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Oral L-Citrulline Supplementation Improves Fatty Liver and Dyslipidemia in Adolescents with Abdominal Obesity: A Parallel, Double-Blind, Randomized Clinical Trial

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Abstract: Obesity in adolescents is associated with non-communicable risk factors and diseases like metabolic-associated fatty liver disease (MAFLD), which is the liver manifestation of metabolic syndrome. L-citrulline is a non-protein amino acid that has shown positive effects on the degree of steatosis in animals with non-alcoholic fatty liver disease (NAFLD). The aim of the study was to evaluate the effect of oral L-citrulline supplementation on liver function and cardiovascular risk factors in adolescents with abdominal obesity and MAFLD. A prospective, double-blind clinical trial in adolescents with abdominal obesity was randomized into two groups: forty-two adolescents were supplemented with L-citrulline (6 g of L-citrulline/day) (n = 22) and placebo (n = 20) for eight weeks. The variables evaluated were anthropometry, blood pressure, glucose, insulin, HOMA-IR, L-citrulline, L-arginine, malondialdehyde, lipid profile, liver profile, urea, uric acid, and hepatic steatosis by ultrasound. After supplementation, the L-citrulline group had a decrease in liver fat accumulation (p = 0.0007); increases in body weight (p = 0.02), glucose (p = 0.03), and HOMA-IR (p = 0.03); and decreases in BMI (p = 0.002), total cholesterol (p = 0.001), HDL-C (p = 0.01), LDL-C (p = 0.002), and alkaline phosphatase (p = 0.05). L-citrulline for eight weeks decreases hepatic fat accumulation and LDL-C levels in adolescents with abdominal obesity and MAFLD.

Keywords: L-citrulline; abdominal obesity; MAFLD; dyslipidemia

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1. Introduction

Obesity in children and adolescents is associated with increased morbidity and mortality in adulthood [1]. Obesity has been associated with non-communicable risk factors and diseases like hypertension, dyslipidemias, prediabetes, metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), and metabolic-associated fatty liver disease (MAFLD) [2,3]. Globally, the prevalence of MAFLD in children and adolescents has increased from 19.34 million in 1990 to 29.49 million in 2017 [4]. The prevalence of MAFLD comorbidities such as MS and T2DM were the highest in the USA, Mexico, Puerto Rico, Costa Rica, Chile, Venezuela, and Guyana in a review including 356 reports from 2000 to 2013 [5]. In 2020, an international expert consensus panel suggested a redefinition of non-alcoholic fatty liver disease (NAFLD) in adults to the term MAFLD [6,7], and, given the accumulating evidence for the validity of the MAFLD criteria in adults [8], an international panel of experts proposed and adapted the criteria for MAFLD for the pediatric population [9]. MAFLD has become the most common chronic liver disease in children and adolescents, and patients with childhood onset have higher risk of progressive disease [10,11].

The combination of several lifestyle factors, including diets rich in simple carbohydrates, saturated fatty acids, and fructose or a hypercaloric diet with low fiber added to sedentary behavior, leads to obesity, insulin resistance, dyslipidemia, and hepatoxicity [12–14]. The proposed criteria for a diagnosis of pediatric MAFLD are based on liver histology (biopsy sample), imaging, blood biomarkers, and evidence of intrahepatic fat accumulation (steatosis), in addition to one of the three criteria: excess adiposity, presence of prediabetes or type 2 diabetes, or evidence of metabolic dysregulation [9]. Ultrasonography can detect the degree of steatosis when it comprises more than 20% of the liver mass and is inexpensive and user friendly, making it the most used imaging modality for the detection of liver steatosis in the pediatric population. It provides a good estimate of the degree of steatosis in patients with suspected MAFLD [3,15]. The high prevalence of pediatric obesity and MAFLD has led to the investigation of the use of various amino acids, including L-citrulline, to attenuate the effect of obesity on the liver.

L-citrulline is a non-protein amino acid considered a metabolite of the urea cycle [16]. The effect of L-citrulline has been studied in pathologies where L-arginine has beneficial effects [17]. However, L-arginine is poorly absorbed from the small intestine, leading to abdominal discomfort and even diarrhea when given in high doses [18]. In addition, oral L-arginine provides reduced circulating L-arginine due to the activation of arginase enzymes that degrade it to ornithine in the enterocytes and hepatocytes [19]. Conversely, L-citrulline is effective, safe, and results in high L-arginine bioavailability [19]. Oral L-citrulline is well absorbed in the intestine, not taken up by the liver, and metabolized in the kidney to L-arginine [19]. Thus, oral L-citrulline is more efficient to increase circulating L-arginine than a similar dose of oral L-arginine [19,20].

