

Article type : Letter to the Editor

Isoleucine and Atopic dermatitis

To the Editor,

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases, especially during childhood, with an estimated prevalence of 11% in Latin America (1). The complexity of AD clinical phenotypes occurs due to the existence of different physiopathological pathways for the illness, including changes in innate and adaptive immune responses, skin barrier dysfunction and allergic sensitization (2).

Lipid precursors such as ceramides and different proteins compose a natural moisturizing factors (NMF) that play an important role for skin barrier function. Besides that, an intense reduction in the production of antimicrobial peptides of the skin, mainly β -defensin, is a hallmark of dermatitis. Bacterial colonization is one of the consequences of this reduction, accentuating the severity of the disease (3).

Leucine, isoleucine (IsL) and valine are essential branched-chain amino acids (BCAA) found especially in meats, cow's milk, and legumes. It is inferred that a diet rich in these amino acids may reduce the loss of muscle mass due to a reduction in their catabolism, including during periods of higher metabolic activity, as happens in the presence of acute infections. Oral supplementation of leucine and IsL works as an important non-pharmacological tool for modulating protein turnover, as these amino acids increase signaling for protein synthesis and have anti-inflammatory activity (4).

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In diseases which require a hypoproteic diet, such as *Maple syrup urine disease* (MSUD), another evidence of a link between IsL and skin health is observed. IsL restriction may trigger a severe dermatological condition, with skin dryness and eczematous lesions, especially in flexures and periorificial areas, similar to what is found in enteropathic acrodermatitis, and may also cause bacterial infections (5).

Thereby, we set out to assess the effects of oral IsL supplementation in patients with AD.

This was a prospective pilot study during sixty days. After approval by the Research Ethics Committee, the first twenty AD patients monitored in a specialized outpatient clinic (aged between five and eighteen years old) have been invited to participate in the study. Patients with food allergies (due to biases related to poor IsL ingestion), kidney or liver disease, difficulty in food swallowing, or using corticosteroids or oral immunosuppressors, have been excluded from the study.

The patients were evaluated in five medical visits at fortnightly intervals. During their first visit, they were evaluated by the SCORAD index (SC) (6) applied independently by two examiners (mean scores) and were submitted to tests for kidney and liver function, blood count, quantitative HPLC (high performance liquid chromatography) of serum amino acids and measurement of specific IgE antibody to a broad spectrum of allergen (ImmunoCAP® ISAC Thermo Fisher – Uppsala - Sweden). Patients have been guided to maintain previously prescribed treatments (moisturizers and topical anti-inflammatories) without any changes. Supplementation with powdered IsL (10-30mg/kg/day) was then initiated, based on twice the recommendations on dietary reference intake (DRI).

During the second, third and fourth visits, patients were clinically assessed with a checklist of medications in use. During the fifth visit, a new SC index was obtained (by the same two examiners who applied the initial test), along with new liver and kidney assessments and a new amino acid chromatography. Photographs of the patients and a 24 h diet recall (24R) were obtained in all visits.

Only one of the twenty patients admitted to the study did not complete it, since the child did not agree to ingest the powdered amino acid. Among the other nineteen

patients, 4 (21%) were males; Mean age was 9 years (range: 5-17). According to SC results, 4 patients (21%) had mild AD, 9 moderate AD (47%), and 6 severe AD (32%). All patients reported adequate diet and IsL intake for their age, according to 24R mean. The other tests were normal for all patients.

In the ImmunoCAP ISAC, all patients (100%) presented sensitization to aeroallergens, with mites (nDerp1 and nDerp2) and cat (rFeld1) being the most prevalent components. No patient was sensitized to food components.

Serum IsL values, SC, objective SCORAD (OSC) and pruritus grades (1 to 10; being ten, the worse pruritus possible) before and after oral IsL supplementation are shown in Table 1. All patients showed statistically significant clinical improvement after supplementation, based on SC and OSC scores. (Figure 1). The most remarkable reduction was noticed in pruritus scores (P). Despite supplementation, no statistically significant difference was found for serum IsL concentrations. No significant correlation (Spearman's rank correlation) was found between initial and final values of IsL and SC, OSC or P. The reduction in scores after IsL supplementation did not depend on AD severity (Table 2). Statistical analyses have been carried out using the software SPSS (Statistical Package for the Social Sciences) version 20.0.

