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RESEARCH PAPER

Liver lipid metabolism, oxidative stress, and inflammation in glutamine-supplemented *ob/ob* mice

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Abstract

Glutamine availability may be reduced in chronic diseases, such as type 2 diabetes mellitus (T2DM)-induced by obesity. Herein, the antioxidant, anti-inflammatory and lipid metabolism effects of chronic oral glutamine supplementation in its free and dipeptide form were assessed in *ob/ob* mice. Adult male C57BL/6J *ob/ob* mice were supplemented with L-alanyl-L-glutamine (DIP) or free L-glutamine (GLN) in the drinking water for 40 days, whilst C57BL/6J Wild-type lean (WT) and control *ob/ob* mice (CTRL) received fresh water only. Plasma and tissue (skeletal muscle and liver) glutamine levels, and insulin resistance parameters (*e.g.*, GTT, ITT, insulin) were determined. Oxidative stress (*e.g.*, GSH system, Nrf2 translocation), inflammatory (*e.g.*, NFkB translocation, TNF-α gene expression) and lipid metabolism parameters (*e.g.*, plasma and liver triglyceride levels, SRBP-1, FAS, ACC, and ChRBP gene expression) were also analyzed. CTRL *ob/ob* mice showed lower glutamine levels in plasma and tissue, as well as increased insulin resistance and fat in the liver. Conversely, chronic DIP supplementation restored glutamine levels in plasma and tissues, improved glucose homeostasis and reduced plasma and liver lipid levels. Also, Nrf2 restoration, reduced NFkB translocation, and lower TNF-α gene expression was observed in the DIP group. Interestingly, chronic free GLN only increased muscle glutamine stores but reduced overall insulin resistance, and attenuated plasma and liver lipid metabolic biomarkers. The results presented herein indicate that restoration of body glutamine levels reduces oxidative stress and inflammation in obese and T2DM *ob/ob* mice. This effect attenuated hepatic lipid metabolic changes observed in obesity.
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Keywords: Amino acids; Anti-inflammatory; Antioxidant; Diabetes; Non-alcoholic fatty liver disease; Obesity.

1. Introduction

The prevalence of obesity and associated chronic diseases, such as type 2 diabetes mellitus (T2DM), continue to rise worldwide, reaching pandemic levels [1]. Obesity is a major health challenge, and it significantly increases the risk for the development of associated non-communicable diseases, including insulin resistance, T2DM, and metabolic dysfunction-associated steatotic liver disease (MASLD) [2]. Although multiple factors are involved in the complex pathogenesis of T2DM induced by obesity, the literature extensively describes the involvement of oxidative stress and inflammation. For instance, lipids accumulation and chronic hyperglycaemia result in the overproduction of oxidative free radicals and associated reactive oxygen species (ROS) [3], which in turn leads to an increase in circulating pro-inflammatory cytokines levels [4]. Elevated ROS and cytokines promote cellular dysfunction in critical organs, such as the liver, leading to MASLD, and contributing to the progressive impairment of glucose homeostasis and lipid fluxes into and from the liver [5].

Glutamine, the most abundant amino acid in the body, is known to have antioxidant and anti-inflammatory properties. This is particularly important in catabolic situations, such as critical illness and sepsis [6–8], post-trauma/surgery [9–11], and exhaustive exercise [12–14], where glutamine concentration can be outside the average range due to increased metabolic demand and a concomitant reduction in its synthesis [15]. Therefore, glutamine exogenous supply has been part of clinical nutrition therapies for

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many years. However, more recent studies have shown that a wide range of metabolites can be altered in chronic diseases, such as T2DM induced by obesity, including glutamine [16–18]. For instance, in 1 study, the gene expression of glutamine synthetase, which uses glutamate as a substrate to produce glutamine, was dramatically reduced in the white adipose tissue (WAT) of high-fat diet (HFD) fed mice and obese subjects [17]. Other studies found that abnormal glutamine metabolism and concentration are altered in high adiposity conditions [19,20], which may result in the progression of the disease. For example, Rhee and Jung [16] reported that low glutamine levels contribute to diabetic retinopathy in T2DM patients, whilst Papandreou and Hernández-Alonso [21] reported that low glutamine concentration was associated with an increase in the development of heart failure in patients with high risk for cardiovascular disease. Moreover, Dollet and Kuefner [22] reported that lower plasma glutamine levels are associated with high body mass index (BMI) and HOMA-IR index in men.

As a conditionally essential amino acid, glutamine is an important regulator of cell metabolism and functions through a range of mechanisms, including the synthesis of tricarboxylic acid (TCA) cycle intermediates, nitrogen donor for purines/pyrimidines, a substrate for the hexosamine biosynthetic pathway and antioxidant defence mediated by the glutathione (GSH) system [23,24]. These systems dynamically interact with the body's immune-inflammatory response. Hence, a drop in glutamine availability can contribute to the low-grade inflammation and oxidative stress of obesity [25,26].

In clinical nutrition, glutamine is frequently administered parenterally, by itself, or as part of total parenteral nutrition (TPN), increasing the availability of total body glutamine [27]. Nevertheless, this administration is very invasive and often limited to hospitalized patients. Oral administration of glutamine is desirable since its supply can be a co-adjuvant therapy to delay or prevent the onset of obesity-induced T2DM. However, due to its high metabolization in the enterocytes [28], oral glutamine supplementation in its free form has often been controversial [29]. Supplementation of glutamine dipeptides, such as L-alanyl-L-glutamine (DIP), can be a very effective and stable way to increase the body's glutamine concentration [7,30]. We investigated herein the antioxidant, anti-inflammatory and lipid metabolism effects of oral glutamine supplementation in its free and DIP form in the liver and skeletal muscle of obese *ob/ob* mice. We hypothesized that glutamine supplementation could restore body glutamine levels, reduce oxidative stress and inflammation, and attenuate the hepatic lipid accumulation observed in obesity-associated T2DM.

2. Material and methods

2.1. Animals

Adult (90±3 days) male C57BL/6J wild-type and *ob/ob* male mice were kept at the animal facility of the Institute of Biomedical Sciences of the University of Sao Paulo, Brazil. The initial breeders of *ob/ob* mice (000632 B6. Cg-Lepob/J) were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). Genotype was conducted after weaning (21±3 days) using a polymerase chain reaction of genomic DNA isolated from tail tips, as demonstrated in the supplementary material S3. At all times, mice were maintained under controlled and standard conditions of light (12 h light/dark cycle), temperature (22±2°C), and relative humidity (55±15%). The supplementation protocol started 90±3 days after weaning and consisted of 40 days of supplementation. At the end of the supplementation period, mice were fasted for 2 hours before euthanasia. Euthanasia was conducted using ketamine (90 mg/kg) and xylazine (10 mg/kg), followed by cervical dislocation. Blood was collected in microtubes and centrifuged (2,500 g, 4°C, 15 min), and plasma was then separated and stored at −80°C. Liver and gastrocnemius muscles were removed, immediately frozen in liquid nitrogen, and stored at −80°C. The experimental protocol used was approved by the Ethics Committee on the Use of Animals of the Institute of Biomedical Sciences, University of São Paulo (protocol number 134/2015) and was performed according to the ethical guidelines adopted by the Brazilian College of Animal Experimentation.

2.2. Amino acid supplementation

Obese *ob/ob* mice were supplemented with L-alanyl-L-glutamine (DIP group, n=6–8) or free L-glutamine (GLN group, n=6–8) for 40 days. DIP was provided by Fresenius Kabi S.A., Bad Homburg, HE, Germany, and free GLN was provided by Labsynth, São Paulo, SP, Brazil. As previously described, both supplements were diluted at 4% in the drinking water [26]. At the same time, both untreated groups, *i.e.*, wild-type lean (WT, n=6–8) and control *ob/ob* mice (CTRL, n=6–8) received fresh water only. All animals had free access to water and standard laboratory chow (Nuvilab cr1; Nuvital Nutrientes LDTA, PR, Brazil). Water intake was monitored and assessed daily, while food intake was evaluated twice a week. The amino acids were added to the drinking water. The water bottles were cleaned, and the supplementation was replaced daily.

2.3. Body weight and composition

The animals were weighed at the beginning of the experiment and once a week during the experiment. The body composition assessment (body fat, lean mass, and blood fluids) was carried out using a Minispec LF50 mq7.5 nuclear magnetic resonance device (Bruker Corporation, Massachusetts, USA). The body composition evaluation was assessed in the last week of supplementation.

