Orignal Article

Nutraceutical Immunomodulation on Acute Inflammation by Abdominal Plastic Surgery

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ABSTRACT

Background: Abdominal plastic surgery is characterized as a surgical trauma which promotes systemic inflammation. Preoperative nutrition can minimize postoperative complications and improve the surgical outcome. This article aims to review the role of nutrition on inflammation in the patients submitted to abdominal plastic surgery.

Material and methods: In the present study, patients were offered two oral nutritional supplements (ONS) once a day for 7 days in the preoperative period. The ONS1 (negative control) consists of a dairy drink, while the ONS2 (positive control) was a drink with immunomodulatory nutraceuticals. Each patient ingested 200 ml of the supplements (ONS1 or ONS2) mixed with 50 grams of diet ice cream. Twenty-five adult patients submitted to a full abdominoplasty were randomly divided into the ONS1 and ONS2 groups. The clinical and laboratory evaluations were performed at three different stages: (T0) on the first day of start of supplementation; (T1) on the anesthetic induction of the surgery; (T2), 24 h after the procedure. The evaluated parameters were: HSP27, HSP70, IL-1β, IL-6, CRP and TNF-α.

Results: There were no statistically significant differences between the experimental groups for all analyzed supplementation times.

Conclusion: Therefore, the oral nutritional supplementation using L-alanyl-glutamine, arginine, and oil mixtures with both high ω-9:ω-6 ratio and low ω-6:ω-3 ratio and containing ω-3 fatty acids (ALA, EPA and DHA) has no nutraceutical preconditioning effect on the shock proteins and inflammatory mediators of surgical trauma that were studied.

Keywords: abdominoplasty, amino acids, fatty acids glutamine, heat shock protein.

Introduction

Abdominal plastic surgery consists of lower abdominal lipectomy, associated with muscle-facial repair or "plastic", through the plication of the aponeurosis of the rectus abdominis muscles. Preoperative nutrition has led to a greater critical impact of postoperative complications and improvements in surgical results. The administration of nutraceutical formulas has been widely used to improve the recovery of patients undergoing surgical conditions. These formulas contain nutrients that are not synthesized by the human body, which are referred to as "essential nutrients" [1]. It is well-known in the surgical field that clinical nutrition plays an important role in surgical outcome [2].

A postoperative immunonutrition performed aggressively incapable of preventing immunosuppression in the first week after the procedure. It is only able to improve the immune and catabolic response to trauma, not to reverse it. Therefore,

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starting immunonutrition in the preoperative period seems to be more effective, as it improves the nutritional status and reduces postoperative complications. This observation strengthens the current concept that nutrients capable of modulating immunological functions must be administered before surgery, so that there is already an increase in tissue and plasma levels at the time of trauma [3].

Currently, there is a wide variety of nutritional supplementation therapy that can be used clinically. The development of an immunomodulatory diet has reversed many of the immune-mediated changes seen after the surgery [4,5]. These balanced nutritional formulations supplemented with specific nutrients (arginine, glutamine, ω-3 fatty acids, and antioxidants, i.e., ascorbic acid and selenium) are able to improve the immune function and modulate inflammation [2].

Therefore, studies investigating the impact of the administration of solutions containing amino acids and fatty acids before the establishment of the inflammatory process must be carried out in order to elucidate the various mechanisms involved in the development of inflammation and its secondary manifestations. Thus, this paper seeks to review the role of nutrition on inflammation in abdominal plastic surgery patients.

Material and methods

Ethical approval

This study was conducted with the approval of the Ceará Federal University (COMEPE 176/11) and São Carlos Hospital (CONEP/CNS – MS 5043). All participants provided a written consent of their willingness to participate in this study.

Subjects and type of study

The clinical study is a prospective, randomized, controlled, double-blind study with 25 patients submitted to abdominal plastic surgery.

Inclusion criteria

For the present study, we used the following inclusion criteria for the patients: (a) must be over 18 years of age; (b) must be hemodynamically stable; (c) must have a platelet count within the normal range; (d) must have signed the informed consent form of the doctor and family members/guardians of the patient.

Exclusion criteria

The study excluded patients with: (a) immunosuppressive therapy; (b) are thrombocytopenic and/or is a chronic user of potent antiplatelet drugs such as Clopidogrel, Heparin, Aspirin, etc.; (c) diabetes mellitus; (d) renal failure; (e) liver failure; (f) heart disease and/or undergoing stent angioplasty; (g) dyslipidemic or are using statins; (h) liver disease; (i) pancreatitis.

