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L-glutamine supplementation improves the inflammatory profile of exercised obese elderly

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ABSTRACT

OBJECTIVE

To elucidate the effect of oral L-glutamine (Gln) supplementation, associated or not with the regular practice of combined-exercise training (CET), on glycemic and lipid profile and systemic inflammatory status in elderly subjects.

METHODS

84 elderly subjects, non-practitioners (NP, n=31) and practitioners of CET (n=53), were supplemented with Gln [0.3g/kg of weight plus 10g of maltodextrin, groups: NP-Gln (n=14), and CET-Gln (n=26)], or placebo [10g of maltodextrin, groups: NP-PL (n=17), and CET-PL (n=27)]. Anthropometric and physical data were assessed. Blood sampling was collected pre and post-30 days of supplementation.

RESULTS

NP subgroups showed higher BMI and serum IL-6 levels than CET subgroups before and post-supplementation. Higher serum levels of IL-10 with lower IL-6 and IL-6/IL-10 ratio were observed post-supplementation in the CET-Gln subgroup than pre-supplementation. When the volunteers were separated according to their BMI, higher IL-6 levels were found in all obese (OB) subgroups than in the adequate weight (AW) subgroups before supplementation. This difference was not maintained between OB CET-Gln and AW CET-Gln subgroups post-supplementation. Higher levels of IL-10 with lower IL-6 and IL-6/IL-10 ratio were found in the OB CET-Gln subgroup post-supplementation than pre-supplementation. No differences were found in the glycemic and lipid profile.

CONCLUSIONS

Oral Gln supplementation when associated with the regular practice of CET can modulate the systemic inflammatory status, especially in obese elderly subjects.

DESCRIPTORS

Aging, Inflamm-aging, Body mass index, Obesity, Cytokines, Glycemic and Lipid profile.

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RESUMO

OBJETIVO

Elucidar o efeito da suplementação oral de L-glutamina (Gln), associada ou não à prática regular de exercícios combinados (REC), sobre o perfil glicêmico e lipídico e o estado inflamatório sistêmico de idosos.

MÉTODOS

84 idosos, não praticantes (NP, n = 31) e praticantes de REC (n = 53), foram suplementados com Gln [0,3g / kg de peso mais 10g de maltodextrina, grupos: NP-Gln (n = 14), e CET-Gln (n = 26)], ou placebo [10g de maltodextrina, grupos: NP-PL (n = 17) e CET-PL (n = 27)]. Dados antropométricos e físicos foram avaliados. Amostras de sangue foram coletadas antes e após 30 dias de suplementação.

RESULTADOS

Os subgrupos NP mostraram maiores IMC e níveis séricos de IL-6 do que os subgrupos REC antes e após a suplementação. Níveis séricos mais elevados de IL-10 com menor proporção de IL-6 e IL-6 / IL-10 foram observados após a suplementação no subgrupo REC -Gln do que na pré-suplementação. Quando os voluntários foram separados de acordo com seu IMC, níveis mais elevados de IL-6 foram encontrados em todos os subgrupos de obesos (OB) do que nos subgrupos de peso adequado (AW) antes da suplementação. Esta diferença não foi mantida entre os subgrupos OB REC -Gln e AW REC -Gln pós-suplementação. Níveis mais elevados de IL-10 com IL-6 e razão IL-6 / IL-10 mais baixos foram encontrados no subgrupo OB REC -Gln pós-suplementação do que na pré-suplementação. Não foram encontradas diferenças no perfil glicêmico e lipídico.

CONCLUSÃO

A suplementação de Gln oral quando associada à prática regular de REC pode modular o estado inflamatório sistêmico, principalmente em idosos obesos.

DESCRITORES

Envelhecimento, Inflamação-envelhecimento, Índice de massa corporal, Obesidade, Citocinas, Perfil Glicêmico e Lipídico.

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INTRODUCTION

Aging is a natural and multifactorial process that, in a general way, is characterized by an evident progressive loss of several systems activities, which affect the physiological ability to maintain homeostasis, resulting in cognitive, physical, psychological, and social deterioration. The consequences of aging for the individual and the society have been widely discussed, especially due to the increase in the growth rates of the elderly population in the world[1].

In this sense, studies have shown the manifestation of a chronic, systemic, sterile low-grade inflammation associated with aging, which according to Franceschi et al.[2], is a phenomenon named inflamm-aging. It is noteworthy to mention that, whereas pro-inflammatory cytokines are essentials to prevent the occurrence of infections during many stages of life, particularly in elderly subjects, the systemic elevation of the same cytokines plays an important role in increasing the risk of the development of diseases and comorbidities. For instance, it has been demonstrated that inflamm-aging can favor the loss of lean mass, cognitive decline, the development of atherosclerosis, insulin resistance, among other diseases[3,4].

Based on the literature, the elevation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and the interleukin (IL)-6 in elderly subjects is often associated with the evident increase in the accumulation of body fat, mainly in the central, abdominal, and visceral adipose tissue. In this respect, World Health Organization (WHO) warns that significant alterations in the body mass index (BMI) due to the body fat accumulation in the elderly are closely related to the manifestation of diseases and comorbidities[5].

To prevent the deleterious effect of aging in the health of the elderly subject, which includes the delay in the inflamm-aging development, some interventions such as a nutritionally adequate diet, protein or amino acids supplementation, as well as the regular practice of physical exercises has stood out in recent decades[1].

Particularly in terms of the regular practice of physical exercises, especially at moderate intensity, is worth citing that it can act by increasing the levels of anti-inflammatory cytokines, such as IL-10. Thus, it is evident that this remarkable capacity of exercise training to induce an anti-inflammatory status consequently provides a reduction in inflamm-aging, which leads to a decrease in the triggering of chronic diseases and comorbidities clearly seen in the elderly[2].

As former cited, in addition to exercise training, amino acid supplementation is an intervention routinely applied in clinical nutrition, in special for elderly subjects. Among the several amino acids, glutamine, which is the most abundant and versatile amino acid in the body, shows the property to improve the recovery of patients, as well as to restore immune functions in elite athletes and modulating the inflammatory response[6]. Furthermore, recently our group has been demonstrated that L-glutamine supplementation was able to improve the antibody response to Influenza virus vaccination in a population of elderly subjects[7,8]. It is noteworthy to highlight that glutamine is widely utilized by several cells, in special enterocytes and immune cells, as a substrate for the synthesis of the nucleotides (both purines and pyrimidines), nicotinamide adenine dinucleotide phosphate (NADPH) and antioxidants, as well as for many other biosynthetic and energetic pathways involved in maintaining cellular integrity and function[6].