2.4. Glucose (GTT) and insulin (ITT) tolerance tests

Glucose and insulin tolerance tests were performed as described by Vinué and González-Navarro [31]. Mice were subjected to a glucose tolerance test (GTT) in the fourth week of supplementation/experimental protocol and an insulin tolerance test (ITT) in the fifth week. In both GTT and ITT, mice were fasted for 4 h, and blood was collected from the tail vein at 0, 20, 40, 60, 90, and 120 min after an intraperitoneal injection of glucose (1 g glucose/kg of body weight) or insulin (0.75 IU for WT or 2 IU for *ob/ob* per kg of body weight), respectively. Blood glucose was measured using a 1 Touch Ultra glucometer (Johnson & Johnson). Insulin plasma levels were measured using a commercial Rat/Mouse Insulin ELISA kit (EZRMI-13K, Merck Millipore, Burlington, Massachusetts, USA). Absorbance at 450 nm and 590 nm was read in a microplate reader (Synergy H1 Hybrid wavelength, BioTek, Winooski, Vermont, USA).

2.5. Glutamine and glutamate measurements

Glutamine and glutamate were measured in the liver, skeletal muscle, and plasma using a commercial kit (Glutamine and Glutamate Determination Kit, #GLN1, Sigma–Aldrich, St. Louis, Missouri, USA). Absorbance was measured at 340 nm in a microplate reader (Synergy H1 Hybrid wavelength, BioTek). The results of plasma glutamine and glutamate levels are as mmol/L. Tissue glutamine and glutamate content are expressed as μmol/g of tissue fresh weight.

2.6. Measurements of plasma triglycerides and cholesterol levels and hepatic enzyme activities

Plasma levels of triglycerides, total cholesterol, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (Gamma GT) were measured using commercial ELISA kits (Labtest, Lagoa Santa, MG, Brazil). Absorbance for each parameter was measured in a microplate reader (BioTek) following the manufacturer's instructions and as described by Finamor, Perez [32]. Results of plasma AST, ALT, and Gamma GT are as U/L and triglycerides and total cholesterol as mg/dL.

2.7. Liver histological analysis (Hematoxylin/Eosin - H and E Staining)

The histological analysis of the liver was performed following the method described by Levene, Kudo [29], where the tissue was fixed in 10% formaldehyde solution for 8 hours. Subsequently, the fixed samples were kept overnight in 70% ethanol. Samples were then dehydrated through baths in 95% ethanol, 100% ethanol, and xylene. Subsequently, the samples were embedded in paraffin at 60°C. A microtome (Zeiss, Jena, Germany) was used to cut the samples into 5-micron slices. Slices were stained with H and E, and cellular morphology was evaluated considering the presence of intracellular vacuoles. About 10 images from each animal were obtained for qualitative evaluation using a Nikon Eclipse Ti-U microscope at 20X magnification coupled with a Nikon DS-R1 digital camera and NIS-Elements BR 3.1 software. The images were projected onto a high-resolution liquid crystal display (LCD) monitor.

2.8. Liver total lipids (Oil Red O - ORO Staining)

Liver samples were embedded in tissue-tek (Thermo Fisher Scientific, Waltham, Massachusetts, USA), placed in isopropanol, and immediately frozen in liquid nitrogen. Twelve-micron slices were prepared using a cryostat (Microm H560, Thermo Fisher Scientific). About 3 slices from different parts of the samples were disposed per slide, and 2 slides per animal were used. Slides were stained with ORO and Mayer's hematoxylin. About 10 images from each animal were obtained using a microscope with 20X objective magnification. The identification and quantification of ORO-stained areas were performed using the ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). This quantification represents the abundance of hepatocyte lipid droplets, as previously described [33].

2.9. Measurements of glutathione system components

Reduced glutathione (GSH) and oxidized glutathione (GSSG) contents in the liver and skeletal muscles were measured using a commercial kit (Quantification kit for oxidized and reduced glutathione, #38185, Sigma-Aldrich) following the manufacturer's instructions and as described by Nakagawa, Umemura [34]. Results are expressed as μmol/g of fresh tissue.

2.10. Thiobarbituric Acid Reactive Substances (TBARS) assay

The TBARS assay was performed on samples from the liver and skeletal muscle to evaluate the amount of malondialdehyde (MDA), a major lipid peroxidation product in animal tissues. As previously described by Draper and Hadley (1990) [35], measurements were carried out at 535 nm using a microplate reader (Synergy H1 Hybrid wavelength, BioTek). Results expressed as MDA/g of fresh tissue.

2.11. Activities of nuclear factor erythroid 2–related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB) p65 subunit

Nuclear and cytosolic fractions were extracted from samples of the liver and skeletal muscle using a commercial kit (Nuclear Extraction Kit, #10009277, Cayman Chemical, Ann Arbor, Michigan, USA). The Nrf2 nuclear translocation was evaluated by the Nrf2 transcription factor assay kit (#600590, Cayman Chemical). The NF-κB (p65) transcription was evaluated by the NF-κB (p65) Transcription Factor assay Kit (#10007889, Cayman Chemical). Measurements were made at 450 nm using a microplate reader (Synergy H1 Hybrid wavelength, BioTek) as described by the manufacturer and MacDowell, Munarriz-Cuezva [36]. Results expressed in absorbance/mg protein.

2.12. Real-time PCR assay

Total RNA extraction from the liver and skeletal muscle homogenates was performed using TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA samples were reverse transcribed to cDNA, and specific primers, described in the supplementary material S1 were used to evaluate mRNA expressions through real-time RT-PCR using a Rotor-Gene 6000 equipment and SYBR GREEN (Thermo Fisher Scientific) as a fluorescent dye. mRNA expression was evaluated in a Rotor-Gene 6000 Software by the 2-$\Delta\Delta$Ct method [34] using β2-microglobulin (β2M) as a housekeeping gene. All gene melting curve diagrams can be found in the supplementary material S2.

2.13. Statistical analyses

Results are presented as mean±SEM of at least 3 independent experiments. Statistical analyses were performed in Windows using GraphPad Prism (La Jolla, California, USA). Data was first analyzed for normality using the Kolmogorov-Smirnov. Subsequently, comparisons between groups were evaluated by 1-way ANOVA followed by Tukey posthoc test. About 2-way ANOVA followed by Bonferroni posthoc-test was used in GTT and ITT analyses. Confidence intervals were set to a 5% significance level (P<.05).

3. Results

3.1. Amino acid supplementation effects on body weight and composition, food, and water intake

Obese ob/ob mice are hyperphagic due to leptin deficiency [37]. Control ob/ob (CTRL) mice exhibited higher food intake (by 67%), body weight (by 84%), body weight gain (by 7-fold), body fat percentage (by 5-fold) and percentage of body fluids (by 44%), and lower lean mass percentage (by 43%) than WT animals (Table 1). Similar results have been reported by others using genetically modified obese animals [38,39].

Obese ob/ob mice receiving free glutamine showed a decrease in food intake (by 20%) and an increase in water intake (by 24%) when compared to control (untreated) ob/ob animals (P<.05, Table 1). Obese ob/ob mice supplemented with DIP exhibited a similar increase in water intake (by 28%) and a non-significant decrease trend in food intake (P<.12). This effect was possible because solutions containing amino acids (*i.e.*, DIP and free glutamine) offered daily in the drinking water increased circulating amino acid concentration and promoted hypothalamic satiety signals [40–42].

The daily water intake did not indicate significant differences in amino acid ingestion among the supplemented groups. Although

Table 1
Body weight and composition, food intake and water intake in wild-type and *ob/ob* mice

		ob/ob groups		
Measurements/groups	WT	CTRL	DIP	GLN
Food intake (g/day)	3.80 ± 0.27	6.33 ± 0.17*	5.74 ± 0.17	5.06 ± 0.16#
Water intake (ml/day)	7.04 ± 0.55	6.08 ± 0.19	7.78 ± 0.28#	7.53 ± 0.41#
Initial body weight (g)	24.89 ± 0.60	45.9 ± 1.55*	45.9 ± 1.55	45.19 ± 0.86
Total body weight gain (g)	0.99 ±0.36	7.15 ± 0.98*	6.90 ± 0.95	5.21 ± 0.53
% body weight gain	4.65 ±1.64	14.97 ± 2.51*	16.46 ± 2.34	12.38 ± 1.34
% of body fat	9.20 ± 0.98	45.55 ± 1.3*	49.67 ± 1.48	46.31 ± 0.95
% of lean mass	77.23 ± 1.71	43.99 ± 0.95*	40.94 ± 1.05	44.31 ± 0.74
% of body fluids	6.26 ± 0.12	8.99 ± 0.21*	9.78 ± 0.24#	9.08 ± 0.14

Groups are: C57BL/6 lean (WT), untreated control) ob/ob (CTRL), ob/ob supplemented with dipeptide (DIP) or L-glutamine (GLN). Animals were supplemented for 40 days in the drinking water (with 4% solution containing amino acids. Data are mean ± SEM (n=6). * Indicates difference versus WT (p<0.05); # indicates difference versus CTRL untreated ob/ob (p<0.05). One-way ANOVA followed by Tukey.