Nutraceutical immunomodulatory formula

1. Oral Nutritional Supplementation 1 (ONS1): Negative control - Dairy drink with 0% fat and consisting of 64% carbohydrate (100% maltodextrin) and 36% protein (100% calcium caseinate). Each 100 ml of the beverage contains 37.4 kcal of total calories, and each patient consumed 200 ml of the supplement per day, blended with 50 g of diet ice cream.
2. Oral Nutritional Supplementation 2 (ONS2): Hyperlipidic supplement composed of 14% carbohydrate (25% maltodextrin and 75% fructose), 25% protein (60% L-alanyl-glutamine and 40% L-arginine), and 61% lipids (mixture of high oleic sunflower, canola, fish, and TCM oils). These oils consists of a medium-chain triglycerides containing ω-9, ω-6, and ω-3 fatty acids, the latter being in the form of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The ω-9:ω-6 ratio is of 3.2:1 (antioxidant action) and the ω-6:ω-3 ratio is of 1.4:1 (anti-inflammatory action). The total calories are 200kcal per 100 ml and the supplement is liquidized with 50 g of diet ice cream. The composition of the ONS1 and ONS2 formulas are presented in Table 1.

ONS1: Oral Nutritional Supplementation 1, ONS2: Oral Nutritional Supplementation 2, ω-3: omega-3 fatty acids, ω-6: omega-6 fatty acids, ω-9: omega-9 fatty acids.

Experimental groups

The participants were randomized and allocated into two different groups: ONS1 (n = 11) and ONS2 (n =14), such that the ONS1 group was supplemented with maltodextrin and calcium caseinate, and the ONS2 group was supplemented with pharmaconutrients in nutraceutical doses based on ω-3 oil mix, ω-6, and ω-9 added with L-arginine and L-alanyl-glutamine (L-Ala-Gln). Blood samples were analyzed at times, T0 (time zero), that is, the time before supplementation (7 days before of the surgical procedure); T1 (time one), that is, the time of the anesthetic preparation of the patient for the surgery (collected inside the operating room); and T2 (time two) that is, 24 h after the procedure (Figure 1).

Table 1: Composition of the ONS1 and ONS2 formulas Composition in 200 mL

Nutrients	ONS1	ONS2
Total calories (kcal)	74,8	400
Carbohydrates (g)	12	14,26
Protein (g)	6,72	25
Fat (g)	0	27
L-arginine (g)	0	10
L-alanyl-glutamine (g)	0	15
ω-3 (g)	0	3,24
ω-6:ω-3 ratio	0	1,4:1
ω-9:ω-6 ratio	0	3,2:1

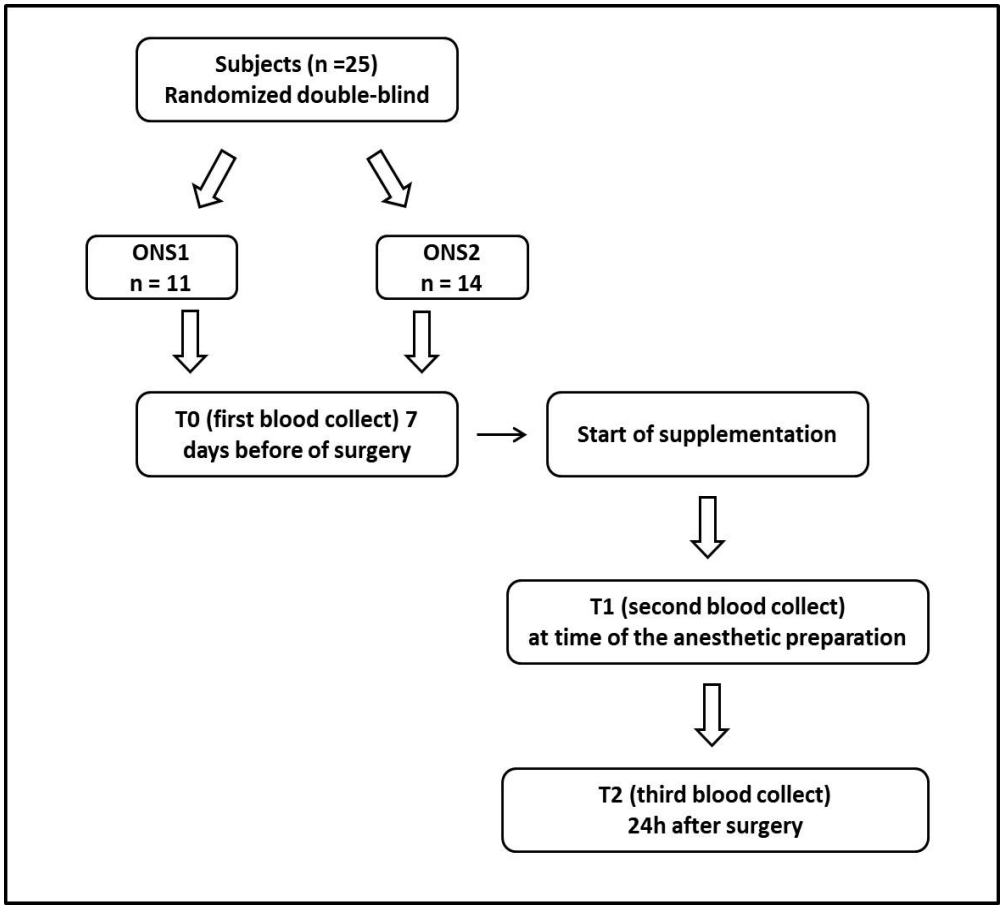

Figure 1. Experimental design protocol. ONS1: Oral Nutritional Supplementation, ONS2: Oral Nutritional Supplementation 2, T0 (time zero) - 7 days before the surgery, T1 (time one) - at the time of the anesthetic preparation, T2 (time two) – 24 h after the surgery.