Considering the previous pieces of information presented, it is evident that inflamm-aging can be considered one of the corollary phenomena that leads to the negative effect of aging on the elderly´s health. Moreover, non-drug intervention such as the regular practice of physical exercise that, besides providing the reduction of body fat, is also characterized as an anti-inflammatory agent, as well as nutritional supplementation with amino acids that can modulate the inflammatory response must be studied in the inflamm-aging context. Therefore, in this study, we aimed to investigate the effect of oral L-glutamine supplementation, associated or not with the regular practice of combined-exercise training, on nutritional status, evaluated by body mass index, lipid profile, and systemic cytokines level in elderly individuals.

METHODS

Subjects and study design

Eighty-four elderly subjects, aged 60 to 85 years, were enrolled in the present study by voluntary adherence. The elderly volunteers were initially separated into 02 groups: non-practitioners of a combined-exercise training program (NP, n=31) and practitioners of a combined-exercise training program (CET, n=53). It is worth clarifying that the elderly participants in this study were the same that participated in the recent article published by our group[7] and in a similar way to previously presented in this report, both the recruitment and the selection of all participants were performed by the co-author CAFS, a geriatric physician and coordinator of the Aging Health Promotion Program belonged to the Discipline of Geriatrics and Gerontology at the Federal University of São Paulo (UNIFESP). It was performed clinical and physical examinations and it was assessed anthropometric characteristics (weight, height, and body mass index (BMI), physical activity level, dietary protein consumption, and amino acids supplementation. None of the elderly volunteers presented chronic infections, cancer, type I diabetes mellitus, thrombosis, renal, liver, and neurological diseases, or other diseases that prevented the regular practice of the exercise training program. All volunteer participants signed the informed consent form previously approved by the National Research Ethics Committee (CAEE number: 18170619.3.0000.5505) and by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP) under number 3.623.247, and Research Ethics Committee of Method Faculty of São Paulo (FAMESP) under number 03/2019. It is also noteworthy to declare that the study agreed with the Ethical Standards defined by Harris and Atkinson[9], and with the Declaration of Helsinki. After that, the volunteer groups were randomly divided into L-glutamine supplemented group (Gln) and placebo supplemented group (PL).

Anthropometric Characteristics and Physical Activity and Nutritional Evaluations

Data concerning height, weight, body mass index (BMI), and body composition by bioimpedance (BIOSCAN 920-2-S Maltron International Limited, UK) were assessed. In addition, data regarding the nutritional consumption of proteins and antioxidants per day, as well as amino acid supplementation were obtained by the Food Frequency Questionnaire and Mini Nutritional Assessment. The physical activity level was obtained using the International Physical Activity Questionnaire (IPAQ), which was validated for the Brazilian population[10,11].

Body mass index (BMI) assessment

The presence of underweight, adequate weight, overweight, and obesity were determined following the cut-off points for the body mass index (BMI, kg/m^2) presented by Adams and collaborators[13], especially for elderly subjects: underweight = BMI <18.5kg/m^2; adequate weight = BMI between 18.5 to 24.9kg/m^2; overweight = BMI between 25 to 29.9kg/m^2; and obesity = BMI> 30kg/m^2.

Combined-Exercise Training (CET) Program

The exercise training program was composed of a combination of aerobic and resistance exercises performed in moderate intensity. As previously reported by our group[7, 12, 13], the exercised elderly volunteers performed their exercise training during 60-75 min per session, 3 times per week, on nonconsecutive days, for at least 12 months. In addition, it is also worth emphasizing that the same experienced physical education professional supervised all the volunteers.

Non-Practitioners (NP) Elderly Group

As former mentioned, the volunteers of the non-practitioners elderly group (NP) were recruited by the co-author CAFS through the database of the Geriatrics and Gerontology Discipline of the Federal University of São Paulo (UNIFESP). After the invitation to participate in the study, the elderly subjects were submitted to the clinical and physical examinations and all of them were inquired concerning the daily routine. Despite they were independent and active, none of them declared to be engaged in a regular exercise training program for at least 12 months. All volunteers were oriented to maintain their normal routine during the study.

Physical Activity Level Assessment

The difference in the physical activity levels between the volunteer groups was assessed using International Physical Activity Questionnaire (IPAQ)[10], previously validated for the Brazilian population[14]. The IPAQ is a useful tool to estimate weekly time spent on physical activities of moderate and high intensity as well as sitting state. The IPAQ results can be presented in METs (metabolic equivalents) or minutes per week. In agreement with WHO recommendations, elderly subjects who present values of more than 150 min per week of physical activity are considered physically active[15].

Supplementation with L-Glutamine or Placebo

Both elderly volunteer groups of this study divided into 2 subgroups in accordance with the type of supplement. In fact, L-glutamine supplementation groups (NP-Gln, n=14; CET-Gln, n=26) were oriented to ingest daily a dose of 0.3g/kg of body weight of L-glutamine (Tongliao Meihua Biological Sci-Tech Co. Ltd., Tongliao, China) plus 10g of maltodextrin (PR Netto Indústria e Comércio de Alimentos Ltda., Sao Paulo, Brazil), whereas the placebo supplementation groups (NP-PL, n=17; CET-PL, n=27) were oriented to ingest daily a dose of 10g of maltodextrin. All the volunteers received 30 sachets containing the supplementation, which presented the same appearance and taste.

Collection of the Blood Samples

Blood sample collection was carried at 7:00-9:00 AM, after a 12 hour fast, on two different occasions: before (pre) and 30 days after (post) of supplementation. It is noteworthy to clarify that the volunteer of CET groups were oriented to perform their last exercise training session 24 h beforehand of blood sampling. Aliquots of 1 mL of plasma and serum, obtained after centrifugation (5 min, 800xg) of blood samples, were stored at -80°C in order to evaluate the glycemia, lipid profile and systemic cytokine levels.

Determination of glycemia and lipid profile

Plasma glucose concentration and the lipid profile (total cholesterol, HDL, and triglycerides) were determined using colorimetric commercial kits (BioClin, São Paulo, Brazil), following the manufacturer's instructions. Intra- and interassay coefficients of variance were 2.5-5% and 5-7%, respectively. In addition, plasma LDL concentrations were determined using Friedewald's formula[8].

Determination of systemic cytokines concentration

The serum concentrations of pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines were determined using the ELISA test (Invitrogen Human Uncoated ELISA kit, California, USA), following the manufacturer's instructions. Intra- and interassay coefficients of variance were 3-5% and 5-7.5%, respectively.