In animal models, oral administration of L-citrulline has shown beneficial effects on the liver and lipid profile, reducing liver damage and dyslipidemia and increasing insulin sensitivity [21–24]. To our knowledge, only one human study has examined the effect of oral L-citrulline (2 g/day) for 12 weeks in adults with MAFLD. A decrease in inflammatory markers, ALT, and hepatic steatosis was found after supplementation in the placebo and citrulline group [25]. MAFLD is a poorly studied condition in the pediatric population and recommendations are based on lifestyle modifications. However, this approach may not be enough to improve liver function. The purpose of this study was to evaluate the effect of oral L-citrulline supplementation on the degree of steatosis in adolescents with abdominal obesity and MAFLD.

2. Materials and Methods

2.1. Subjects

Recruitment was carried out from April to December 2021. Mexican adolescents from the city of Leon, Guanajuato, who were aged between 15 and 19 years with overweight or obesity by body mass index (BMI) for age according to the World Health Organization (WHO), who were stage 5 on the Tanner scale, who had a diagnosis of MAFLD (measuring steatosis by ultrasound) [9], and who had no significant alcohol consumption were included in the study. Participants with L-citrulline intolerance, adherence of less than 80%, or other liver diseases were excluded.

2.2. Ethics Approval

The study was approved by the Ethics Committee for Research of the University of Guanajuato (CIBIUG-P69-2020) and registered in ClinicalTrials.gov with the number NCT04871360. After explaining the research procedure, the participants and their guardians signed an informed consent form.

2.3. Sample Size

The calculation of the sample size was made with the formula for comparative studies of Velasco et al. A minimum of 20 participants per group was required to achieve 80% power to detect greater than 5% change in grade of steatosis in the treatment groups with a

significance level of 0.05. Twenty-three participants were assigned to the citrulline group, twenty-one participants to the placebo group, and twenty-two and twenty participants, respectively, completed treatment.

2.4. Study Design

This randomized, double-blind, placebo-controlled, and parallel design clinical trial was performed from May 2021 to December 2021. All participants were assigned using a random number table to one of two groups by an investigator unrelated to the treatment and follow-up of the participants: the citrulline group with 6 g/day of L-citrulline or the placebo group (maltodextrin) for eight weeks. The supplements were provided in bottles with capsules of the same size and color. They were told to consume 4 capsules in the morning before the first meal and 4 capsules at night after dinner every day of the week without exception. The dose of 6 g/day was adopted based on review of the existing literature [22,26–31]. Follow-up and adherence were assessed every two weeks and were evaluated with the Morisky-Green test [32] and capsule count; using $\geq$80% of the supplements was regarded as compliance with the protocol. Participants were sent daily text messages reminding them to take their supplement and not to change their diet and physical activity habits.

2.5. Anthropometric and Blood Pressure Measures

Anthropometric parameters, including height, weight, and waist circumference, were measured. Weight was measured using a Tanita HD 357 scale, height with a Seca 406 stadiometer, and waist circumference with a Lufkin measuring tape. An average reference value was considered according to the waist circumference percentiles in children and adolescents for the Latino population [33]. BMI was determined by dividing the weight (kg) by the square of the height (m^2). Obesity was considered as a BMI for age more than 2 standard deviations above the median established in the WHO Child Growth Standards [34]. A trained nutritionist performed all the measurements. In addition, blood pressure was measured with an Omron digital sphygmomanometer and the values proposed in the integrated guidelines for cardiovascular health and risk reduction in children and adolescents were considered [35].