Masking method

Both supplements ONS1 and ONS2 were produced by Nutrimed Industrial LTDA, (Fortaleza, Ceará, Brazil), a company specializing in the manufacture of enteral nutrition. The formulations were developed in such a way that it is impossible to identify the compositions of each product before opening the data regarding it. In order to ensure the double-blind nature of the study, all the production and formula controls were carried out by a nutritionist employed by the company who is not directly involved in the study. At the end of data collection and statistical analysis, the real formulation of ONS1 and ONS2 was revealed to analyze the results. After the production, the supplements went through a microbiological analysis, and were then released for administration to patients with the same sensory characteristics and packaging. The only difference between them was the production batch and the impression of ONS1 or ONS2 on the plastic bottle.

Randomization method

The randomization method was carried out by generating random numbers through the website http://www.graphpad.com/quickcalcs/RandMenu.cfm. The randomization process was followed by the concealment procedure, in which no one directly involved in the care of the patient knew what formulation have been used, including the researcher and the patient.

Laboratory evaluation

The laboratory collections were performed with the patients fasting for at least 12 hours. Parameter analysis: ultrasensitive C-reactive protein (CRP), interleucin-IL-1β (IL-1β), interleucin-IL-6 (IL-6), tumor necrosis factors-α (TNF-α), heat shock protein (HSP27), and heat shock protein (HSP70) were performed using blood samples (15 ml) taken at four different times: (1) pre-surgical, (2) in-anesthetic induction, (3) 1st postoperative day, and (4) 3rd postoperative day.

Statistical analysis

The study data were entered into the Excel software for Windows, Microsoft version 2007, and was analyzed using the Graphpad Prism software version 5.0 for Windows. The Shapiro-Wilk test was applied to verify the normality of the data. The variables that followed the normality curve were then subjected to the analysis of variance (ANOVA) and the post-test of multiple comparison of Tukey. The -data were

presented with as mean ± standard error of the mean (mean ± SEM). Comparisons were made within and between the groups. A statistical significance was observed when $p<0.05$ was obtained.

Results

Effect of nutraceutical immunomodulatory solution on IL-1β levels

We did not verify statistically significant differences at the IL-1β levels between the experimental groups ONS1 (T0: 0.37 ng/mL; T1: 0.41 ng/mL; T2: 0.44 ng/mL) and ONS2 (T0: 0.62 ng/mL; T1: 0.53 ng/mL; T2: 0.50 ng/mL) for all analyzed moments, i.e., times T0, T1, and T2 (Figure 2).

Effect of nutraceutical immunomodulation solution on IL-6 levels

Similar to the IL-1β results, no statistically significant differences at the IL-6 levels were observed between the experimental groups ONS1 (T0: 2.46 ng/mL; T1: 1.89 ng/mL; T2: 42.87 ng/mL) and ONS2 (T0: 1.57 ng/mL; T1: 1.27 ng/mL; T2: 30.58 ng/mL) for all analyzed times T0, T1, and T2 (Figure 3).

Effect of nutraceutical immunomodulation solution on TNF-α levels

The nutraceutical immunomodulation formula was unable to show statistically significant differences at the TNF-α levels

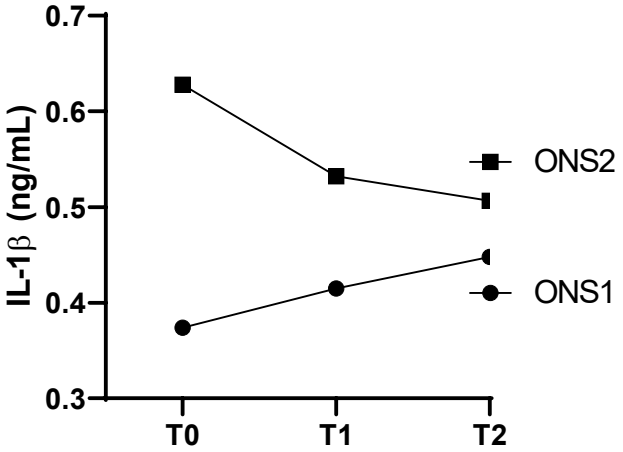

Figure 2. Nutraceutical immunomodulation effect on IL-1β levels. T0 – before supplementation, T1 – at time of the anesthetic preparation, T2 – 24 h after surgery. ONS1: Oral Nutritional Supplementation 1 (control group), n=11. ONS2: Oral Nutritional Supplementation 2 (test group), n=14. Values are means ± SEM, $p > 0.05$ between experimental groups (ONS2 *vs*. ONS1).