Statistical Analysis

The results obtained in this study were initially analyzed using the Kolmogorov-Smirnov test to verify its normality, and the homogeneity of variance was subsequently assessed using the Levene test.

Due to the observation that the data presented a homogeneous or parametric distribution, thus the results were presented as means and standard deviations.

The Student's t-test was used to evaluate the occurrence of statistically significant differences between the results obtained before (pre) and post-supplementation period in both volunteer groups (intragroup analysis). The two-way ANOVA test for repeated measures with Bonferroni´s post hoc test was used to evaluate the occurrence of statistically significant differences in the results obtained before (pre) and the post-supplementation period between the volunteer groups (intergroup analysis).

The Chi-square test was used to determine whether the difference in the number of elderly subjects with adequate weight, overweight, and obesity was significant.

The level of significance was set at 5% (p <, 0.05).

RESULTS

As formerly mentioned, the elderly population enrolled in this study was the same that previously participated in the study published by our group[7]. Therefore, some data showed in Table 1 are similar those presented in this article recently published. It was observed that height and weight found in the NP-Gln group were higher than the other groups. Particularly, higher BMI values were observed in NP-PL and NP-Gln subgroups than the CET-PL and CET-Gln subgroups, respectively. Concerning the glycemic and lipid profile, no differences were found between the subgroups (Table 1).

In addition, Table 1 also presents the results obtained using IPAQ. In this respect, it was found that NP subgroups presented not only lower physical activity levels but also higher sitting times than the values observed in the CET subgroups, respectively. It is worth pointing out that, although the NP subgroups presented lower physical activity levels, the values observed were above 150 min of physical exercise per week (min/w), which classify these volunteers as active elderly subjects.

Table 1 - Anthropometric characteristics [age (years), weight (kg), height (cm), body mass index (BMI, kg/m2)], glycemic index (glucose, in mg/dL), lipid profile [total cholesterol, HDL, LDL, and triglycerides, in mg/dL), and physical activity level (IPAQ, in minutes per week - min/w) of elderly volunteers in the NP and CET groups supplemented with placebo or L-glutamine (Gln). All data are presented in mean and standard deviation statistically evaluated by two-way ANOVA test for repeated measures with Bonferroni´s posthoc test. The level of significance was 5% (p <0.05).

Groups / Variables	NP		CET		p value
	Placebo (n=17)	Gln (n =14)	Placebo (n=27)	Gln (n=26)	
Age (year)	75.1 ± 7.1	72.9 ± 5.4	72.2 ± 5.9	71.4 ± 5.9	NS
Weigth (kg)	66.1 ± 10.7	75.7 ± 14.5*	61.9 ± 10.3	62.8 ±12.7	p<0.05
Height (cm)	154.2 ± 9.5	162.3 ± 8.3	156.0 ± 9.7	156.4 ± 8.9	NS
BMI (kg/M²)	27.7 ± 4.1#	28.4 ± 3.7$	25.4 ± 3.6	25.5 ± 3.9	p<0.05
Total body fat (%)	39.6 ± 10.1	38.1 ± 9.1	35.4 ± 7.7	35.3 ± 7.7	NS
Fat-free mass (%)	60.4 ± 9.3	61.9 ± 9.2	64.6 ± 7.8	64.7 ± 7.5	NS
Skeletal muscle mass (kg)	18.3 ± 4.1	22.3 ± 4.0	19.6 ± 3.6	19.8 ± 3.9	NS
Lipid profile (mg/dL)					
Total cholesterol	210.8 ± 32.1	220.5 ± 37.3	207.2 ± 11.7	196.2 ± 31.3	NS
HDL	34.8 ± 8.1	34.9 ± 6.1	37.9 ± 6.4	38.9 ± 6.1	NS
LDL	146.4 ± 33.7	161.2 ± 37.1	147.2 ± 30.4	134.9 ± 28.34	NS
Triglycerides	122.8 ± 30.8	114.9 ± 14.3	121.8 ± 33.2	110.1 ± 20.3	NS
IPAQ (min/w)					
Physical activity	433.7 ± 76.1#	371.4 ± 69.2$	677.3 ± 60.4	754.1 ± 85.6	p<0.05
Sitting	1685 ± 176.5#	1874 ± 178.6$	1224 ± 112.21	1326 ± 107.74	p<0.05

* statistical difference in relation to the other groups
# statistical difference between NP-PL subgroup and CET-PL subgroup
$ statistical difference between NP-Gln subgroup and CET-Gln subgroup

To respond to our objectives, we evaluated the nutritional status of the participants of each volunteer groups in accordance with their BMI classification [adequate weight (AW) = BMI between 18.5 to 24.9kg/m²; overweight (OW) = BMI between 25 to 29.9kg/m²; and obesity (OB) = BMI> 30kg/m²].

Table 2 shows the number of volunteers presenting adequate weight (AW), overweight (OW), and obesity (OB) in each volunteer group. No differences were found between the values found.

Table 2. Separation of the elderly volunteer groups in accordance to their Body Mass Index (BMI) classification (adequate weight = BMI between 18.5 to 24.9kg/m2; overweight = BMI between 25 to 29.9kg/m2; and obesity = BMI> 30kg/m2). All data are presented in absolute number (n) and it was statistically evaluated by Chi-square test. The level of significance was 5% (p <0.05).

Groups / BMI	Non-practitioners (NP)		Combined-exercise training (CET)	
	Placebo (n=17)	Gln (n=14)	Placebo (n=27)	Gln (n=26)
Adequate Weigth (AW)	5	2	12	15
Overweigth (OW)	7	7	12	7
Obese (OB)	5	5	3	4

Table 3 shows the results obtained in the evaluation of glycemic and lipid profile (total cholesterol, HDL, LDL, and triglycerides) of the volunteer subgroups, classified according to their BMI, in adequate weight (AW), overweight (OW), and obesity (OB), both before (pre) and post-supplementation period. Interestingly, no statistical differences were observed.