Table 2
Glutamine and glutamate in plasma, liver, and skeletal muscle

			ob/ob groups		
Measurements/groups		WT	CTRL	DIP	GLN
Plasma (mmol/L)	Glutamine	1.44 ± 0.07	1.11 ± 0.07*	1.54 ± 0.10#	1.33 ± 0.09
	Glutamate	0.52 ± 0.03	0.53 ± 0.02	0.59 ± 0.04	0.56 ± 0.03
Liver (µmol/g of fresh tissue)	Glutamine	5.20 ± 0.24	3.90 ± 0.41*	5.81 ± 0.41#	5.18 ± 0.18
	Glutamate	1.71 ± 0.12	1.38 ± 0.21	1.75 ± 0.06	1.83 ± 0.16
Skeletal Muscle (µmol/g of fresh tissue)	Glutamine	9.19 ± 0.53	6.75 ± 0.36*	9.83 ± 0.36#	8.98 ± 0.65#
	Glutamate	3.40 ± 0.17	3.56 ± 0.17	3.57 ± 0.20	3.36 ± 0.15

Groups are: C57BL/6 lean (WT), untreated (control) *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in the drinking water with 4% solution containing amino acids. Data are mean ± SEM (n=6 per group). * Indicates difference versus WT (p<0.05); # indicates difference versus CTRL untreated *ob/ob* (p<0.05). One-way ANOVA followed by Tukey.

the DIP group showed a small variation in total body weight gain and percentages of body fat and lean mass, we could not observe significant differences among the supplemented groups (Table 1). Nevertheless, the percentage of free body fluid was higher ($P<.05$) in the DIP (by 8.8%) group when compared to control ob/ob mice (Table 1). While body composition changes can be induced by amino acids intake, the absence of these effects in the GLN-supplemented group may eventually lead to the hypothesis that the presence of L-alanine in the DIP could have been responsible for some metabolic changes [7,43] associated with mild and non-significant body composition variations.

3.2. DIP supplementation raises plasma and tissue glutamine levels in ob/ob *mice*

Plasma glutamine levels may be reduced in catabolic conditions, such as sepsis [7,8] and exhaustive exercise [13,14], and in chronic diseases, such as obesity-induced type 2 diabetes mellitus (T2DM) [18,22]. Herein, control ob/ob mice had significantly lower plasma glutamine levels (by 22%), and glutamine content in the liver (by 25%) and skeletal muscle (by 26%), compared to WT mice (Table 2). DIP supplementation restored ($P<.05$) glutamine levels in plasma (by 38%), liver (by 48%), and skeletal muscle (by 45%, Table 2). In ob/ob animals supplemented with free GLN, the skeletal muscle glutamine levels were restored ($P<.05$), by 33%, as compared to ob/ob controls (Table 2). No statistical differences were found in glutamate in plasma, liver and skeletal muscle among all groups (Table 2).

3.3. DIP and free GLN supplementation improve insulin resistance in ob/ob *mice*

Hyperglycaemia and hyperinsulinemia are commonly associated with obesity [44]. As also reported by others [42,43], *ob/ob* mice showed hyperglycaemia and hyperinsulinemia. Control *ob/ob* mice exhibited 49% higher blood glucose levels, accompanied by plasma insulin levels nearly 10 times higher than in WT animals ($P<.05$, Fig. 1A and B). In *ob/ob* mice supplemented with DIP and free GLN, hyperglycaemia and hyperinsulinemia were attenuated ($P<.05$) by 17% when compared to control *ob/ob* mice.

The glucose tolerance test (GTT) was evaluated after 4 weeks of supplementation, and the insulin tolerance test (ITT) was performed the following week. Control *ob/ob* mice exhibited the expected glucose and insulin intolerance. DIP supplementation improved glycaemic (Fig. 1C and E) and insulinemic (Fig. 1D and F) responses when compared to control *ob/ob* animals ($P<.05$). Although animals supplemented with free GLN showed a non-significant reduction in AUC GTT (Fig. 1C), AUC ITT (Fig. 1D) was lower ($P<.05$), when compared to the *ob/ob* CTRL group. When compared to the DIP group, free GLN showed a mild ($P<.05$) increase in AUC GTT.

3.4. DIP and free GLN supplementation improve lipid metabolism and hepatic stress markers in ob/ob *mice*

Increased plasma triglyceride (TG) levels and ectopic fat deposition in various organs, including the liver, are associated with impaired hepatic function in obesity [45,46]. Control ob/ob mice

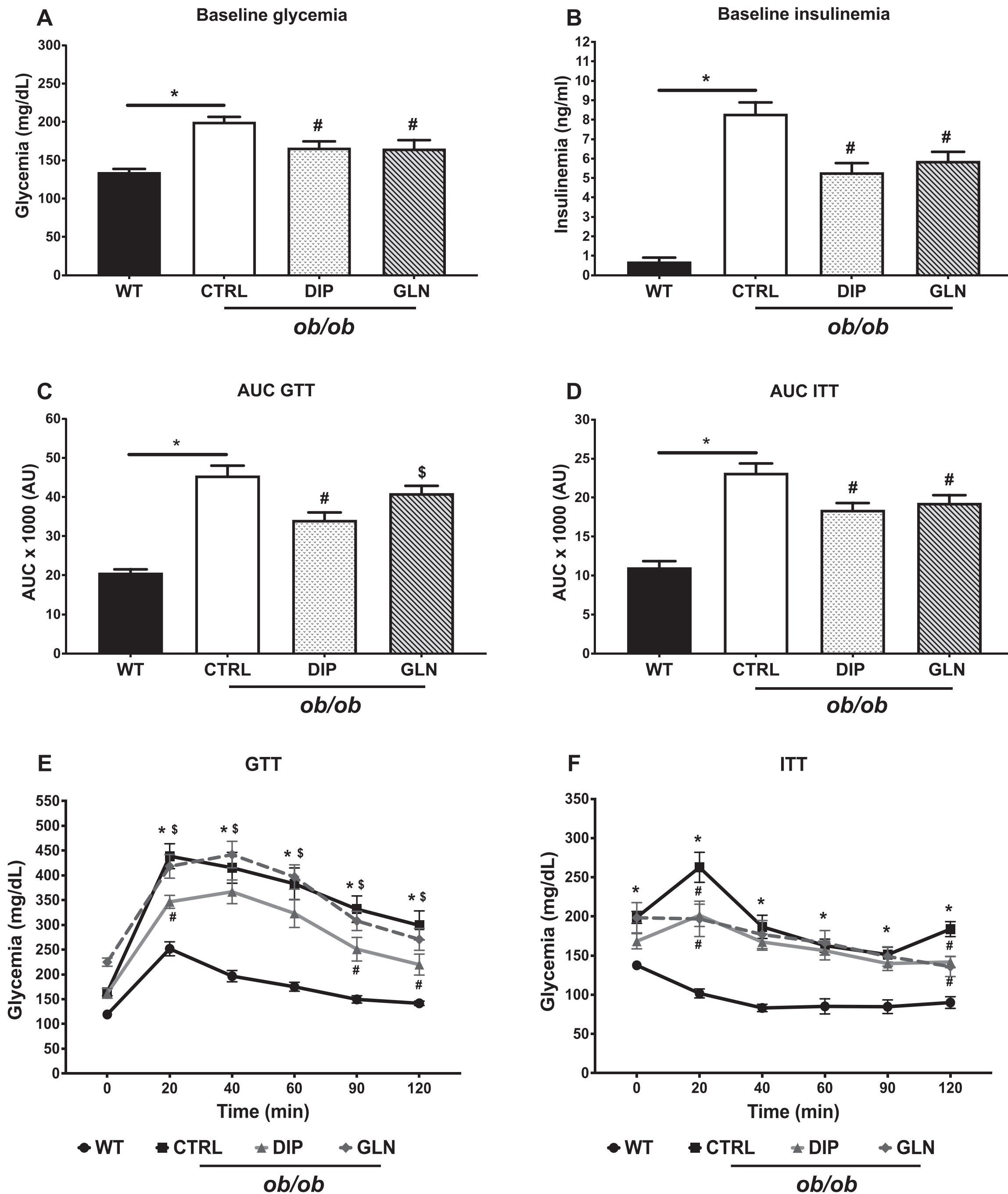

Fig. 1. Biomarkers of glucose homeostasis. Groups are C57BL/6J lean (WT), *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in drinking water with a 4% solution containing amino acids. (A) Baseline fasting glycemia (mg/dL). (B) Insulinemia (mg/dL). (C) Area under the curve (AUC) in Arbitrary Units (AU) of Figure 1E. (D) AUC in AU of Figure 1F. (E) Glycemia (mg/dL) monitoring during Glucose Tolerance Test (GTT). (F) Glycemia (mg/dL) monitoring during Insulin Tolerance Test (ITT). Values are mean±SEM (n=6 per group). * indicates difference *versus* WT ($P<.05$); # indicates difference *versus* CTRL *ob/ob* ($P<.05$); $ indicates difference *versus* DIP ($P<.05$). A, B, D and F: 1-way ANOVA followed by Tukey posthoc test; C and E: 2-way ANOVA followed by Bonferroni posthoc test.

showed increased TG and total plasma cholesterol levels and liver content of TG and fat droplets when compared to WT mice (Fig. 2A, C, B and G, respectively). The supplemented groups showed a decrease ($P<.05$) of approximately 40% in plasma TG levels and 44% in hepatic TG content (Fig. 2A and B, respectively).