IsL is not one of the amino acids frequently found in NMF as occurs with glycine, alanine, and ornithine (7). Therefore, BCAAs do not seem to act on the structure itself, but rather on inflammatory pathways. Based on this, the paradigm of IsL metabolism, previously thought restricted to energy production and protein synthesis, has its meaning extended. Research suggests that BCAAs can indirectly modulate the inflammation of muscle cells by producing glutamine used in the usual metabolism of essential amino acids. Glutamine acts on immune system cells, especially in the differentiation of B-lymphocytes and macrophages, in phagocytosis and in cytokine production, and also in the coordination of neutrophil superoxide production and apoptosis of various cell types (4).

Furthermore, IsL specifically induces the expression of human β -defensin. Antimicrobial peptides are important for innate immunity since they protect the epithelium against damage caused by microorganisms. Studies of the intestinal microbiota have shown that changes in defensins favor inflammatory pathological

situations, as seen in Crohn's disease and cystic fibrosis (8). The action of BCAAs on the skin is not completely understood. A study had shown that topical IsL seems to stimulate the production of β -defensin (9); however, the consequences of oral IsL supplementation had not been assessed at the time that study came out.

The present study found no statistically significant increase in serum IsL levels after supplementation with this amino acid, and both initial and final IsL concentrations in patients were within the normal range (general population). Therefore, despite having assessed a small number of patients, which makes statistical analyses relatively more difficult, we consider that a possible hypothesis to explain the absence of significant increases in serum IsL would be that, in patients with adequate intake, serum IsL levels keep stable and are predominantly directed to the areas with higher consumption. A different situation is found in metabolic diseases with dietary IsL restriction. In these cases, there seems to be greater difficulty in reaching homeostasis, with more extreme variations in serum concentrations of this amino acid (5).

This study has some limitations. A control group of patients with the same severity of AD, treated with placebo, has not been done. Furthermore, frequent medical visits may produce a bias towards better adherence to medications already in use, improving the treatment, even in a short follow-up period.

To conclude, IsL supplementation may be one of the complementary treatments for AD, acting through an anti-inflammatory pathway and regulating one of the pillars of innate immunity in a simple, low-cost way. More studies will be needed to complement this first assessment.

Table 1: Serum isoleucine levels*, SCORAD (SC), objective SCORAD (OSC) and pruritus grades ($\pm$ standard deviation) before and after oral isoleucine supplementation for sixty days.

Parameter	Initial	Final	Difference	p ¹
Isoleucine	52.0 $\pm$ 12.7	58.3 $\pm$ 21.5	6.2 $\pm$ 22.6	0.244
SC	40.2 $\pm$ 19.4	21.0 $\pm$ 13.3	-19.2 $\pm$ 11.4	<0.001
OSC	30.4 $\pm$ 17.1	13.6 $\pm$ 10.1	-16.8 $\pm$ 10.7	<0.001
P	6.6 $\pm$ 2.0	3.3 $\pm$ 1.6	-3.4 $\pm$ 1.5	<0.001

¹ Paired T test; P=Pruritus; SCORAD= Scoring Atopic Dermatitis; *amino acid chromatography (HPLC); Kolmogorov-Smirnov tests show that data for all variables are approximately normally distributed.

Table 2: Percentage of reduction in relation to initial values for SCORAD (SC), objective SCORAD (OSC) and pruritus grades after supplementation with oral isoleucine.

Parameter	*Mild (N=4)	*Moderate (N=9)	*Severe (N=6)	p ¹
SC	-49.9 $\pm$ 12.0	-52.5 $\pm$ 24.4	-41.6 $\pm$ 13.6	0.57
OSC	-59.1 $\pm$ 8.6	-59.4 $\pm$ 25.4	-49.3 $\pm$ 12.3	0.59
P	-60.8 $\pm$ 7.9	-54.7 $\pm$ 22.6	-39.2 $\pm$ 22.0	0.23

¹ ANOVA. P= Pruritus. *AD severity.

Figure 1: Photos of patients before and after oral isoleucine supplementation

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Patient 1



Before



After

Patient 2



Before



After