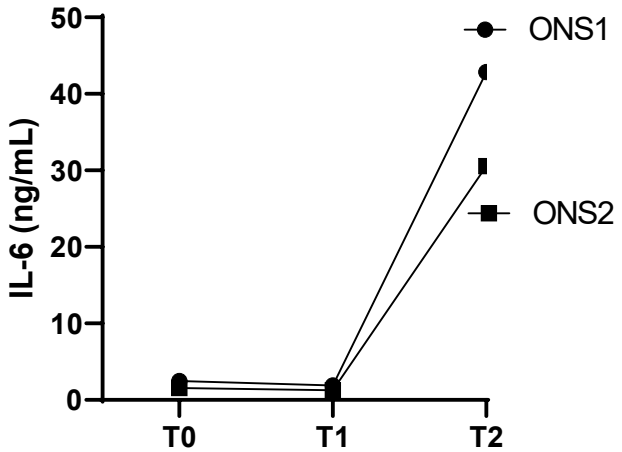

Figure 3. Nutraceutical immunomodulation effect on IL-6 levels. T0 – before supplementation, T1 – at time of the anesthetic preparation, T2 – 24 h after surgery. ONS1: Oral Nutritional Supplementation 1 (control group), n=11. ONS2: Oral Nutritional Supplementation 2 (test group), n=14. Values are means ± SEM, $p > 0.05$ between experimental groups (ONS2 *vs*. ONS1).

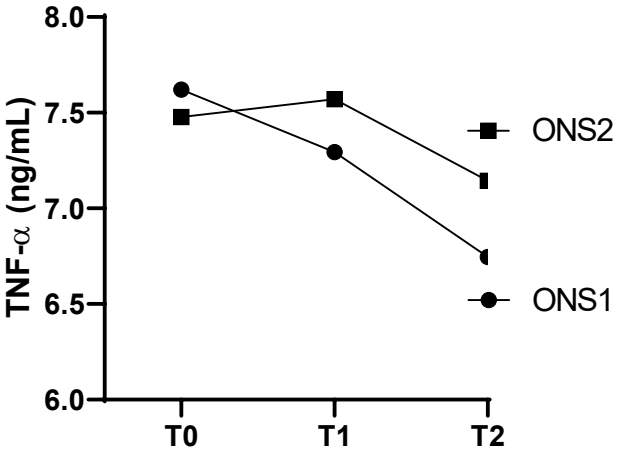

Figure 4. Nutraceutical immunomodulation effect on TNF-α levels. T0 – before supplementation, T1 – at the time of the anesthetic preparation, T2 – 24 h after the surgery. ONS1: Oral Nutritional Supplementation 1 (control group), n=11. ONS2: Oral Nutritional Supplementation 2 (test group), n=14. Values are means ± SEM, $p > 0.05$ between experimental groups (ONS2 *vs*. ONS1).

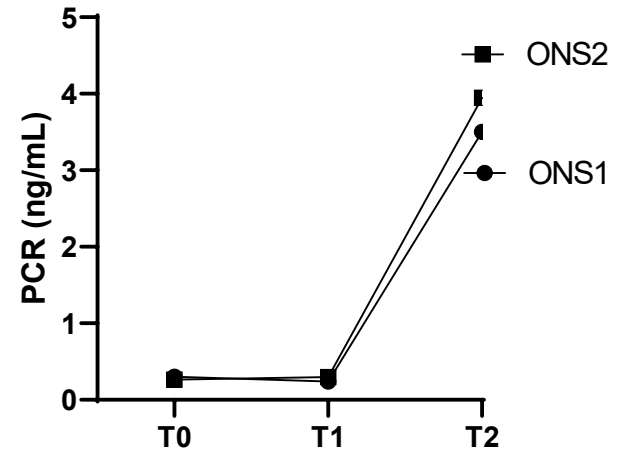

Figure 5. Nutraceutical immunomodulation effect on CRP levels. (T0 – before supplementation, T1 – at time of the anesthetic preparation, T2 – 24h after surgery. ONS1: Oral Nutritional Supplementation 1 (control group), n=11. ONS2: Oral Nutritional Supplementation 2 (test group), n=14. Values are means ± SEM, $p > 0.05$ between experimental groups (ONS2 *vs*. ONS1).

between the experimental groups ONS1 (T0: 7.62 ng/mL; T1: 7.29 ng/mL; T2: 6.74 ng/mL) and ONS2 (T0: 7.47 ng/mL; T1: 7.57 ng/mL; T2: 7.14 ng/mL) for all analyzed times T0, T1, and T2 (Figure 4).