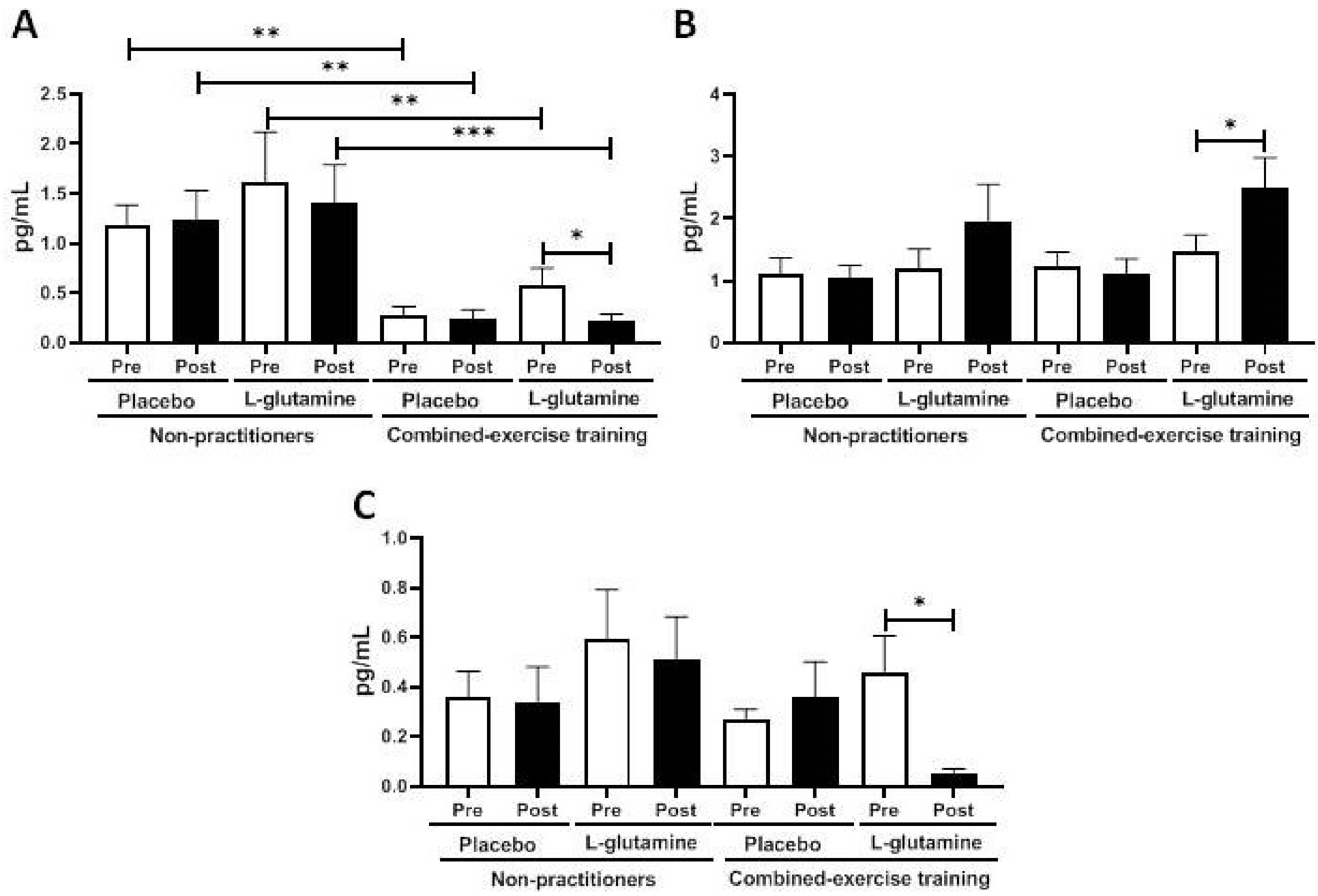
Table 3. Data related to the glycemic and lipid profile of elderly volunteers, from the NP and CET groups, supplemented with placebo (NP-PL and CET-PL) or L-glutamine (NP-Gln and CET-Gln), both before (pre) and after 30 days (post) of supplementation, separated by their BMI classification (adequate weight - AW = BMI between 18.5 to 24.9kg/m2; overweight - OW = BMI between 25 to 29.9kg/m2; and obesity - OB = BMI> 30kg/m2). All data are presented in mean and standard deviation statistically evaluated by two-way ANOVA test for repeated measures with Bonferroni´s posthoc test. The level of significance was 5% (p <0.05)

Groups		Variables (mg/dL)	Glycemia	Total cholesterol	HDL	LDL	Triglycerides
NP-PL	AW	Pre	113.7±27.26	218.1±30.06	41.7±2.83	152.9±36.63	117.8±31.05
		Post	115.5±28.34	191.4±2.41	40.5±0.15	134.5±13.95	125.4±31.48
	OW	Pre	106.9±19.43	208.1±37.28	36.62±7.54	141±36.11	152.1±72.19
		Post	97.67±8.96	212.5±34.82	37.3±7.84	151.7±43.42	117.2±20.51
	OB	Pre	96.29±4.22	207.2±31.89	35.76±6.96	147.3±34.06	120.9±21.16
		Post	89.68±22.42	264.70±81.5	40.17±5.25	201.7±81.87	114±11.57
NP-Gln	AW	Pre	91.40±23.51	185.6±30.61	27.68±0.69	135.6±30.21	111.4±5.51
		Post	123.10±15.68	222.8±54.25	36.48±6.201	160.4±43.04	111.4±5.519
	OW	Pre	96.68±3.71	201.2±38.21	40.83±5.31	138±36.85	129.8±25.05
		Post	102.1±10.2	203.6±43.93	44.05±3.91	137.2±43.08	112.1±12.95
	OB	Pre	105.5±11.37	193.30±23.22	38.93±10.69	130.3±15.48	112.2±18.58
		Post	108.3±13.59	183.20±5.615	44.02±7.58	113.7±5.94	120.3±18.59
CET-PL	AW	Pre	93.07±8.39	196.1±33.8	35.62±5.80	138.3±35.26	116.9±16.33
		Post	104.2±11.04	205.7±21.54	38.16±4.57	145.4±20.94	111±16.62
	OW	Pre	97.39±14.3	212.2±23.57	33.81±5.82	150.6±22.02	105.3±15.35
		Post	90.64±14.98	216.8±18.25	37.41±5.96	163.80±32.82	136.3±49.66
	OB	Pre	96.68±13.14	237.8±37.79	36.77±8.37	171.8±26.39	110.4±19.29
		Post	93.51±23.63	219.2±30.73	39.54±5.40	157.5±32.51	146.3±44.60
CET-Gln	AW	Pre	89.23±10.35	216.6±39.49	36.93±7.93	157.6±39.43	110.8±17.15
		Post	97.32±15.58	207.7±32.97	40.64±1.61	137.8±20.27	110.8±22.72
	OW	Pre	95.8±11.96	247±68.09	37.80±6.46	187±62.55	105.8±16.19
		Post	88.8±14.33	208.6±19.02	40.03±7.62	148.1±20.56	110.8±21.87
	OB	Pre	94.34±5.706	231.20±48.8	40.66±12.32	169.3±51.39	102.3±13.66
		Post	97.40±23.05	250.5±72.12	44.20±12.20	169.3±51.39	106.1±5.54