Plasma activities of hepatic lesion marker enzymes, ALT, AST, and Gamma GT (Fig. 2D–F, respectively), were measured. Control Ob/ob mice exhibited increased ($P<.05$) plasma ALT and AST activities. DIP and free GLN supplementation decreased ($P<.05$) ALT activity but did not change AST and Gamma GT activities ($P<.05$). This is interesting, as it confirms previous findings [47] that increased intake of amino acids did not aggravate hepatic stress induced by obesity. Hepatic fat droplets were more abundant in the control ob/ob group than in WT mice ($P<.05$). The amino acid supplementations decreased by 79% the hepatic fat droplet abundance compared to control ob/ob animals ($P<.05$, Fig. 2G).

We have also measured the mRNA levels of transcription factors and enzymes involved in hepatic lipid metabolism. The lev-

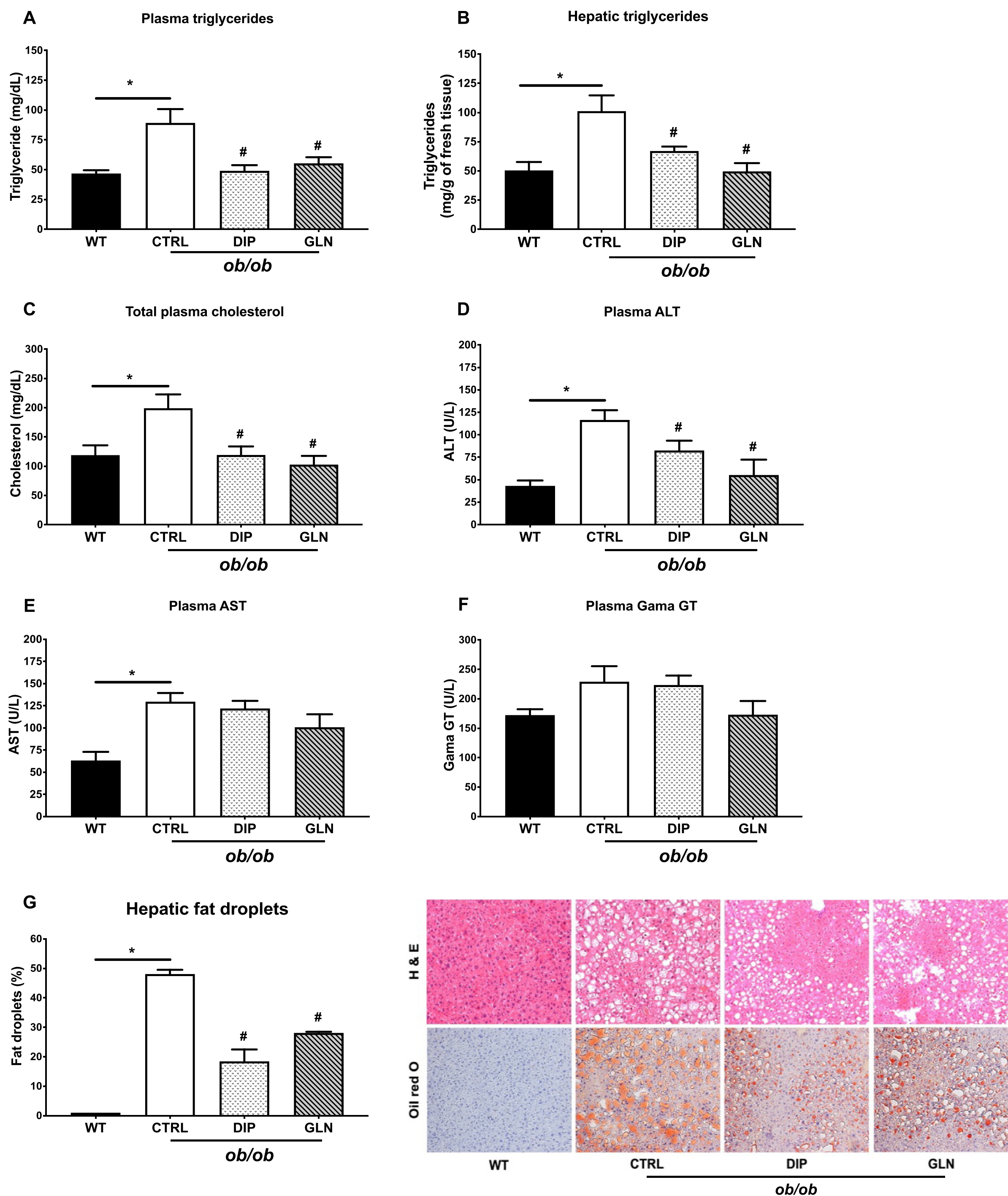

Fig. 2. Triglycerides, cholesterol, and liver biomarkers. Groups are C57BL/6J lean (WT), *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in drinking water with a 4% solution containing amino acids. (A) Plasma triglycerides (mg/dL). (B) Hepatic triglycerides (mg/g fresh tissue). (C) Plasma total cholesterol (mg/dL). (D) Plasma alanine aminotransferase (ALT) levels (U/L). (E) Plasma aspartate aminotransferase (AST) levels (U/L). (F) Plasma gamma-glutamyl transferase (Gamma GT) levels (U/L). (G) Hepatic fat droplets (%) and representative images of Hematoxylin and Eosin (H and E) or Oil Red O staining of liver samples. Values are mean±SEM (n=6 per group). * indicates difference *versus* WT ($P<.05$); # indicates difference *versus* CTRL *ob/ob* ($P<.05$). 1-way ANOVA followed by Tukey posthoc test.

els of the transcription factor SREBP-1c, which activates genes that encode for enzymes required for fatty acid synthesis, were increased (by 21-fold) in control ob/ob mice, compared to WT animals ($P<.05$, Fig. 3A). Increased ($P<.05$) Dgat-α (by 2.5-fold, Fig. 3B), ACC (by 14-fold, Fig. 3C), and FAS (by 10-fold, Fig. 3D) mRNA levels were found in control ob/ob mice as compared to WT animals. DIP and free GLN supplementation decreased SREBP-1c (by 85% and 71%, respectively), Dgat-α (by 53% and 50%, respectively), ACC (by 64% and 62%, respectively), and FAS (by 86% and 40%, respectively) expression compared to control ob/ob mice ($P<.05$). ChREBP plays a key role in regulating glucose metabolism and de novo lipogenesis in metabolism-regulating tissues, such as