2.6. Biochemical Analyses

A venous blood sample was obtained after 12 h of fasting. Serum was processed and centrifuged the same day. Aliquots of 500 µL were taken and frozen at −80 °C until further determination. Glucose was determined by the GOD-PAD Lakeside glucose oxidase method. The lipid profile (triglycerides, total cholesterol, very-low-density lipoprotein (VLDL-C) and high-density lipoprotein (HDL-C)) was measured with the modified Huang method using the Spinreact brand. LDL-cholesterol was calculated using the Friedwald formula. The atherogenic index was calculated by dividing total cholesterol by HDL-C. Urea levels were obtained by the GLDH Urease kinetic method and uric acid by peroxidase enzymes and colorimetry. In turn, to assess liver function, plasma AST and ALT enzymes were measured by the UV enzymatic kinetic method, alkaline phosphatase by the optimized kinetic method, and direct bilirubin by the DMSO method (dimethyl sulfoxide Malloy–Evelyn reaction). The reference values described by Lira et al. [36] were considered. L-citrulline, L-arginine, and malondialdehyde (MDA) were determined by ELISA kits from MyBioSource (San Diego, CA, USA); insulin was determined by ELISA kit from ALPCO (Salem, NH, USA). The homoeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as (glucose × insulin)/22.5.

2.7. Steatosis Screening

The degree of hepatic steatosis was assessed with a SonoSite M-Turbo portable ultrasound with a 2–5 MHz multifrequency convex transducer. Fat accumulation and size of the four liver lobes were evaluated by a trained specialist. The diagnosis was made

considering the following ultrasound criteria: (1) increased echogenicity with respect to the kidney; (2) non-compromised areas, defined as specific areas of the liver without fatty infiltration; (3) visualization of the wall of portal vessels and diaphragm; (4) sound attenuation; and (5) hepatomegaly. The degree of steatosis was classified as mild, moderate, or severe according to the criteria proposed by Csendes et al. [37].

2.8. Dietary Assessment

Three 24 h food reminders were carried out at the beginning (two during the week and one at the weekend) and three reminders at the end of the intervention. Both recalls (baseline and post-treatment) were analyzed with Food Processor Software version 23, which determined the average energy intake and macronutrient and fructose consumption.

2.9. Physical Activity

At the beginning and at the end of the intervention, IPAQ was used to estimate the physical activity level of the participants. The metabolic equivalent score was determined to categorize the physical activity level of the participants.

2.10. Statistical Analysis

The Kolmogorov–Smirnov test was run to assess the normality of the data distribution. The data that presented a normal distribution were expressed as means and standard deviations; data that had a non-parametric distribution were expressed as medians and interquartile ranges. A comparison of the means obtained between the citrulline and placebo groups was performed using the Student's *t* statistic for independent samples. And a Wilcoxon test was performed to compare the medians. A circular migration plot was used to visualize and explore the flows of qualitative variables. This is a new method of visualizing and exploring the flow of data, improving the ability to graphically evaluate patterns and trends, in this case between the severity of fatty liver before and after treatments. A Student's *t*-test for independent samples and an ANOVA of repeated measures was performed to compare the means between groups with parametric distributions. For data that did not present a normal distribution, a Mann–Whitney U test was performed. A Chi-square test was used for categorical variables. To perform the Chi-square test, adjacent categories were combined to avoid having expected frequencies close to 0. The SPSS software version 23 was employed for data analysis. Significant differences were considered with a value of $p < 0.05$ at a confidence level of 95%.

3. Results

A flow diagram depicting the progress of participants in the trial is shown in Figure 1. Of the 55 adolescents evaluated, 5% did not present MAFLD and were excluded, 33% presented mild steatosis, 51% moderate steatosis, and 11% severe steatosis. The volunteers had a treatment adherence of 96.0%, thus no participant was excluded by this criterion. No participant reported adverse effects from taking the supplement.

Table 1 shows the comparison of the baseline and final values within the groups. All participants presented abdominal obesity according to the waist circumference for age [28]. No significant differences were observed between the groups in all measurements at baseline (Table 1). No changes in height were observed, body weight increased significantly ($p = 0.02$), and BMI decreased significantly ($p < 0.001$) in the citrulline group; this was due to the dispersion of the values in the database. The HOMA-IR increased, while total cholesterol and HDL-C decreased significantly in both groups. As expected, L-citrulline decreased LDL-C ($p = 0.002$) and alkaline phosphatase levels, although without reaching statistical significance ($p = 0.05$), and glucose increased ($p < 0.001$). When comparing the baseline values against post-treatment in the placebo group (Table 1), it was found that diastolic pressure decreased significantly ($p = 0.02$). Although the participants were asked not to change their diet, the placebo group had a decrease in fructose consumption after

treatment ($p = 0.04$). No changes were observed in the general characteristics between the groups post-treatment.