In relation to the evaluation of the concentrations of the systemic cytokines found before (pre) and post-supplementation period in the elderly volunteer groups, in figure 1A is possible to observe higher IL-6 levels in the NP subgroups (NP-PL and NP-Gln) than CET subgroups (CET-PL and CET-Gln) both pre- and post-supplementation period, regardless of the supplement. In addition, only the CET-Gln subgroup showed a significant reduction of IL-6 levels post-supplementation as compared to the baseline (pre) values. Concerning systemic IL-10 levels, Figure 1B shows a significant reduction of the levels of this cytokine post-supplementation in the CET-Gln subgroup in comparison to the values found in the pre-supplementation period. To improve the understanding of the effect of L-glutamine supplementation on these cytokines evaluated here, we performed the analysis of the ratio between serum concentrations of cytokines IL-6 and IL-10 (IL-6/IL-10 ratio). As shown in figure 1C, a significant reduction in the ratio between these cytokines was observed post-supplementation in the CET-Gln subgroup as compared to the values found pre-supplementation period.

In addition to the analysis above described, we also evaluated the concentrations of the systemic cytokines found before (pre) and post-supplementation period among the volunteers of each group separated by their BMI. In Figure 2A is showed that before the supplementation began, all the subgroups classified as obese (OB) showed increased IL-6 levels as compared to the values found in the subgroups classified as adequate weight (AW). Interestingly, after the supplementation, it was observed that the subgroups composed of obese elderly subjects (OB) supplemented with placebo maintained higher IL-6 levels than the subgroups with adequate weight (AW), whereas the subgroups composed of obese elderly subjects (OB) and supplemented with Gln, the IL-6 levels observed did not show significant differences as compared to the values found in the subgroups with

Figure 1. Serum concentration of IL-6 (A) and IL-10 (B), as well as the ratio between IL-6 and IL-10 (IL-6/IL-10, C) in the elderly subjects groups, supplemented with placebo (PL) or L-glutamine (Gln) before (pre) and after (post) 30 days of the supplementation. All data are presented in mean and standard deviation and it was statistically evaluated by a two-way ANOVA test for repeated measures with a risk value of 5% (p <0.05). * p <0.05; ** p <0.01, and *** p <0.001.



elderly subjects presenting adequate weight (AW). In addition, a significant reduction in the IL-6 levels was observed in the subgroup with obese volunteers and supplemented with Gln (CET-Gln) post-supplementation in a comparison with the values found pre-supplementation. Concerning the systemic IL-10 concentration, Figure 2B shows a significant increase in the levels of this cytokine post-supplementation period in the obese volunteers of the CET-Gln subgroup as compared to the values obtained pre-supplementation. In a similar way to the previously presented, we performed the analysis of the ratio between serum concentrations of cytokines IL-6 and IL-10 (IL-6/IL-10 ratio). As shown in Figure 2C, a significant reduction in the ratio between these cytokines was observed post-supplementation in the obese volunteers of the CET-Gln subgroup as compared to the values found pre-supplementation.