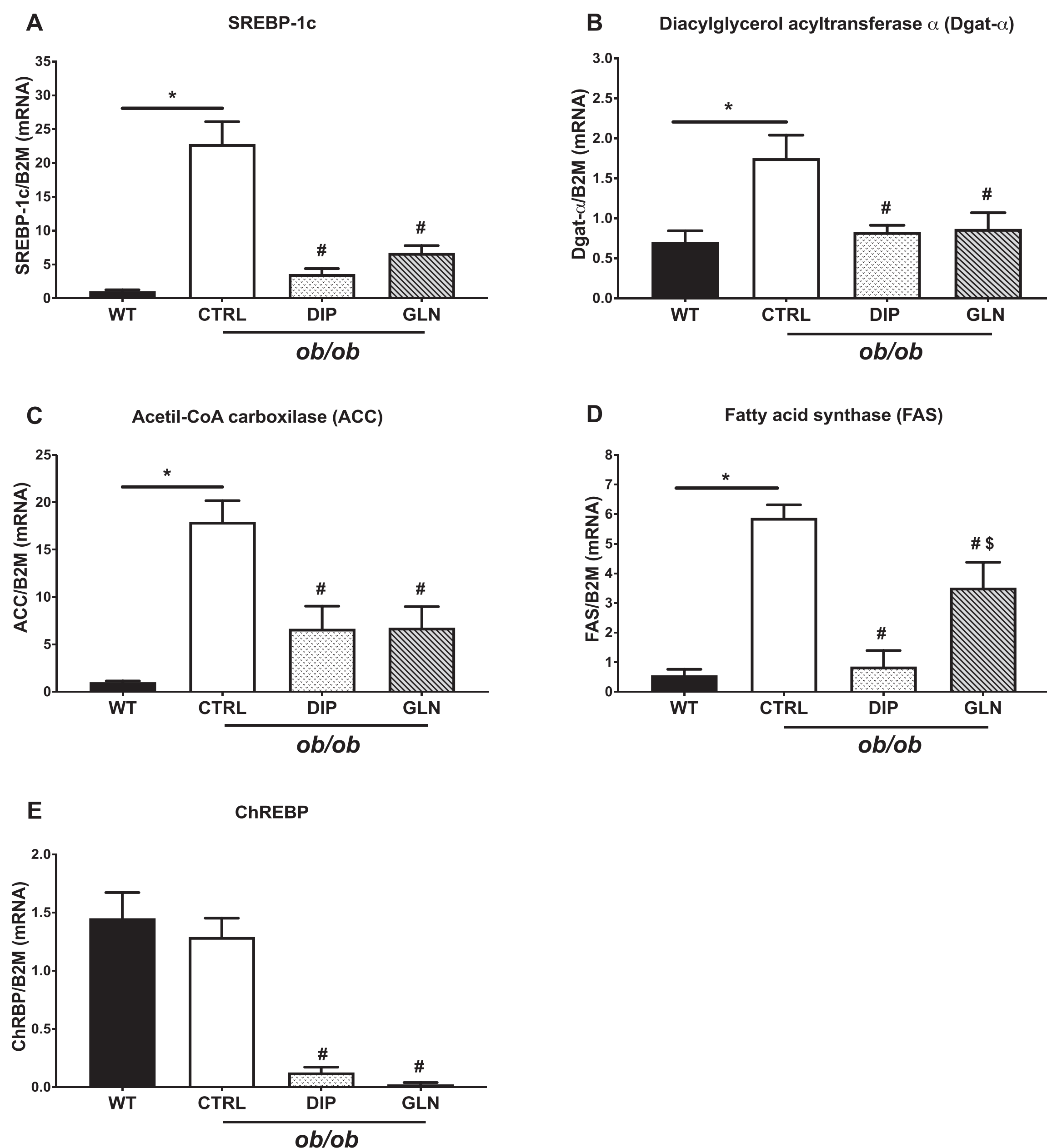

Fig. 3. mRNA expression of genes involved in lipid metabolism in the liver. Groups are C57BL/6J lean (WT), *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in drinking water with a 4% solution containing amino acids. (A) SRBP1c. (B) Diacylglycerol acyltransferase α (Dgat-α). (C) acetyl-CoA carboxylase (ACC). (D) fatty acid synthase (FAS). (E) (ChREBP). β2-microglobulin (B2M) was used as housekeeping. Values are mean±SEM (n=6 per group). * indicates difference *versus* WT (P<.05); # indicates difference *versus* CTRL *ob/ob* (P<.05); $ indicates difference *versus* DIP (P<.05). 1-way ANOVA followed by Tukey posthoc test.

the liver [48]. There were no significant differences in ChREBP expression between control ob/ob and WT mice (Fig. 3E). Conversely, DIP and free GLN groups exhibited a decreased (by 90% and 98%, respectively) expression of ChREBP compared to control ob/ob mice (P<.05, Fig. 3E).

3.5. Antioxidant effects mediated by the supplemented amino acids

The prolonged caloric surplus and consequently chronically elevated circulating glucose and fatty acids in obesity increase the production of reactive oxygen species (ROS), leading to oxidative stress in tissues and organs, such as the liver and skeletal muscles [49–51]. Since glutamine has antioxidant properties [7,14,52], we investigated the effects of glutamine supplementation on oxidative stress and antioxidant status indicators. Control ob/ob mice increased by 55% and 86% liver and skeletal muscle TBARS contents, respectively, compared to the WT group (P<.05, Table 3). DIP and free GLN reduced TBARS contents by around 40–50% in the skeletal muscle and liver of ob/ob mice as compared to the control ob/ob group (P<.05, Table 3).

We have also measured the transcription factor Nrf2 translocation, as it controls the gene expression of several antioxidant enzymes under normal and stressed conditions [53]. Nrf2 translocation was reduced by 87% and 43% (P<.05) in control ob/ob mice, compared to WT in both liver (Fig. 4A) and skeletal muscle (Fig. 4B), respectively. DIP and free GLN supplementation sig-

Table 3
TBARS in liver and skeletal muscle.

Measurements/groups	WT	*ob/ob* groups		
		CTRL	DIP	GLN
Liver (MDA/g fresh tissue)	2.90 ± 0.4	4.51 ± 0.55*	2.45 ± 0.40#	2.41 ± 0.35#
Skeletal muscle (MDA/g fresh tissue)	2.52± 0.29	4.71 ± 0.67*	2.75 ± 0.46#	2.71 ± 0.35#

Groups are: C57BL/6 lean (WT), untreated (control) ob/ob (CTRL), ob/ob supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in the drinking water with 4% solution containing amino acids. Data are mean ± SEM (n=6). * Indicates difference versus WT (p<0.05); # indicates difference versus CTRL untreated ob/ob (p<0.05). One-way ANOVA followed by Tukey.

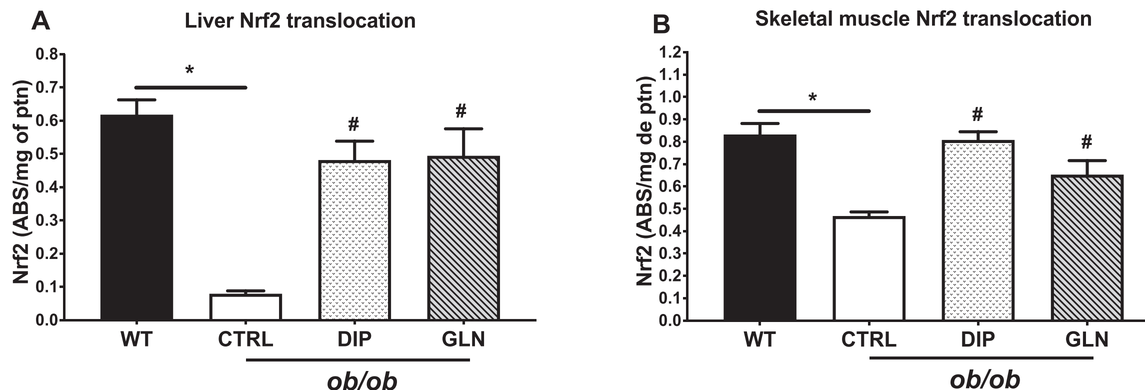

Fig. 4. Nrf2 translocation. Groups are C57BL/6J lean (WT), *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in drinking water with a 4% solution containing amino acids. Nrf2 translocation in (A) liver and (B) skeletal muscle. Values are mean±SEM (n=6 per group). * indicates difference *versus* WT (P<.05); # indicates difference *versus* CTRL *ob/ob* (P<.05). 1-way ANOVA followed by Tukey posthoc test.

nificantly raised (by 6-fold and 6.2-fold, respectively) liver Nrf2 translocation. The marked effect of the amino acids in obese mice almost returned the Nrf2 translocation levels to those observed in lean WT animals. Skeletal muscle Nrf2 translocation also showed an increase (by 73% and 36%, respectively) due to DIP and free GLN supplementation (P<.05, Fig. 4B).

The mRNA levels of the antioxidant enzymes were also measured (Fig. 5). In the liver, there was no difference in catalase and GCLC mRNA levels found among ob/ob and WT animals (Fig. 5A and E, respectively). Interestingly, in the skeletal muscle of ob/ob mice, expressions of catalase and GCLC were significantly reduced (by 96%) when compared to WT animals (P<.05, Fig. 5B and F, respectively). In the same tissue, DIP and free GLN supplementation abolished the decreased catalase mRNA levels observed in control ob/ob mice (P<.05, Fig. 5B). GPx1 mRNA levels in the liver were markedly increased (1.4-fold) in GLN-supplemented animals when compared to control ob/ob mice (P<.05, Fig. 5C). DIP supplementation increased GPx1 mRNA levels in the liver (by 44%, Fig. 5C) and muscle (by 1.8-fold) when compared to control ob/ob mice (P<.05, Fig. 5D).

Nrf2 has also been involved in maintaining the redox state of the cell through the glutathione system [54]. The redox state of the skeletal muscle was markedly different in control ob/ob compared to WT mice: reduction of 40% in total glutathione and 50% in reduced glutathione (GSH), whereas the GSSG/GSH ratio was increased by 50% (Table 4). There was no difference in the liver glutathione system components of control ob/ob mice compared to WT. However, DIP and free GLN raised the total glutathione content in the liver (by 53% and 40%, respectively) and in the skeletal (by 83% in both) when compared to control ob/ob mice. DIP and free GLN also increased GSH content in the liver (by 63% and 59%, respectively) and in skeletal muscle (by 100% and 166%, respectively). The GSSG/GSH ratio was reduced due to DIP (by 25%) and GLN (by 35%) treatments in the liver, whereas in skeletal muscle, both decreased by around 60% (P<.05, Table 4).