Effect of nutraceutical immunomodulation solution on CRP levels

Statistically significant differences at CRP levels between groups ONS1 (T0: 0.30 ng/mL; T1: 0.23 ng/mL; T2: 3.50 ng/mL) and ONS2 (T0: 0.26 ng/mL; T1: 0.29 ng/mL; T2: 3.94 ng/mL) for times T0, T1, and T2 (Figure 5) was not observed.

Effect of nutraceutical immunomodulation solution on HSP27 levels

The female participants supplemented with the immune-enhancing solution did not show any statistically significant differences at their HSP27 levels when comparing the groups ONS1 (T0: 13.924 ng/mL; T1: 11.627 ng/mL; T2: 11.022 ng/mL) and ONS2 (T0: 10.816 ng/mL; T1: 12.619 ng/mL; T2: 11.418 ng/mL) at all times T0, T1, and T2 (Figure 6).

Effect of nutraceutical immunomodulation on HSP70 levels

No statistically significant differences were verified at HSP70 levels between the groups ONS1 (T0: 4.711 ng/mL; T1: 4.916 ng/mL; T2: 3.912 ng/mL) and ONS2 (T0: 4.538 ng/mL; T1: 7.892 ng/mL; T2: 3.210 ng/mL) for times T0, T1, and T2 (Figure 7).

Discussion

We investigated the effect of an oral immune-enhancing nutritional supplement on the preoperative host defense on inflammation profile in abdominal plastic surgery. However, we did not observe any significant change in the concentration of postoperative inflammatory mediators when the patients' supplemental diet is modified.

The general physiological (or pathophysiological) result of the inflammatory response depends on which cells are present, the nature of the stimulus, the timing of the generation, and concentrations of eicosanoids [6]. Therefore, diets rich in arginine, glutamine, ω-3 PUFAs, and other nutritional immunomodulators may be used as strategies to reduce postoperative inflammatory markers.

The improvement by immune-supplementation diet seems to occur with some delay [7]. Several studies have confirmed its advantage of treatment before injury the host [8-12]. Therefore, several reports have found that supplementation with arginine could have a clinical benefit in immune responses [13–15] or have no positive effects [10,16]. ω-3 PUFAs also presented anti-inflammatory and immunostimulatory properties when the synthesis and release of eicosanoids are modulated [11,17-20].

It is known that glutamine improves protein synthesis and inhibits protein degradation [21]. This amino acid is very important during times of severe stress caused by surgery or illness as its concentration later tends to decrease within skeletal muscles [21-23].

The nutraceutical action of the supplement used in this study does not seem be effective for modulating the inflammatory condition generated in the post-surgical period of a total abdominoplasty. Nevertheless, our study seems to be one of the pioneers in the literature to investigate the effects of preoperative supplementation with a high ω-9:ω-6 ratio, a low ω-6: ω-3 ratio, and containing ω-3 acids (ALA, EPA, and DHA), L-Ala-Gln, and L-arginine on the inflammatory response modulation of a surgical trauma resulting from abdominoplasty.

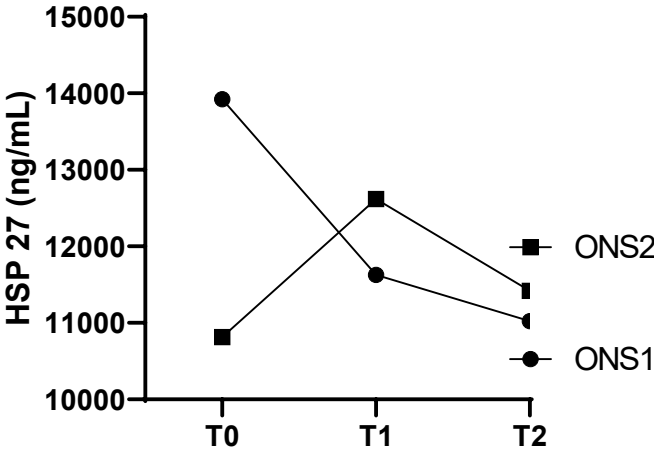

Figure 6. Nutraceutical immunomodulation effect on HSP-27levels. T0 – before supplementation, T1 – at the time of the anesthetic preparation, T2 – 24h after the surgery. ONS1: Oral Nutritional Supplementation 1 (control group), n=11. ONS2: Oral Nutritional Supplementation 2 (test group), n=14. Values are means ± SEM, p > 0.05 between experimental groups (ONS2 *vs.* ONS1).