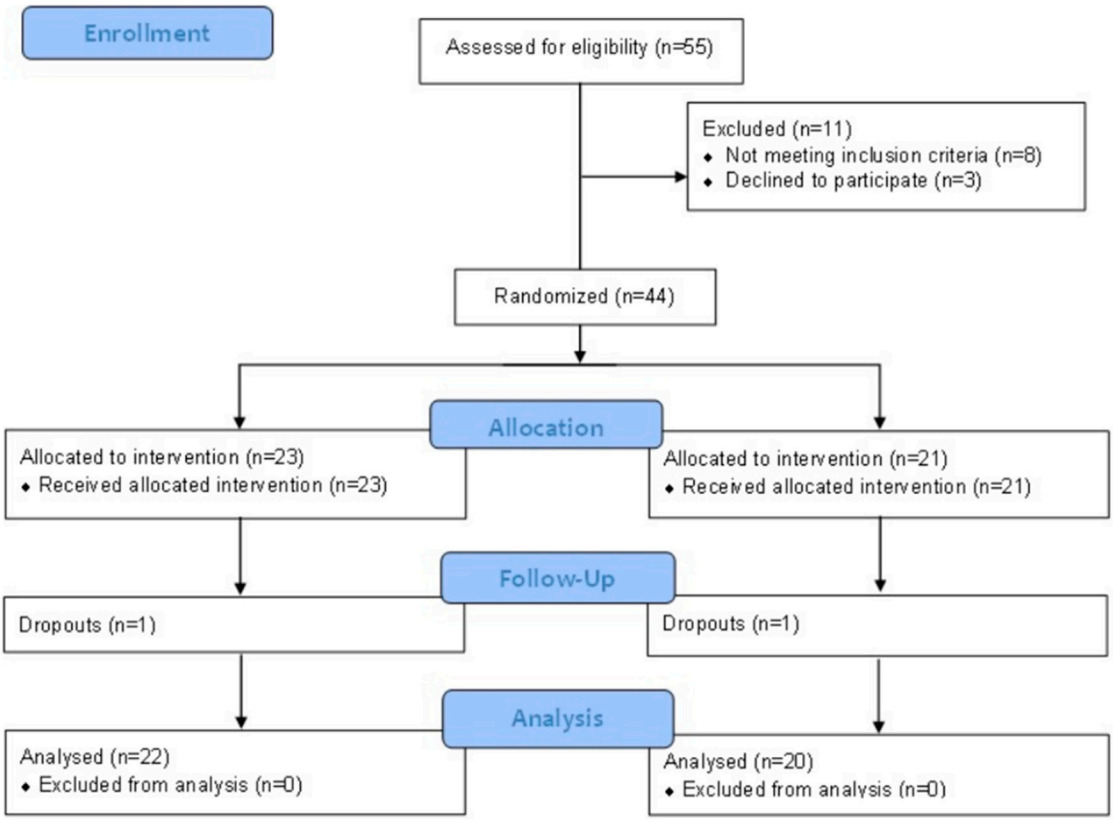

Figure 1. CONSORT flow diagram.

Table 1. Within-group comparisons from baseline to post-treatment (n = 42).