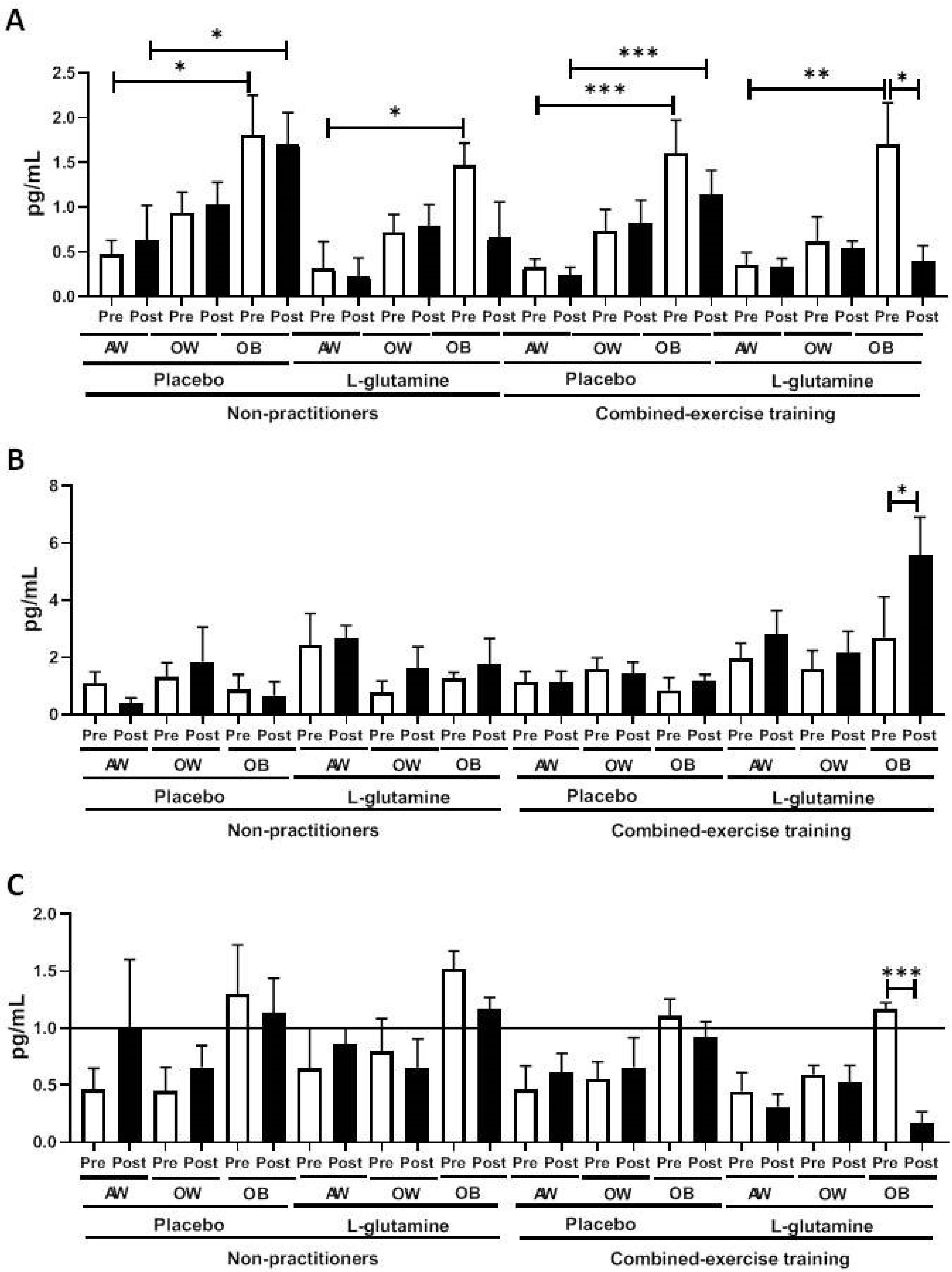


Figure 2. Serum concentration of IL-6 (A) and IL-10 (B), as well as the ratio between IL-6 and IL-10 (IL-6/IL-10, C) in the elderly subjects groups, supplemented with placebo (PL) or L-glutamine (Gln) before (pre) and after (post) 30 days of the supplementation, and separated by their BMI classification in adequate weight (BMI between 18.5 to 24.9kg/m²), overweight (BMI between 25 to 29.9kg/m²), and obesity (BMI> 30kg/m²). All data are presented in mean and standard deviation and it was statistically evaluated by a two-way ANOVA test for repeated measures with a risk value of 5% (p <0.05). * p <0.05; ** p <0.01, and *** p <0.001.

DISCUSSION

In order to investigate whether the oral L-glutamine (Gln) supplementation would be able to modulate the metabolic profile as well as the systemic inflammatory status in elderly subjects groups who practice or not of a combined-exercise training program, which were subsequently separated in: adequate weight (AW), overweight (OW), and obese (OB) in accordance with their BMI, in this study we evaluated the glycemic and lipid profile and also systemic concentrations of IL-6 (a pro-inflammatory cytokine), and IL-10 (an anti-inflammatory cytokine), as well as the ratio between these cytokines. The results obtained by us showed that the elderly volunteer groups (NP and CET groups) did not show differences in the glycemic and lipid profile both pre- and post-supplementation period, regardless of the supplement provided. In contrast, it was found significant differences in the systemic inflammatory status. In this sense, higher IL-6 levels were observed in NP subgroups than CET subgroups before and after the supplementation period, whereas, specifically, the CET-Gln showed not only higher IL-10 levels but also lower IL- 6 levels with a significant reduction of IL-6/IL-10 ratio post-supplementation as compared to the baseline values found. In terms of the results obtained when the elderly volunteer subgroups were separated in accordance with their BMI, higher IL-6 levels were found in all OB subgroups than in AW subgroups in the pre-supplementation period. However, after the supplementation period, this significant difference in IL-6 levels was not maintained between OB and AW subgroups supplemented with Gln. In addition, only the obese elderly subjects who composed the CET-Gln subgroup showed not only a significant reduction of IL-6 levels but also an increase in IL-10 levels post-supplementation period as compared to the values pre-supplementation. These last observations had a direct impact on the ratio between these cytokines leading to a significant decrease of the IL-6/IL-10 ratio in the CET-Gln subgroup composed of obese elderly subjects.

Based on the literature, is broadly accepted that IL-10 is a classical anti-inflammatory cytokine, which presents the main function of the regulation of the immune system, particularly by inhibiting the expression and/or synthesis of pro-inflammatory cytokines[16]. Interestingly, it was reported that individuals with adequate weight presented IL-10 secretion from immune cells, especially by macrophages with M2 profile, that infiltrating adipose tissue2. In fact, the production and release of IL-10 from adipose tissue to the circulation can act regulating the systemic inflammatory status and consequently minimize the development of chronic inflammatory diseases, including in elderly subjects[17].

In a different way from that observed in individuals with adequate weight, the accumulation of fat in adipose tissue, the main factor that leads to obesity, is closely associated with the induction of a systemic inflammatory status and the manifestation of several disorders, such as insulin resistance, hypercholesterolemia, dyslipidemia, vascular disturbances[18]. Although we did not evidence differences in relation to glycemic and lipid profile in the volunteer subgroups, which can putatively be explained by the fact that all volunteers enrolled in this study were physically active, a significant increase in IL-6 levels was found in all subgroups composed of obese elderly subjects in relation to the values observed in all subgroups composed of elderly subjects presenting adequate weight.

According to the literature, elderly subjects who present BMI above 30 kg/M^2 are classified as obese[19] and the increased fat accumulation in adipose tissue promotes atrophy of adipocytes due to the intense increase in their cell volume[20,21]. In consequence, the death of the adipocyte elicits the infiltration and activation of immune cells, especially macrophages, in the adipose tissue in order to eliminate the dead cell[22,24]. However, as shown in histological sections, there is a prominent presence of macrophages around the dead cells, originating an arranged cell known as "crown-like structures - CLS"[25, 26]. In response to this infiltration and activation of immune cells inside the adipose tissue, there is a significant increase in the secretion of pro-inflammatory cytokines, such as IL-6 to the circulation, which chronically, can induce a remarkable alteration in the systemic inflammatory status from a regulatory profile to a pro-inflammatory status[1].

Interleukin 6 not only is one of the most studied cytokines but also it is widely accepted that this cytokine has an immunoregulatory function with evident pro-inflammatory and endocrine actions[29], depending on the origin and situation by which its production and secretion was stimulated. According to Guimarães[30], IL-6 shows the ability to act in different situations in several tissues, both in peripheral tissues and in the central nervous system. Particularly depending on its systemic concentration, the IL-6 can influence the maintenance of body weight, energy homeostasis, sensitivity insulin, and the development of atherosclerosis[30]. Regarding the latter influence, for instance, it was reported that the chronicity of high systemic levels of IL-6 acts enhancing the formation of foam cells and atheromatous plaque due to its inhibitory action on the enzyme lipoprotein lipase, an enzyme responsible for the hydrolysis of the triglycerides of very-low-density lipoproteins (VLDL), as well as by favoring increased lipid uptake by macrophages[31-33]. Therefore, taking into account these pieces of information, there is no doubt that decreasing serum IL-6 concentrations, especially in obese individuals, is extremely important.

Beyond its remarkable pro-inflammatory action, another IL-6 hallmark is the ability to act on body energic regulation. By the way, this metabolic action is closely associated with the practice of physical exercise, in which it was demonstrated that the plasma concentration of IL-6 can increase up to 100 times during and after an exhaustive physical exercise session[34]. There is a consensus in the literature showing that systemic elevations of IL-6 observed after an exhaustive exercise session occurs in response to the skeletal muscle fibers contraction[35]. Especially in this situation, the IL-6 is classified as a myokine, and its secretion from skeletal muscle has metabolic actions not only in local, by eliciting the increase of glucose uptake by skeletal muscle, but also systemically, by inducing glycogenolysis in liver and lipolysis in adipose tissue [36], which increases the bioavailability of glucose and fatty acid to the skeletal muscles, besides can contribute to reducing the fat body accumulation[37-39]. Interestingly, the myokine IL-6 is also associated with the regulation of inflammation, since this cytokine precedes the release of anti-inflammatory cytokines, such as IL-1ra and IL-10, during and after the physical exercise session[34,40]. Therefore, it is noteworthy to point out that the elevations in the systemic anti-inflammatory cytokines in response to physical exercise sessions are associated with the well-accepted anti-inflammatory property of regular practice of exercise training[34, 36].