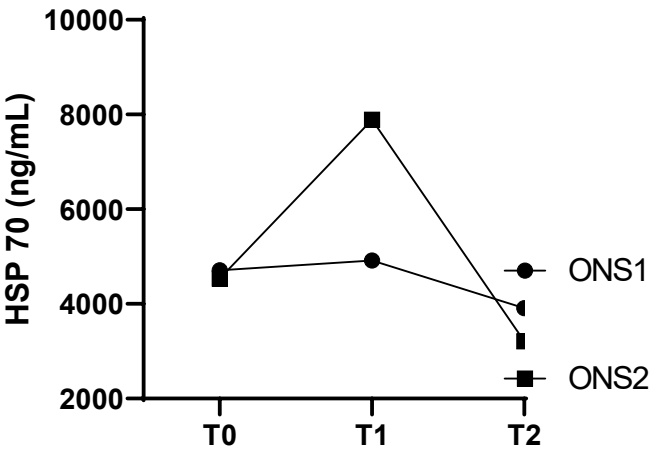

Figure 7. Nutraceutical immunomodulation effect on HSP-70 levels. T0 – before supplementation, T1 – at the time of the anesthetic preparation, T2 – 24 h after surgery. ONS1: Oral Nutritional Supplementation 1 (control group), n=11. ONS2: Oral Nutritional Supplementation 2 (test group), n=14. Values are means ± SEM, p > 0.05 between experimental groups (ONS2 *vs.* ONS1).

Systemic inflammation is one of the aspects that occur in patients undergoing surgery, although inflammation is essential for a successful recovery after an injury, an uncontrolled systemic inflammatory response can cause adverse outcomes [24].

The increased expression of the bearing molecules on the surface of endothelial cells, and selectins in response to pathogens or cytokines are mainly produced by activated macrophages TNF-α and IL-1β [25]. In our study, the supplemented patients were evaluated at three different moments and did not show a considerable difference at their IL-1β levels between experimental groups ONS1 and ONS2 at any evaluation time. These results show that inflammations from plastic surgery could not be controlled or minimized by using nutraceutical formulas.

It has been reported that injury increases the release of pro-inflammatory cytokines such as TNF-a, and IL-6 levels [25]. Among other molecules, IL-6 is a pleiotropic cytokine with a wide range of biological activities, among which are the regulation of immune and inflammatory responses [26]. The kinetics of inflammatory IL-6 showed a sharp increase after surgery, but to a lesser extent for the supplemented group in comparison to the control group. Furthermore, the concentration of IL-6 returned to the preoperative values for the supplemented group within eight days after the surgery, an effect that was not observed for the control group [7].

Later, they also observed that the perioperative administration of an enteral diet enriched with arginine, ω-3 PUFAs, and RNA reduces the plasma levels of IL-6. Another study also verified a decrease in the serum levels of IL-6 before and after the surgery by means of the preoperative and perioperative supplementation [27]. An *in vitro* study showed the presence of dermal fibroblast cells incubated with ω-3 PUFAs, which increases the inhibition of IL-6 expression [28]. Thus, this inhibitory action on IL-6 expression may be important for therapeutic applications on scar tissue, since a high expression of IL-6 in hypertrophic scars was reported [29].

In contrast, the patients supplemented with the nutraceutical formulas evaluated in this paper did not present any significant difference at the IL-6 levels between experimental groups ONS1 and ONS2 for any experimental time. These results suggest that the immune-enhancing diet did not affect the IL-1β and IL-6 levels of women submitted to plastic surgery. On the other hand, studies on inflammation levels of gastric cancer patients saw an increase in the IL-6 cytokine [30-32]. The IL-6 levels of the patients saw a rise after the oral intake (600 mL/d) of an immune-modulatory supplementation liquid containing 600kcal, 33.6g of proteins, 7.5g of arginine, 0.7g of EPA, 1.1g of DHA, and 1.2g of nucleotides (Impact; Nestle, Sao Paulo, Brazil) [30].

An arthritis model also reported that the inhibition of IL-6 improves collagen-induced arthritis, which mimics a disease that causes an acute inflammation, generating hypernociception [33]. Studies have shown that IL-6 induces the IL-1β production in inflammatory mechanical hypernociception in rats. In addition, it was demonstrated that the IL-6 inhibition, caused by the use of antibodies against IL-6, prevents the TNF-α production, which is responsible for plantar mechanical hypernociception. The antinociceptive effect stems from the inhibition of the cytokine cascade underlying IL-6 [34]. Thus, it appears that both IL-1β and IL-6 levels sequentially precede the release of eicosanoids in order to induce hypernociception in rats [33]. Moreover, a positive correlation ($p < 0.05$) was found between IL-1β and IL-6 levels for experimental group ONS2, which received a nutraceutical pre-immunomodulation, at the time of the anesthetic preparation of the patient for the surgery (T1) and 24 h after the surgery (T2).