3.6. Anti-inflammatory effects of the supplemented amino acids

Fatty acid and cholesterol metabolism changes are widely reported in obesity and are likely intricately linked to the development and sustainment of metabolic inflammation and insulin resistance [55]. We measured the translocation of NFkB p65 in the liver and skeletal muscle (Fig. 6A and B, respectively). There was an increase (by 2-fold and 2.5-fold, respectively) in translocation of NFkB p65 in the untreated (control) *ob/ob* compared to the WT group in liver and skeletal muscle. The DIP supplementation reduced (P<.05) the translocation of p65 in the liver.

Regarding the expression of inflammatory cytokine genes, TNF-α gene expression was higher (by 3.3-fold and 95%, respectively) in the liver and skeletal muscle of control *ob/ob* than in WT (Fig. 6C and D, respectively). Conversely, DIP and free GLN supplementations reduced (by around 75%) TNF-α gene expression in the liver (P<.05). DIP and free GLN also reduced (by around 80%) TNF-α gene expression in skeletal muscle (P<.05). Control *ob/ob* mice exhibited lower IL-6 (by 80%) gene expression in the skeletal muscle (P<.05, Fig. 6F) when compared to WT. Although amino acid-treated *ob/ob* animals showed an increased trend in muscle IL-6 gene expression, this was not statistically significant.

4. Discussion

The present study investigated the effects of free (GLN) and dipeptide (DIP) forms of L-glutamine supplementation in obese ob/ob mice. The results presented herein confirm our hypothesis that body glutamine levels restoration reduces oxidative stress and inflammation in obese ob/ob mice. This effect attenuated the

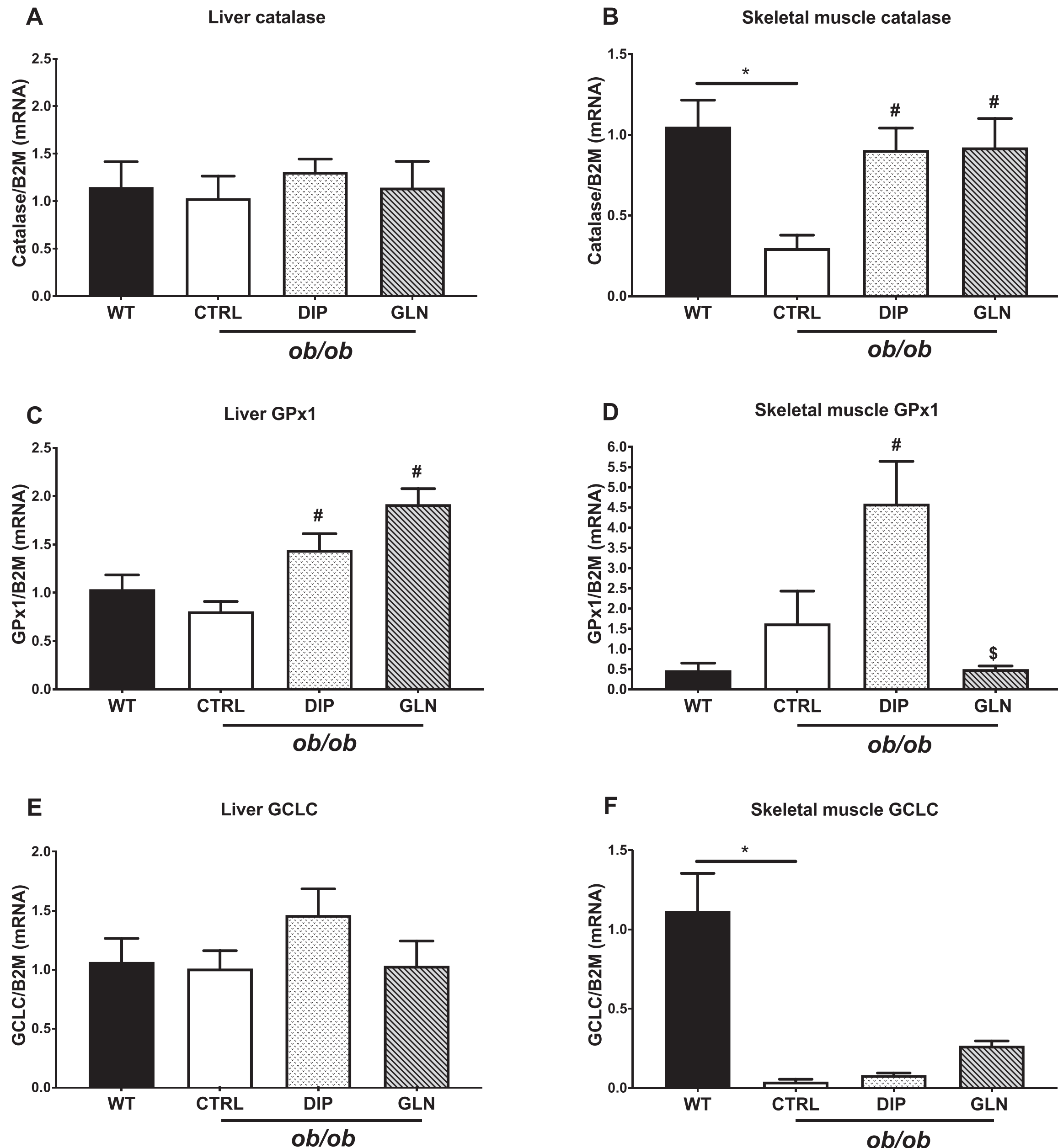

Fig. 5. Antioxidant enzymes mRNA expression in liver and skeletal muscle. Groups are C57BL/6J lean (WT), *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in drinking water with a 4% solution containing amino acids. (A) Catalase in the liver. (B) Catalase in skeletal muscle. (C) Glutathione peroxidase 1 (GPx1) in the liver. (D) GPx1 in skeletal muscle. (E) Glutamate-cysteine ligase (GCLC) in the liver. (F) GCLC in skeletal muscle. β2-Microglobulin (B2M) was used as housekeeping. Values are mean±SEM (n=6 per group). * indicates difference *versus* WT (P<.05); # indicates difference *versus* CTRL *ob/ob* (P<.05). $ indicates the difference *versus* DIP (P<.05). 1-way ANOVA followed by Tukey posthoc test.

hepatic lipid metabolic changes commonly associated with MASLD development; a disease frequently observed in T2DM induced by obesity.

Modern lifestyle often promotes an imbalance between calories consumed and calories expended. The excessive intake of calories, associated with sedentarism, leads to an increased accumulation of body fat, as the surplus of energy is stored as triglycerides in WAT [2]. In this study, we have observed that ob/ob mice presented an increase (P<.05) in food intake, as well as a high % of body fat and a low % of lean body mass when compared to WT animals (Table 1). High WAT and low lean body mass are signs of overweight and obesity [56], which favours the development of other chronic diseases, including T2DM and MASLD [2,57,58]. For instance, excessive WAT mass enlarges fat cells (*i.e.*, hypertrophy) and triggers a persistent state of low-grade oxidative stress and inflammation [17,59]. Here, control ob/ob mice showed increased skeletal muscle and liver biomarkers of oxidative stress (Table 4) and inflammation (Fig. 6). This condition was accompanied by impaired insulin sensitivity, as indicated by glucose homeostatic parameters, like baseline glycemia, insulinemia, and GTT/ITT responses (Fig. 1A, B, C/E, and D/F, respectively). Although the precise mechanisms remain unclear, there is increasing evidence that oxidative stress and inflammation play a crucial role in the etiology of obesity and the development of T2DM [3,4].

In tissues such as the skeletal muscle and liver, chronic oxidative stress and inflammation promote perturbations in the trans-

Table 4
Levels of total, reduced (GSH) and oxidized (GSSG) glutathione and the ratio (GSSG/GSH) in liver and skeletal muscle.

		ob/ob groups		
measurements/groups	WT	CTRL	DIP	GLN
LIVER				
Total glutathione (μmol/g fresh tissue)	0.49 ± 0.04	0.43 ±0.04	0.62 ± 0.02#	0.60 ± 0.03#
GSH (μmol/g fresh tissue)	0.32 ± 0.03	0.28 ± 0.02	0.44 ± 0.05#	0.43 ± 0.04#
GSSG (μmol/g fresh tissue)	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
GSSG/GSH ratio	0.29 ± 0.06	0.28 ± 0.04	0.23 ± 0.06	0.20 ± 0.03
SKELETAL MUSCLE				
Total glutathione (μmol/g fresh tissue)	0.080 ± 0.003	0.033 ± 0.005*	0.080 ± 0.012#	0.104 ± 0.028#
GSH (μmol/g fresh tissue)	0.063 ± 0.006	0.030 ± 0.003*	0.068 ± 0.006#	0.087 ± 0.019#
GSSG (μmol/g fresh tissue)	0.015 ± 0.001	0.016 ± 0.002	0.015 ± 0.001	0.016 ± 0.002
GSSG/GSH ratio	0.241 ± 0.019	0.581 ± 0.123*	0.231 ± 0.019#	0.215 ± 0.048#

Groups are: C57BL/6 lean (WT), untreated (control) ob/ob (CTRL), ob/ob supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in the drinking water with 4% solution containing amino acids. Data are mean ± SEM (n=6). * Indicates difference versus WT (p<0.05); # indicates difference versus CTRL untreated ob/ob (p<0.05). One-way ANOVA followed by Tukey.

membrane insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1R) via downstream pathways, including IκB kinase (IKKβ)/NF-κB, protein kinase B (PKB/Akt), and c-Jun N-terminal kinase (JNK) [60]. These signaling pathways lead to the phosphorylation of IRS-1/IRS-2 by serine kinases, which disrupts insulin signaling leading to insulin resistance [4,61]. This condition is particularly important in the skeletal muscle, as this tissue is a major regulator of systemic glucose homeostasis, managing around 75% of whole-body insulin-stimulated glucose uptake to synthesize glycogen and generate ATP [62]. The liver is also a key glucose metabolic regulator, as it orchestrates the balance of glucose uptake and storage via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis during fasting periods [23,63]. In addition, high hepatic triglycerides and fat droplet accumulation (Fig. 2B and G, respectively) observed in our study can impair mitochondrial function, which further contributes to ROS production and inflammation, leading to insulin resistance and MASLD induced by obesity [64,65].