	Citrulline (n = 22)					Placebo (n = 20)				
	Baseline		Post-Treatment			Baseline		Post-Treatment		
	Mean	SD	Media	SD	*p*	Mean	SD	Media	SD	*p*
Anthropometry										
Height (m)	1.64	0.09	1.64	0.09	0.99	1.69	0.20	1.69	0.11	0.99
Weight (kg)	91.5	16.8	92.8	17.5	0.02	94.2	15.0	95.7	14.9	0.17
BMI (kg/m^2)	34.1 (27.2, 43.5)		33.7 (27.6, 46.4)		0.00	33.0	3.3	33.5	2.9	0.08
Waist circumference (cm)	105.9	12.3	106.3	13	0.62	104.9	9.4	104.5	10.8	0.71
BP (mmHg)										
Systolic	115.9	12.2	116.7	14.2	0.44	119.3	16.5	116.1	13.6	0.10
Diastolic	73.6 (64.6, 96.3)		74.1 (62.3, 95.3)		0.15	76.5	11.4	73.0	9.9	0.02
Metabolic profile										
Glucose (mg/dL)	93.8	8.5	101.5	11.9	0.00	97.1	8.9	102.9	13.3	0.08
Insulin (μU/mL)	43.1	20.0	50.9	17.1	0.73	38.0	18.8	47.4	21.5	0.09
HOMA-IR	10.1	5.3	12.5	4.3	0.03	8.9	21.5	12.2	6.4	0.03
Urea (mg/dL)	25.0 (13.0, 58.0)		22.5 (16.0, 35.0)		0.16	24.7	8.3	22.6	7.8	0.36
BUN (mg/dL)	11.7 (6.1, 27.1)		10.5 (7.5, 16.4)		0.17	11.5	3.9	10.5	3.6	0.36
Uric acid (mg/dL)	5.8	1.2	6.3	1.5	0.06	6.4	1.7	6.5	1.5	0.77
Lipid profile										
Total cholesterol (mg/dL)	165.0 (106.0, 349.0)		149.5 (111, 237)		0.001	177.3	41.4	155.1	31.4	0.03
HDL-C (mg/dL)	39.5 (25.0, 65.0)		33.0 (12.0, 55.0)		0.011	40.2	10.8	37.2	8.6	0.04
LDL-C (mg/dL)	111.5 (59.0, 254.0)		84.0 (44.0, 171.0)		0.002	104.5 (57.0, 196.0)		90.0 (57.0, 196.0)		0.05
VLDL-C (mg/dL)	26.5 (10.0, 44.0)		27.0 (13.0, 52.0)		0.67	21.0 (12.0, 64.0)		24.5 (12.0, 103.0)		0.90
Atherogenic Index	4.5 (2.8, 11.0)		4.0 (2.8, 16.5)		0.28	4.5	1.1	4.3	1.3	0.43
Triglyceride, mg/dL	132.0 (50.0, 222.0)		135.0 (66.0, 258.0)		0.62	105.5 (60.0, 318.0)		121.5 (60.0, 516.0)		0.85
Hepatic profile										

Table 1. *Cont.*

	Citrulline (n = 22)					Placebo (n = 20)				
	Baseline		Post-Treatment			Baseline		Post-Treatment		
	Mean	SD	Media	SD	*p*	Mean	SD	Media	SD	*p*
Direct bilirubin (mg/dL)	0.02 (0.01, 0.19)		0.02 (0.01, 0.30)		0.21	0.03 (0.01, 4.03)		0.02 (0.01, 0.30)		0.05
AST (U/L)	23.0 (13.0, 61.0)		25.5 (16.0, 53.0)		0.58	27.5 (18.0, 68.0)		27.0 (15.0, 75.0)		0.22
ALT (U/L)	23.5 (10.0, 78.0)		25.5 (10.0, 114.0)		0.06	42.5	27.2	37.8	25.6	0.11
Alkaline phosphatase (U/L)	236.7	93.3	192.6	68.4	0.05		87.5	236.7	87.3	0.58
L-citrulline (nmol/mL)	2.0 (0.0, 14.2)		2.2 (0.0, 23.7)		0.40	2.5 (0.0, 15.2)		3.2 (0.0, 13.4)		0.82
L-arginine (µg/mL)	3.3	1.7	2.8	1.5	0.10	2.7	1.7	3.0	1.7	0.37
MDA (ng/mL)	1849	239	1845	278	0.92	1689	278	1684	251	0.89
Lifestyle										
Kcal	2472.7	649.8	2598.2	818.1	0.63	2482.5	905.2	2379.1	929.	0.30
Protein (g)	107.3	29.6	97.8	24.8	0.22	110.9	44.6	99.9	35.8	0.17
Carbohydrates (g)	310.0	94.1	347.0	123.4	0.24	300.2	86.0	297.5	113.3	0.05
Lipids (g)	92.7	34.7	92.7	35.4	0.88	96.4	48.4	90.7	47.6	0.09
Total fructose (g)	34.5 (13.1, 90.9)		28.4 (11.8, 83.5)		0.08	36.2	19.8	24.8	15.7	0.04
Physical activity (METs)	888.0 (0.0, 10590.0)		763.5 (0.0, 7542.0)		0.93	371.2 (99.0, 6684.0)		594.0 (66.0, 3096.0)		0.64

BMI: body mass index; BP: blood pressure; HOMA-IR: homoeostasis model assessment of insulin resistance; BUN: urea nitrogen; HDL-C: high density lipoproteins; LDL-C: low density lipoproteins; VLDL-C: very-low-density lipoproteins; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde. Data are presented as means ± SD when a parametric distribution was presented and medians (interquartile ranges) when they presented a non-parametric distribution. *p*: comparison of means or medians within groups.