A decrease in the production of IL-1 **β**, Il-6 and TNF- α by macrophages has been reported after endotoxin injection, burns and surgeries [34,35]. In addition, studies in healthy humans showed a significant immunomodulatory effects related to the ω-3 PUFAs. It was verified that there was a decrease in the production of inflammatory parameters such as IL-1β, IL-6, and TNF-α, when administrating more than 2.3 g EPA plus DHA per day [34].Glutamine improves the tissue metabolism and reduces oxidative stress and pro-inflammatory cytokine such as TNF-α. A study reported a reduction in the TNF-α concentration after using ω-3 PUFAs supplement [36]. In addition, a positive correlation ($p < 0.05$) was observed between TNF-α and IL-6 levels for the experimental group ONS1, which did not receive the nutraceutical pre-immunomodulation at the time of the anesthetic preparation of the patient for the surgery (T1) and 24 h after the surgery (T2).

An i*n vitro* study using over 2.3 g of EPA + DHA/day showed a reduction in the TNF-α production by mononuclear cells [36]. In contrast, the study noted that patients supplemented with the evaluated nutraceutical formula did not present a pronounced difference in their TNF-α levels between experimental groups ONS1 and ONS2 at any experimental time. Similarly, Nakamura *et al.* observed low levels of TNF-α before and after the surgery. Also, no significant difference was verified between the supplement and control groups regarding the evaluation of the influence of preoperative administration of immune-enhancing diet on inflammatory and immune responses in patients undergoing major surgery for cancer [37].

According to them, the reason for this discrepancy may be due to the short biological half-life of TNF-α in circulation [37]. Thus, the similarity we observe between the ONS1 and ONS2 experimental groups concerning the plasma levels of TNF-α may be due to the local character of its expression, its short half-life or differences in the sensitivity and specificity of the assays used in the studies. The amount of nutrients used might also be relevant since studies in humans use less fish oil than most animal studies. However, for studies using nutraceutical formulas that do not affect immunological

or inflammatory activities, the different conclusions may be related to the different experimental protocols used, particularly those involving cell preparation and culture, or cytokine assays as well as the different characteristics of the participants (gender, age, usual diet) [34].

C-reactive protein (CRP) is an acute phase protein synthesized by the liver in response to circulating inflammatory cytokines, such as IL-6, and to infectious processes, being also associated with the risk of postoperative complication [38,39]. In a routine analysis, the CRP levels are from 0.4 to 0.5 mg/dL, while ultra-sensitivity methods can detect it until 0.09mg/dL [40]. Several reports noted high CRP levels following injuries that decrease after nutraceutical supplementation. Some studies observed that a perioperative administration of an enteral diet enriched with immunonutrients reduce the CRP plasma levels [41,42]. A study investigating inflammation levels in gastric cancer patients saw an increase in the CRP levels [31]. The rising levels of CRP, over 0.8 mg/dL in cancer patients were associated with postoperative complications and a shorter 30-day survival[43]. In addition, patients submitted to percutaneous endoscopic gastrostomy, and those with high CRP levels (over 1.0 mg/dL) had a mortality rate of 20.5% in comparison to 2.6% among the patients with normal values. Another study evaluated that the risk of death increases by seven times [44]. Also, for some researchers, it is evident that the increase in CRP is induced by the increase in IL-6 levels [43,45].

In a randomized study with indication for surgery for repair of cleft lip and palate for children, a 20% L-Ala-Gln solution (administrated by intravenous infusion three hours before the surgery) was able to decrease the high CRP levels found in the postoperative period [46]. Our results reveal that patients supplemented with the nutraceutical formula did not show a considerable difference in their CRP levels between ONS1 and ONS2 experimental groups for any of the analyzed times.

Heat shock proteins (HSP) are known for their cytoprotective capacity, and their concentration increases after stressful events [47]. Glutamine metabolism is closely related to the optimal regulation of the HSP response at both transcriptional and post-transcriptional levels [48]. Several studies have shown that glutamine induces HSP production, and increases the cell survival against various stress conditions observed *in vitro* and in Ischemia/Reperfusion models [49-51]. The small 27-kDa HSP (HSP27) is involved in a wide range of cellular protection processes against denaturation. This protein binds directly to certain structural proteins in the sarcomeres in order to protect them against denaturation, and it may eventually reduce cell apoptotic pathway activation [58,59]. Also, the HSP27 can reduce the uncontrolled activation of the nuclear factor-kβ pathway, attenuating the expression of the pro-inflammatory cytokine genes [54,55].

Previous studies have shown that the pretreatment with ω-3 PUFAs induces an increase in the expression of the HSPs [56,57]. This increased expression is known to reduce the organ injury, attenuate the pro-inflammatory response and improve survival in experimental models for sepsis, shock, critical illness, and injury [53]. A study using fish oil decreases the antibody titers related to HSP27, which is mainly related to general anti-inflammatory effects of ω-3 PUFAs supplements [60].

