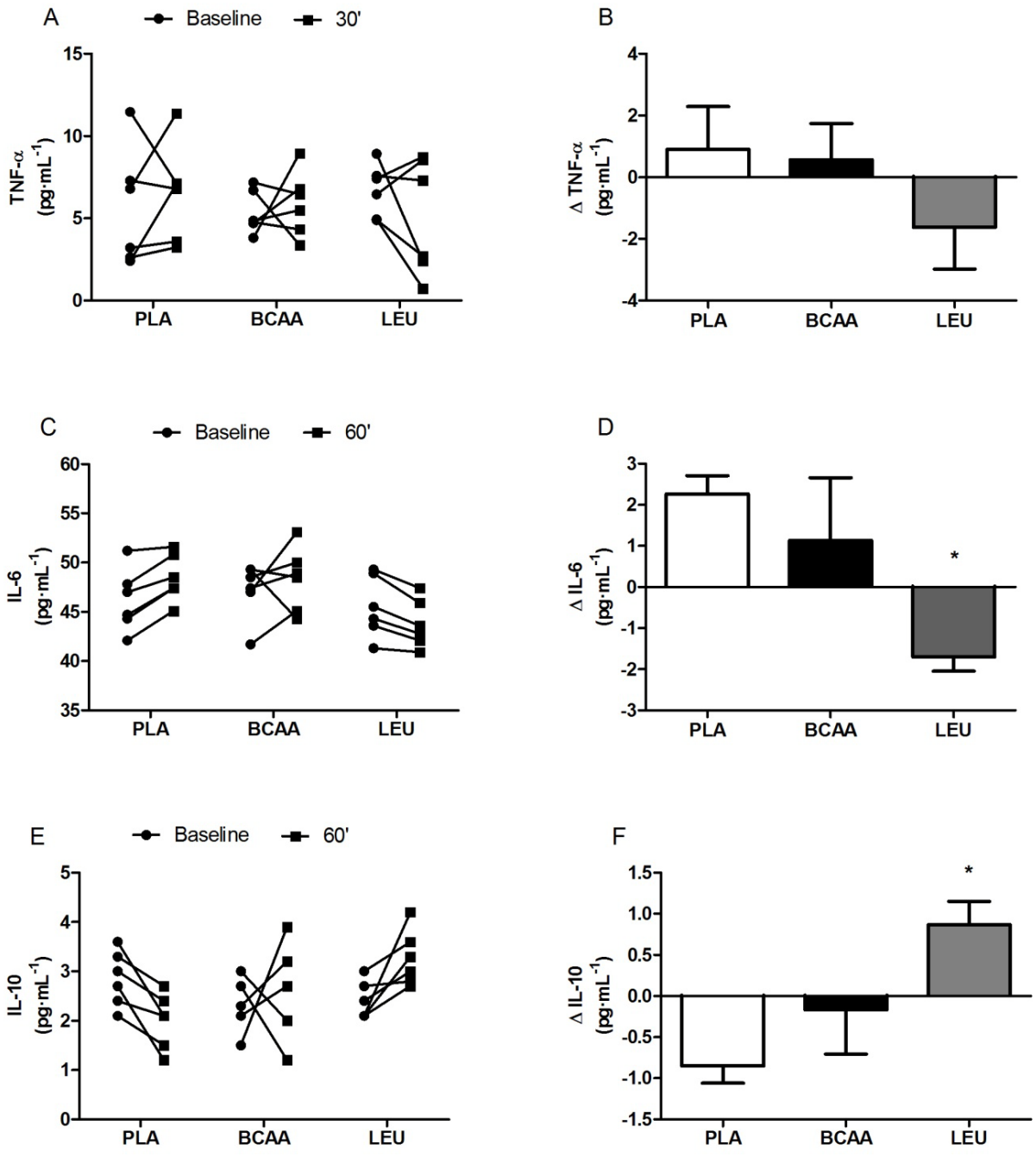

Figure 1: Serum TNF-α (*A*), delta of serum TNF-α (*B*) before and after 30 minutes of supplement intake; Serum IL-6 (C), delta of serum IL-6 (*D*), serum IL-10 (*E*), and delta of serum IL-10 (*F*)before and after 60 minutes of supplement intake; Serum TNF-α/IL-10 ratio (*G*) and delta of serumTNF-α/IL-10 ratio (*H*). Values are mean ± SEM and represent 6 individual independent determinations; * $p < 0.05$ vs. PLA group.

BCAAs promoted high variability in serum IL-6 levels 60 minutes after ingestion (Figure 1D; 1.1 ± 1.5 pg•mL-1) when compared to the other interventions.

At baseline, serum IL-10 levels were similar in all the interventions (Figure 1E; PLA 2.9 ± 0.2 pg•mL-1, BCAAs 2.4 ± 0.2 pg•mL-1, and LEU 2.4 ± 0.2 pg•mL-1) but significantly increased 60 minutes after LEU intake when compared to both PLA and BCAAs ingestions (Figure 1E: PLA 2.0 ± 0.2 pg•mL-1, BCAAs 2.3 ± 0.4 pg•mL-1, and LEU 3.3 ± 0.2 pg•mL-1; $p < 0.05$). The delta values measured demonstrated that 60 minutes after LEU intake, serum IL-10 concentration was significantly increased when compared to BCAAs and PLA ingestions (Figure 1F; PLA 2.9 ± 0.2 pg•mL-1, BCAAs 2.4 ± 0.2 pg•mL-1, and LEU 2.4 ± 0.2 pg•mL-1; $p < 0.05$). BCAAs also promoted high variability in serum IL-10 concentration 60 minutes after ingestion (Figure 1F; PLA -0.9 ± 0.2 pg•mL-1, BCAAs -0.2 ± 0.5 pg•mL-1, and LEU 0.9 ± 0.3 pg•mL-1; $p < 0.05$).

Conclusions

These preliminary results demonstrated that free leucine supplementation, but no within a mixture of BCAAs can influence serum cytokine pattern, in order to promote a more anti-inflammatory patter, in heathy humans. Further work is required to test this branched-chain amino acid in other situations of moderate and severe inflammation for instance in pathological and catabolic conditions characterized by systemic and muscle pro-inflammatory status associated with muscle wasting.

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