Table 2 shows the proportion of steatosis in its different degrees according to the treatment group before and after the administration of L-citrulline and placebo. In the L-citrulline group, an association was found between the baseline and final MAFLD with steatosis degree. In the placebo group, no association was found between baseline and final MAFLD with steatosis degree. There was no significant difference between the proportion of degrees of steatosis at the end of study between the treatment groups ($p = 0.58$).

Table 2. Degree of steatosis in participants with MAFLD at baseline and post-treatment by groups.

Citrulline n = 22		Final Steatosis Normal	Mild	Moderate	Severe	Total	Chi-Square	*p*
Baseline steatosis	Mild	8	2	0	0	10	11.589	0.0007
	Moderate	1	1	6	0	8		
	Severe	0	0	2	2	4		
	Total	9	3	8	2	22		
Placebo n = 20		Final steatosis Normal	Mild	Moderate	Severe	Total	Chi-square	*p*
Baseline steatosis	Mild	3	3	0	0	6	1.633	0.2013
	Moderate	3	3	6	0	12		
	Severe	0	0	1	1	2		
	Total	6	6	7	1	20		

A Chi-square test was performed to assess the relationship between the variables. To perform the Chi-square test, adjacent categories were combined to avoid having expected frequencies close to 0.

Figure 2 shows the migration graph for the treatment with L-citrulline: 80% (n = 8) of the adolescents who presented MAFLD with mild steatosis at baseline significantly changed to normal post-treatment. In adolescents with MAFLD and moderate steatosis, 13% (n = 1), 13% (n = 1), and (75%, n = 6) changed to a mild or normal degree or remained unchanged, respectively. In adolescents with MAFLD and severe steatosis, 50% (n = 2) improved to moderate and the other 50% (n = 2) remained unchanged. Figure 3 shows the migration graph of adolescents supplemented with placebo. After treatment, 50% (n = 3) of adolescents with MAFLD and mild steatosis improved to normal and 50% remained in the mild degree. In participants with MAFLD and moderate steatosis, 25% (n = 3) and 25% changed to mild and normal (25%, n = 3) degrees, respectively, while 50% remained

unchanged (50%, n = 6). In adolescents with MAFLD and severe steatosis, 50% (n = 1) remained unchanged and 50% (n = 1) changed to a moderate degree. However, these changes were not statistically significant post-treatment ($p = 0.20$).