Therefore, patients supplemented with the nutraceutical formula evaluated in this study did not show a significant difference at their HSP27 levels between ONS1 and ONS2 experimental groups for any experimental time. In addition, a negative correlation ($p < 0.05$) was observed in the ONS1 experimental group (the one not receiving the nutraceutical pre-immunomodulation) for HSP27 *versus* IL-1β levels at the beginning of the supplementation a week before surgery (T0), and for HSP27 *versus* TNF-α at 24 h after the surgery (T2). Likewise, clinical data have shown a correlation between the increased levels of serum HSP70 and improved patient survival after a severe trauma [61]. Studies have shown that HSP27 is regulated by the L-glutamine availability in the tissues under stress [62, 63].

Other data indicated that glutamine can increase the occurrence of deficient lung tissue HSP70 after sepsis [51]. They showed that the lung HSP70 expression was enhanced when GLN was administrated 1 h after the initiation of the sepsis induced in rats, attenuating the tissue metabolic dysfunction observed after sepsis. Furthermore, it led to a significant reduction in the 4 day mortality induced by the sepsis. A previous research revealed a small deficiency of HSP70 expression in the lungs after a sepsis [64]. An *in vitro* model observed that extracellular HSP70 can signal a pro-inflammatory activity through Toll-like receptor-4-dependent pathways [65].

A study using coronary artery bypass grafting vs. patients studied "off-pump" showed that these patients presented low levels of serum HSP70 and high levels of IL-6 [66]. However, HSP70 seems to be part of an immunoregulatory response that potentially down-regulates the inflammatory response [51]. Similar to HSP27, studies have shown that the L-glutamine availability in the tissues under stressful conditions also has an influence on HSP70 [63,67].

Thus, the perioperative administration of an immune-enhancing diet rich in glutamine can attenuate inflammations and postoperative complications. Another report states that glutamine is efficient in inducing the HSP-72 expression in human blood monoclonal cells, improving the TNF-α production in response to the endotoxin [50]. Also, it was verified by a randomized double-blind study that the administration of parenteral glutamine induces HSPs in critical patients [58]. Therefore, despite the experimental results *in vivo* and *in vitro*, patients supplemented with the nutraceutical formula we studied did not show a pronounced difference at their HSP70 levels between experimental groups ONS1 and ONS2 for any experimental time. Also, a positive correlation ($p < 0.05$) was observed between HSP70 and TNF-α levels in the ONS2 experimental group (the one receiving the

nutraceutical immunomodulation) at the beginning of the supplementation, that is, a week before the surgery (T0).

Various researches that use preconditioning nutraceuticals reveal that immunonutrients with an anti-inflammatory effect can be used in countless diseases and acute inflammatory conditions. Our study used a solution with L-Ala-Gln, L-arginine, and fatty acids for 7 days before the surgical procedure and this solution had no effect on inflammation. Previous studies also observed contrasting results [68-70].

Bower *et al.* noted no considerable effects on the outcome in patients receiving <800 mL/d of their immune-enhancing diet [68]. Heslin *et al.* could not find any difference in the postoperative complications when comparing patients treated with immunonutrition or with crystalloid fluid support [69]. Tavares *et al.* found no statistically significant differences for IL-1β, IL-6, TNF-α and HSP27 variables when using immune-supplementation.[70] All these studies suggested that postoperative immunonutrition needs an adequate dosage and administration time in order to be effective, particularly in the first days after the surgery, when the injury-induced depression of the immune defense mechanisms are maximal. The optimal situation should be the opposite that is, guaranteeing sufficient concentrations of the immune-enhancing nutrients before the surgery. Thus, the most rational approach should be the administration of these modified diets in the preoperative period.

Although we carried out a comprehensive assessment, including nutrition, inflammatory, and immune parameters before and after the surgery, our study has its limitations. In fact, the study is a pilot study performed with a small number of participants. Therefore, the confirmation of these results for a higher number of abdominal plastic surgery patients may be required. In addition, the effects of an Ala/Gln/fatty acids supplementation also needs to be evaluated in the dietary intake throughout the study period. A randomized analysis should be performed, as well as the verification of whether a similar effect is obtained under the conditions in which the postoperative protocol is different. Among these mechanisms, there are the nitrergic route by iNOS activity, and the role of inducing inflammation factors, such as NF-kB, which has been shown to be important for experimental studies in acute inflammation.

Conclusion

We investigated the effects of the oral consumption preconditioning of a supplementation consisting of L-Ala-Gln, L-arginine and oil mix with high ω-9:ω-6 ratio and low ω-6:ω-3 ratio, and containing ω-3 acids (ALA, EPA, and DHA) in abdominal plastic surgery patients. Our results indicate that an oral immune-enhancing diet does not extraordinarily improve the acceleration of the inflammatory and immune responses at the early stages after the surgery.

In conclusion, the administration of a supplemented diet significantly improved the outcome for our studied patients in comparison to a conventional treatment. Therefore, we suggest that in a future study, a nutritional analysis should be performed and that surgical patients should be separated according BMI. This approach will enable a better evaluation of the risk of postoperative complications and the impact of the immune-supplementation.

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