Traditionally, many studies have explored carbohydrate and lipid metabolism disturbances in the context of T2DM induced by obesity [3,60]. However, much less is known about the role of amino acids. For instance, it has been shown that among the 20 amino acids detailed in the mammal's genetic code, the L-α-amino acid glutamine may be associated with the progression of T2DM [18,19,66–68]. Using ob/ob mice as a model of obesity, we confirm previous findings that lower (P<.05) plasma and/or tissue (*i.e.*, skeletal muscles and liver) glutamine levels (Table 2) can be observed in obesity. Although the pathophysiology of abnormal glutamine levels is not completely understood, a significant number of studies have identified that glutamine levels in the body rely on a series of homeostatic mechanisms affecting the activity of glutamine synthetase, a key glutamine synthesis enzyme, leading to impairments in glutamine synthesis/release. At the same time, there is an increase in glutaminase activity, a glutamine-degrading enzyme, which increases the uptake/consumption of glutamine [24,28,69]. In obesity, it seems that the greater glutamine consumption by inflammatory cells is superior to the body's ability to meet these requirements due to the dramatic reduction in glutamine production, especially in the skeletal muscle and liver. Indeed, as observed in Table 1, glutamine levels in CTRL ob/ob mice were dramatically reduced, when compared to lean animals. While WAT is abundant in obesity, and these cells can produce and utilize glutamine, it has a minor contribution to the body's glutamine levels and/or stores [70,71].

The fall of body glutamine in catabolic scenarios has been driving glutamine supplementation studies. Herein, the chronic provision of glutamine in the DIP form to ob/ob mice restored (P<.05) plasma and tissue glutamine levels (Table 2). This effect may have reduced (P<.05) the oxidative stress induced by obesity in the liver and skeletal muscle tissues (Table 3) via the glutamine-GSH axis (Table 4) and Nrf2. Despite the increase in plasma and liver glutamine stores were not statistically significant, free GLN promoted several positive effects, like DIP. DIP forms of glutamine supply are generally known to be more effective, producing better results in several catabolic situations [7,30]. However, based on the results presented herein, the effectiveness of oral glutamine supply may not only rely on its biochemical form (*i.e.*, free *vs.* conjugated with another amino acid), but also on its frequency of ingestion. Indeed, previous studies showed that chronic free GLN and DIP have a similar impact on glutamine metabolism and were associated with antioxidant and anti-inflammatory effects in animal models [43,72].

GSH (γ-L-glutamyl-L-cysteinylglycine) is a nonenzymatic antioxidant compound ubiquitously distributed in eukaryotes that can directly react with ROS, generating oxidized GSH (GSSG). The ratio between the intracellular concentration of GSSG and GSH, generally expressed as [GSSG]/[GSH] is an indicator of the redox state of the cell [73]. The first step in the de novo synthesis of GSH begins with the action of glutamate-cysteine ligase (GCLC), an enzyme responsible for catalyzing the initial and rate-limiting reaction to form the dipeptide γ-glutamylcysteine from cysteine and glutamate [74]. Hence, the reduction (P<.05) in skeletal muscle GCLC (Fig. 5F) gene expression of control ob/ob mice has the potential to affect the de novo synthesis of GSH, resulting in higher (P<.05) biomarkers of oxidative stress (Tables 3 and 4). Nevertheless, the non-significant changes in liver GCLC (Fig. 5E) might be associated with the severity of MASLD. Some studies found significant reductions in GCLC only in severe cases of liver failure, rapidly leading to death [75]. This is due to the vital role of the liver in GSH synthesis and export to the rest of the organism [76]. Indeed, the restoration of muscle GSH may be due to the increased efflux of GSH by the liver. Alternatively, the lack of muscle GCLC changes in DIP and GLN groups sheds light on possible non-canonical GCLC

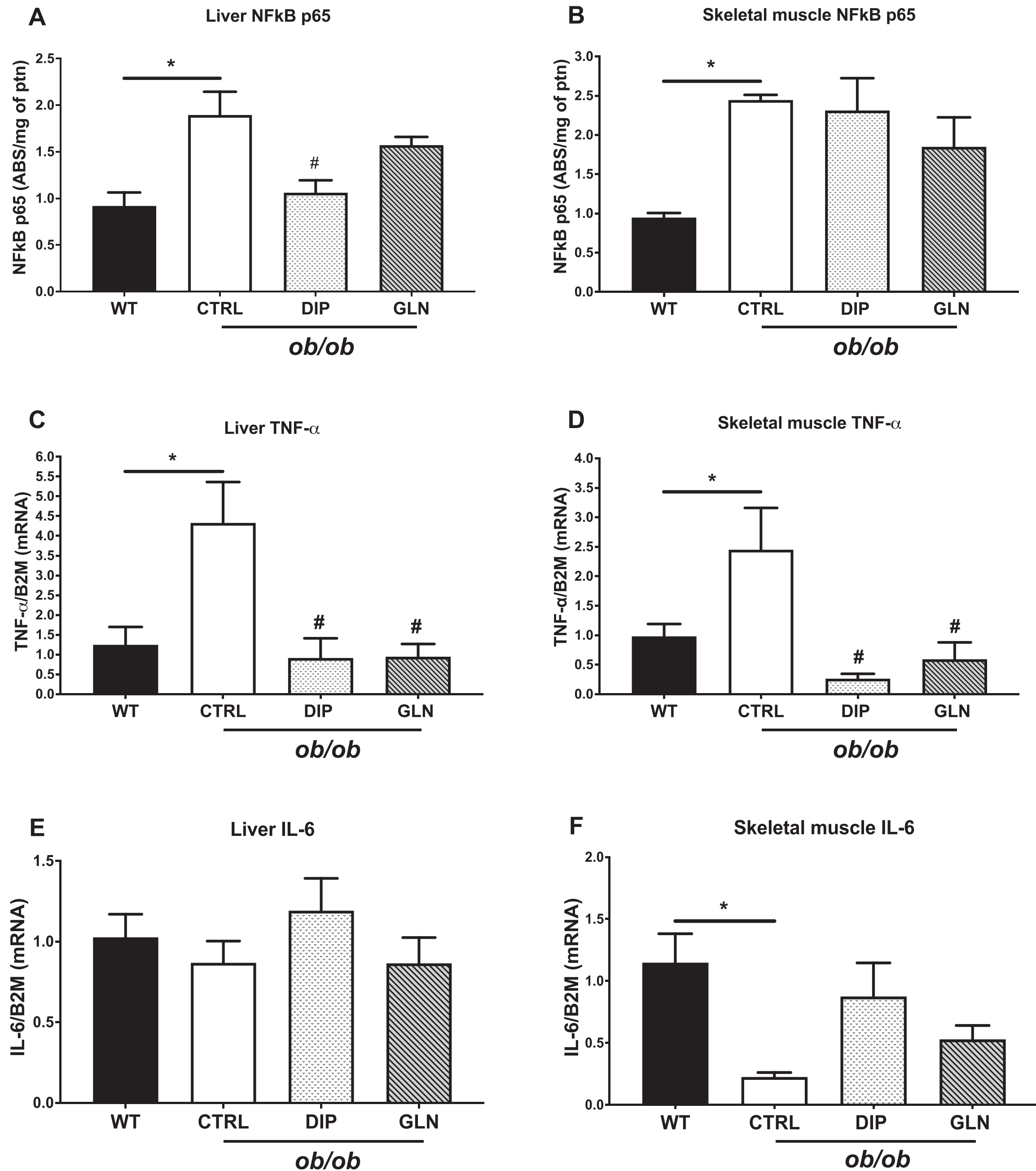

Fig. 6. Anti-inflammatory effects in liver and skeletal muscle. Groups are C57BL/6J lean (WT), *ob/ob* (CTRL), *ob/ob* supplemented with dipeptide (DIP) or free L-glutamine (GLN). Animals were supplemented for 40 days in drinking water with a 4% solution containing amino acids. (A) NFkB p65 translocation in the liver. (B) NFkB p65 translocation in skeletal muscle. (C) TNF-α mRNA expression in the liver. (D) TNF-α mRNA expression in skeletal muscle. (E) IL-6 mRNA expression in the liver. (F) IL-6 mRNA expression in skeletal muscle. Values are mean±SEM (n=6 per group). * indicates difference *versus* WT (P<.05); # indicates difference *versus* CTRL *ob/ob* (P<.05). 1-way ANOVA followed by Tukey posthoc test.

regulations [76]. More studies are required to understand the regulation of muscle GCLC, as well as its relationship with interorgan glutamine metabolism.