Based on these pieces of information, our findings that CET subgroups presented lower BMI and systemic IL-6 levels than NP subgroups, both before and after the supplementation period, regardless of the supplement, not only reinforces the literature[13] but also allow us to suggest that the regular practice of combined-exercise training by elderly subjects is able to minimize the development of inflamm-aging.

Interestingly, when the elderly volunteers in each subgroup (NP-PL, NP-Gln, CET-PL, and CET-Gln) were separated in accordance with their BMI classification, a statistically significant difference was found between the obese and adequate weight subgroups in relation to the systemic IL-6 levels before the supplementation period. These observations were expected and corroborate our former pieces of information in which the accumulation of body fat is related to the induction of a systemic pro-inflammatory status by increasing circulating levels of pro-inflammatory cytokines, such as IL-6[31, 41]. Particularly in this study, the higher IL-6 levels found in the obese subgroups can putatively indicate that, in those elderly subjects, the inflamm-aging phenomenon is in course.

Beyond the evaluation of the influence of regular combined-exercise training in the inflamm-aging occurrence, as former cited it is widely accepted that amino acids supplementation can ameliorate the deleterious effect of aging, including by preventing or delaying the inflamm-aging development[6,42,43].

Regarding the literature, the association of Gln supplementation with the practice of physical activities can act as a regulating factor for inflammatory processes. It is well-known that Gln is the most abundant non-essential amino acid in the circulation. By the way, although its production and secretion occur in different tissues, the major source of Gln is mainly associated with its synthesis by the skeletal muscle. However, in situations of catabolism, such as in reduced consumption of carbohydrates, development of diseases, stress, and obesity, plasma Gln concentration is reduced[6].

Among many effects of Gln[44,4], it has been reported that this amino acid has the capacity to modulate cytokine production by immune cells, both in vitro and in vivo. For instance, the production of TNF-a by mononuclear cells obtained from peripheral blood and stimulated with LPS was suppressed by the addition of Gln[46]. On the other hand, it was documented that Gln increased the IL-1 beta and IL-6 production by peritoneal macrophages stimulated with LPS[47]. In relation to the Gln, physical exercise, and immune response, it was demonstrated that during a strenuous and prolonged physical exercise session, plasma glutamine concentrations are reduced by 20-25%[48]. Furthermore, recently our group reported that oral Gln supplemented was able to improve not only the salivary redox indexes[13], as well as the antibody responses to Influenza virus vaccination and lymphocytes activation[7], in these same group of elderly subjects practitioners of the combined-exercise training program participating in the present study.

Here, our findings that oral Gln supplementation not only significantly decreased the systemic IL-6 levels but also increased systemic IL-10 levels, which directly impacted the reduction of the IL-6/IL-10 ratio, only in the CET-Gln subgroup, corroborate the data showing that Gln is able to modulate inflammatory processes, especially when associated with regular practice of exercise training. Interestingly, when the elderly subjects subgroups were separated in accordance with their BMI classification, this prominent capacity of Gln to modulate the inflammatory processes when associated with exercise training was highlighted, since it was observed that only the CET-Gln subgroup composed of obese elderly subjects showed a significant reduction of IL-6 levels in opposite to significant elevation of IL-10 levels. It is utmost to point out that, although it was not statistically different, the systemic levels of IL-6 and IL-10 found in the NP-Gln subgroup also composed of obese elderly subjects followed the same trend of IL-6 reduction and IL-10 elevations post-supplementation period. These last observations allow us to putatively suggest that an oral Gln supplementation for a period longer than 30 days by obese and active elderly subjects, but non-practitioners of a regular exercise training program, could lead to a remarkable regulation of systemic inflammatory status.

Taken together, the results presented in this study showed, for the first time, that oral Gln supplementation for 30 consecutive days when associated with the regular practice of combined-exercise training can induce a systemic anti-inflammatory status, which can prevent or delay the development of inflamm-aging, especially in obese elderly subjects.

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REFERENCES

1. GALLEA F, BRESCIANI Y. Modulación antioxidante y antiinflamatoria del ejercicio físico durante o envejecimiento Revista Española de Geriatría y Gerontología2018. p. 279-84.
2. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev. 2007;128(1):92-105.
3. Liu YZ, Wang YX, Jiang CL. Inflammation: The Common Pathway of Stress-Related Diseases. Front Hum Neurosci. 2017;11:316.
4. Ma L, Chan P. Understanding the Physiological Links Between Physical Frailty and Cognitive Decline. Aging Dis. 2020;11(2):405-18.
5. Adams KF, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R, et al. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. N Engl J Med. 2006;355(8):763-78.
6. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. Nutrients. 2018;10(11).
7. Monteiro FR, Roseira T, Amaral JB, Paixão V, Almeida EB, Foster R, et al. Combined Exercise Training and l-Glutamine Supplementation Enhances Both Humoral and Cellular Immune Responses after Influenza Virus Vaccination in Elderly Subjects. Vaccines (Basel). 2020;8(4).
8. Paixão V, Almeida EB, Amaral JB, Roseira T, Monteiro FR, Foster R, et al. Elderly Subjects Supplemented with L-Glutamine Shows an Improvement of Mucosal Immunity in the Upper Airways in Response to Influenza Virus Vaccination. Vaccines (Basel). 2021;9(2).
9. Harriss DJ, Atkinson G. Ethical Standards in Sport and Exercise Science Research: 2016 Update. Int J Sports Med. 2015;36(14):1121-4.
10. Craig C, Marshall A, Sjöström M. International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise*; 2003. p. 1381–95.
11. Matsudo SMM. Envelhecimento, atividade física e saúde. BIS Boletim do Instituto de Saúde (Impresso). 2009(47):76-9.
12. Bachi ALL, Barros MP, Vieira RP, Rocha GA, de Andrade PBM, Victorino AB, et al. Combined Exercise Training Performed by Elderly Women Reduces Redox Indexes and Proinflammatory Cytokines Related to Atherogenesis. Oxid Med Cell Longev. 2019;2019:6469213.
13. Almeida EB, Santos JMB, Paixão V, Amaral JB, Foster R, Sperandio A, et al. L-Glutamine Supplementation Improves the Benefits of Combined-Exercise Training on Oral Redox Balance and Inflammatory Status in Elderly Individuals. Oxid Med Cell Longev. 2020;2020:2852181.
14. Benedetti TRB, Antunes PdC, Rodriguez-Añez CR, Mazo GZ, Petroski ÉL. Reprodutibilidade e validade do Questionário Internacional de Atividade Física (IPAQ) em homens idosos. Revista Brasileira de Medicina do Esporte. 2007;13(1):11-6.
15. World OH. Global Recommendations on Physical Activity for Health. World Health Organization: Geneva, Switzerland; 2010.
16. Speretta GF, Rosante MC, Duarte FO, Leite RD, Lino AD, Andre RA, et al. The effects of exercise modalities on adiposity in obese rats. Clinics (Sao Paulo). 2012;67(12):1469-77.