Based on the results presented herein, we believe that glutamine exogenous supply increased glutaminolysis to feed the glutamine-GSH axis, attenuating the pro-oxidant scenario induced by obesity. These results agree with previous findings in animals [7,72] and humans [9,77]. While low (P<.05) Nrf2 translocation was detected in the liver (Fig. 4A) and skeletal muscle (Fig. 4B) of control ob/ob mice, the increase (P<.05) in Nrf2 translocation induced by DIP and free GLN in the liver, and skeletal muscle sheds light into the involvement of a broader antioxidant mechanism. The transcription factor Nrf2 exerts a central role in the redox state of the cell by binding to the antioxidant response element (ARE) and positively regulates the expression of various cellular defense mechanisms against toxic and oxidative stressors [78]. This condition includes catalase (Fig 5A and B) and GPx1 (Fig 5C and D) gene expression. Several nutritional compounds have shown antioxidant effects via Nrf2 in chronic diseases associated with obesity, including sulforaphane [79], curcumin [80], garlic by-products [81], and, more recently, glutamine [82].

Nrf2 nuclei translocation occurs in the cytosol by dissociating from Kelch-like-ECH-associated protein 1 (KEAP1) [83]. In physiological situations, ROS can react with KEAP1 sensor cysteine

residues, thus promoting the activation of antioxidant responses to maintain homeostasis. However, during stress, especially induced by chronic hyperglycemia and hyperlipidemia, Nrf2 gene expression is downregulated [83–85]. The lack of antioxidant responses associated with the loss of Nrf2 is likely to alter many cellular signaling pathways, including the inflammatory response mediated by NF-κB [78]. This observation agrees with our findings, where control ob/ob animals presented an increase (P<.05) in NF-κB translocation in the liver and skeletal muscle (Fig. 6A and B), which possibly increased tissue TNF-α gene expression (Fig. 6C and D). On the other hand, in the liver, DIP supplementation restored (P<.05) Nrf2 translocation (Fig. 4A and B) values to a similar level observed in WT animals, and at the same time reduced (P<.05) NF-κB translocation in ob/ob mice (Fig. 6A). These results associated with lower (P<.05) TNF-α gene expression suggest that the anti-inflammatory properties of glutamine could be mediated by the Nrf2-NF-κB pathway. Mechanistic studies are required to confirm this hypothesis. Interestingly, free GLN also induced similar (P<.05) antioxidant responses via Nrf2, leading to lower TNF-α gene expression in the liver (Fig. 6C) and skeletal muscle (Fig. 6D). However, this effect was independent of p65 NF-κB translocation. It is possible that higher data variance in this group or alternative inflammatory pathways (*e.g.*, Jak-Stat), not included in this study could be associated with glutamine anti-inflammatory effects.

The interleukin-6 (IL-6) released by skeletal muscle has pleiotropic effects in several cells and tissues, including adipose tissue and liver. IL-6 also exhibits autocrine actions in the skeletal muscle cells where it is produced, and these effects may vary with this myokine level. For instance, low levels of IL-6 activate satellite cells and regeneration of myotube, whereas prolonged elevated levels cause skeletal muscle wasting [86]. Moreover, it has been described that IL-6 is a tissue-specific molecule with pro-inflammatory and anti-inflammatory roles and an energy sensor that regulates insulin-sensitive tissues, such as skeletal muscle and adipose tissue. In the skeletal muscle, IL-6 also potentiates adaptation to endurance training, improving its function in old mice [87]. Herein, both the reduction (P<.05) in ob/ob compared to control mice and the increased trend, although non-significant, induced by the amino acids in muscle IL-6 gene expression (Fig. 6E) in the ob/ob mice require further studies. So far, the latter findings are not aligned with the inflammation state described herein, and more research is required to clarify the role of IL-6 in the skeletal muscle of ob/ob mice and the response to glutamine supplementation.

Obesity is a multifaceted metabolic disorder that becomes a metabolic disease once the adaptive lipid-storing function is impaired. For instance, increased lipid storage in key metabolic organs, such as the liver, further aggravates insulin resistance and may lead to MASLD [2,88]. In this study, control ob/ob animals showed a rise (P<.05) in circulating lipids (Fig. 2A and C) and hepatic fat deposition (Fig. 2B and G), which is likely to be associated with further adverse health effects, such as insulin resistance (Fig. 1). However, our data showed that hepatic biomarkers of lipogenesis and the de novo synthesis of fatty acids, such as SREBP1, Dgat-α, ACC, and FAS (Fig. 3A–D, respectively) have been reduced by amino acids supplementation, which possibly explain why there was a lower (P<.05) accumulation of fat droplets (Fig. 2G). This effect on the liver has important clinical perspectives, as it could attenuate the progression of MASLD via reductions in fibrosis [89]. A decrease in hepatic ChREBP (Fig. 3E) reduces the expression of enzymes involved in glycolysis, glucose production and lipogenesis and, through unknown mechanisms, also reduces body weight and insulin resistance [48]. Although our and other [47] findings indicate that these effects can be mediated by a reduction in oxidative stress and inflammation, we cannot discard the possibility that a higher intake of amino acids may have increased TCA cycle intermediates, such as pyruvate for further metabolism in the mitochondria [43,72].

DIP and GLN directly contribute to plasma and tissue glutamine levels. DIP allows a rapid increase in glutamine concentration via its preferred absorption mediated by Pept-1. Although free GLN is normally highly metabolized by enterocytes, its increased and chronic ingestion may spare tissue glutamine stores. Indeed, high glutamine ingestion can overcome the enterocyte glutamine utilization rates, allowing this amino acid to reach the blood and raise its circulating levels [7,90]. The administration of glutamine in a dipeptide form is a well-known successful strategy to prevent glutamine from its metabolization in the enterocytes [28,43,91]. This may explain differences reported in the effects of free glutamine with the results obtained in DIP-supplemented mice. It is important to note that the kinetic of oral L-glutamine supplementation (free and bond forms) ranges from around 30 to 120 minutes after ingestion. Defense mechanisms, on the other hand, including the antioxidant and immune/inflammatory systems have a quick metabolic turnover, and hence it is plausible that in 24 hours, chronic doses may have an advantage over single doses [24]. However, in animal models, chronic supplementation via drinking water increases the daily amino acid intake [43,72], which could be difficult to extrapolate to humans, and hence this can be considered a limitation of this study. Although we have not seen changes in liver damage biomarkers, the effects described herein highlight the importance of new studies in humans.

5. Conclusion

Evidence is herein presented that chronic glutamine supplementation, either in the free or dipeptide forms, can restore skeletal muscle glutamine levels, reduce oxidative stress and inflammation, and attenuate the hepatic lipid metabolic changes observed in *ob/ob* mice. Although more studies are required, especially in humans, these findings support the proposition that oral glutamine supplementation may improve the usual comorbidities associated with obesity and T2DM, such as fatty liver, which is a severe and highly deadly condition.

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Availability of data and materials

The data used and analyzed in this study are available from the corresponding author upon request.

Declaration of competing interest

The authors have no conflicts of interest to declare regarding the execution of this study nor manuscript for publication.

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Formal analysis, Data curation. **Eloisa Aparecida Vilas-Boas:** Writing – review & editing, Writing – original draft, Visualization, Resources, Formal analysis, Data curation. **Hilton K. Takahashi:** Validation, Software, Resources, Methodology, Investigation, Formal analysis. **Ana Cláudia Munhoz:** Validation, Software, Resources, Methodology, Investigation, Formal analysis. **Layanne C.C. Araújo:** Validation, Resources, Methodology, Investigation, Formal analysis. **Carla Roberta Carvalho:** Validation, Supervision, Resources, Methodology, Investigation, Formal analysis. **Jose Donato Jr:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Rui Curi:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Investigation, Formal analysis, Data curation. **Angelo Rafael Carpinelli:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Data curation. **Vinicius Cruzat:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2025.109842.

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