17. Juge-Aubry CE, Somm E, Pernin A, Alizadeh N, Giusti V, Dayer JM, et al. Adipose tissue is a regulated source of interleukin-10. Cytokine. 2005;29(6):270-4.
18. Rosini TC, Silva AS, Moraes C. Diet-induced obesity: rodent model for the study of obesity-related disorders. Rev Assoc Med Bras (1992). 2012;58(3):383-7.
19. Deurenberg P, Yap M. The assessment of obesity: methods for measuring body fat and global prevalence of obesity. Baillieres Best Pract Res Clin Endocrinol Metab. 1999;13(1):1-11.
20. Hoffstedt J, Arner E, Wahrenberg H, Andersson DP, Qvisth V, Löfgren P, et al. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. Diabetologia. 2010;53(12):2496-503.
21. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med. 2011;364(25):2392-404.
22. Sun S, Ji Y, Kersten S, Qi L. Mechanisms of inflammatory responses in obese adipose tissue. Annu Rev Nutr. 2012;32:261-86.
23. Fischer-Posovszky P, Wang QA, Asterholm IW, Rutkowski JM, Scherer PE. Targeted deletion of adipocytes by apoptosis leads to adipose tissue recruitment of alternatively activated M2 macrophages. Endocrinology. 2011;152(8):3074-81.
24. Herrero L, Shapiro H, Nayer A, Lee J, Shoelson SE. Inflammation and adipose tissue macrophages in lipodystrophic mice. Proc Natl Acad Sci U S A. 2010;107(1):240-5.
25. Huh JY, Park YJ, Ham M, Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol Cells. 2014;37(5):365-71.
26. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem. 2007;282(48):35279-92.
27. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. 2008;8(4):301-9.
28. Quail DF, Dannenberg AJ. The obese adipose tissue microenvironment in cancer development and progression. Nat Rev Endocrinol. 2019;15(3):139-54.
29. Flynn MG, Markofski MM, Carrillo AE. Elevated Inflammatory Status and Increased Risk of Chronic Disease in Chronological Aging: Inflamm-aging or. Aging Dis. 2019;10(1):147-56.
30. Guimarães JPT, Filgueiras LR, Martins JO, Jancar S. Leukotriene Involvement in the Insulin Receptor Pathway and Macrophage Profiles in Muscles from Type 1 Diabetic Mice. Mediators Inflamm. 2019;2019:4596127.
31. Engin A. The Pathogenesis of Obesity-Associated Adipose Tissue Inflammation. Adv Exp Med Biol. 2017;960:221-45.
32. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc. 2001;60(3):349-56.
33. Greenberg AS, Nordan RP, McIntosh J, Calvo JC, Scow RO, Jablons D. Interleukin 6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin 6 in cancer cachexia. Cancer Res. 1992;52(15):4113-6.
34. Walsh NP, Gleeson M, Shephard RJ, Woods JA, Bishop NC, Fleshner M, et al. Position statement. Part one: Immune function and exercise. Exerc Immunol Rev. 2011;17:6-63.
35. Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Pedersen BK. IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. Am J Physiol Endocrinol Metab. 2002;283(6):E1272-8.
36. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev. 2008;88(4):1379-406.
37. Pedersen BK. IL-6 signalling in exercise and disease. Biochem Soc Trans. 2007;35(Pt 5):1295-7.
38. Hoene M, Weigert C. The role of interleukin-6 in insulin resistance, body fat distribution and energy balance. Obes Rev. 2008;9(1):20-9.
39. Smith RL, Soeters MR, Wüst RCI, Houtkooper RH. Metabolic Flexibility as an Adaptation to Energy Resources and Requirements in Health and Disease. Endocr Rev. 2018;39(4):489-517.
40. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003;285(2):E433-7.
41. Eder K, Baffy N, Falus A, Fulop AK. The major inflammatory mediator interleukin-6 and obesity. Inflamm Res. 2009;58(11):727-36.
42. Witard OC, Ball D. The interaction between nutrition and exercise for promoting health and performance. Proceedings of the Nutrition Society. 2018;77(1):1-3.
43. Lambertucci AC, Lambertucci RH, Hirabara SM, Curi R, Moriscot AS, Alba-Loureiro TC, et al. Glutamine supplementation stimulates protein-synthetic and inhibits protein-degradative signaling pathways in skeletal muscle of diabetic rats. PLoS One. 2012;7(12):e50390.
44. Curi MM, Dib LL, Pinto DS. Management of solid ameloblastoma of the jaws with liquid nitrogen spray cryosurgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;84(4):339-44.
45. Pithon-Curi TC, Schumacher RI, Freitas JJ, Lagranha C, Newsholme P, Palanch AC, et al. Glutamine delays spontaneous apoptosis in neutrophils. Am J Physiol Cell Physiol. 2003;284(6):C1355-61.
46. Pithon-Curi TC, Trezena AG, Tavares-Lima W, Curi R. Evidence that glutamine is involved in neutrophil function. Cell Biochem Funct. 2002;20(2):81-6.
47. Yassad A, Husson A, Bion A, Lavoinne A. Synthesis of interleukin 1beta and interleukin 6 by stimulated rat peritoneal macrophages: modulation by glutamine. Cytokine. 2000;12(8):1288-91.
48. Lagranha VL, Baldo G, de Carvalho TG, Burin M, Saraiva-Pereira ML, Matte U, et al. In vitro correction of ARSA deficiency in human skin fibroblasts from metachromatic leukodystrophy patients after treatment with microencapsulated recombinant cells. Metab Brain Dis. 2008;23(4):469-84.