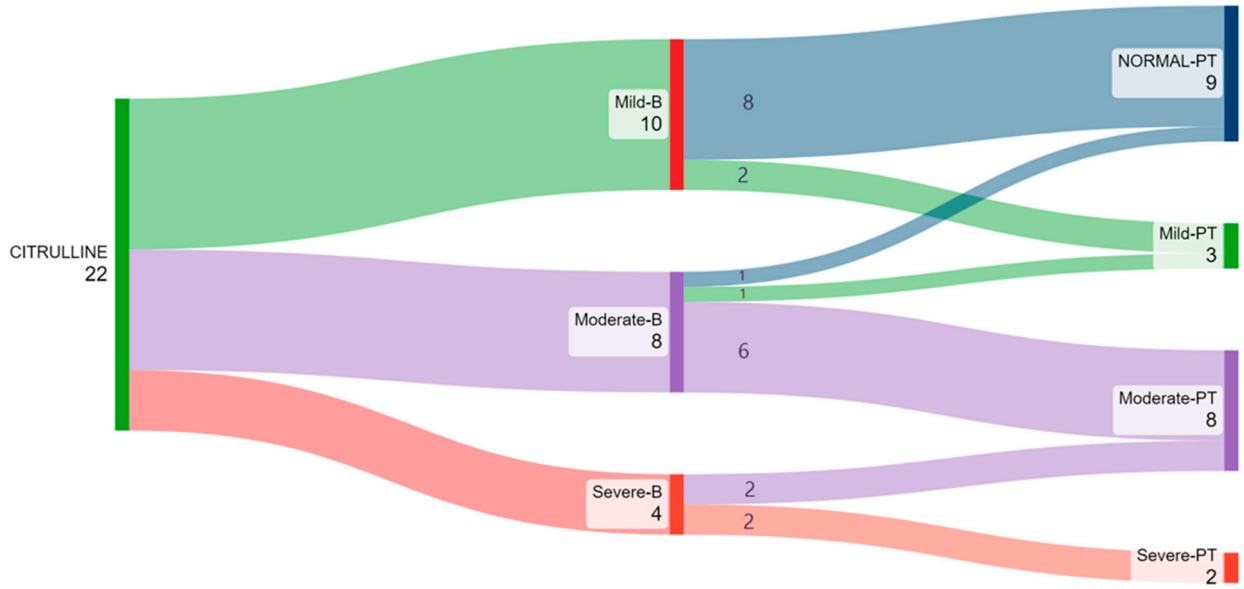

Figure 2. Migration graph of the participants supplemented with L-citrulline. This graph shows the degrees of severity of steatosis before treatment (B) on the left side (MILD—green, MODERATE—purple, SEVERE—red) and on the right side post-treatment (NORMAL—blue).

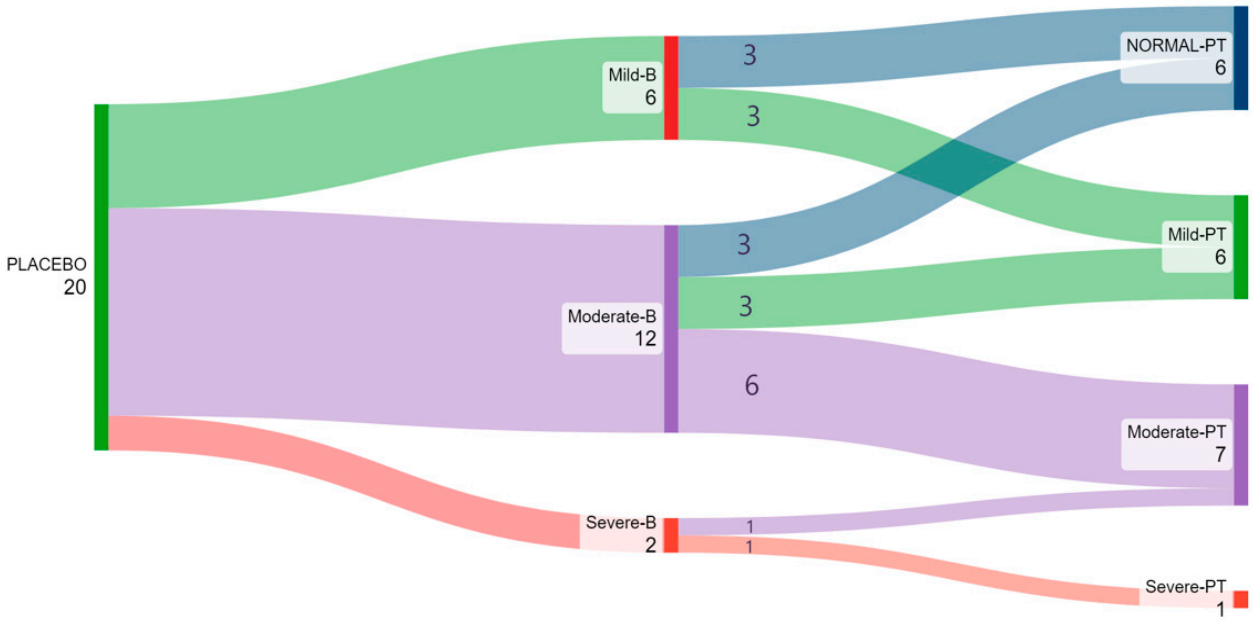

Figure 3. Migration graph of placebo-supplemented participants. This graph shows the degrees of severity of steatosis before treatment (B) on the left side (MILD—green, MODERATE—purple, SEVERE—red) and on the right-side post treatment (NORMAL—blue).

Figure 4 shows liver ultrasound images for the L-citrulline group (A) and placebo group (B) at baseline and after interventions.

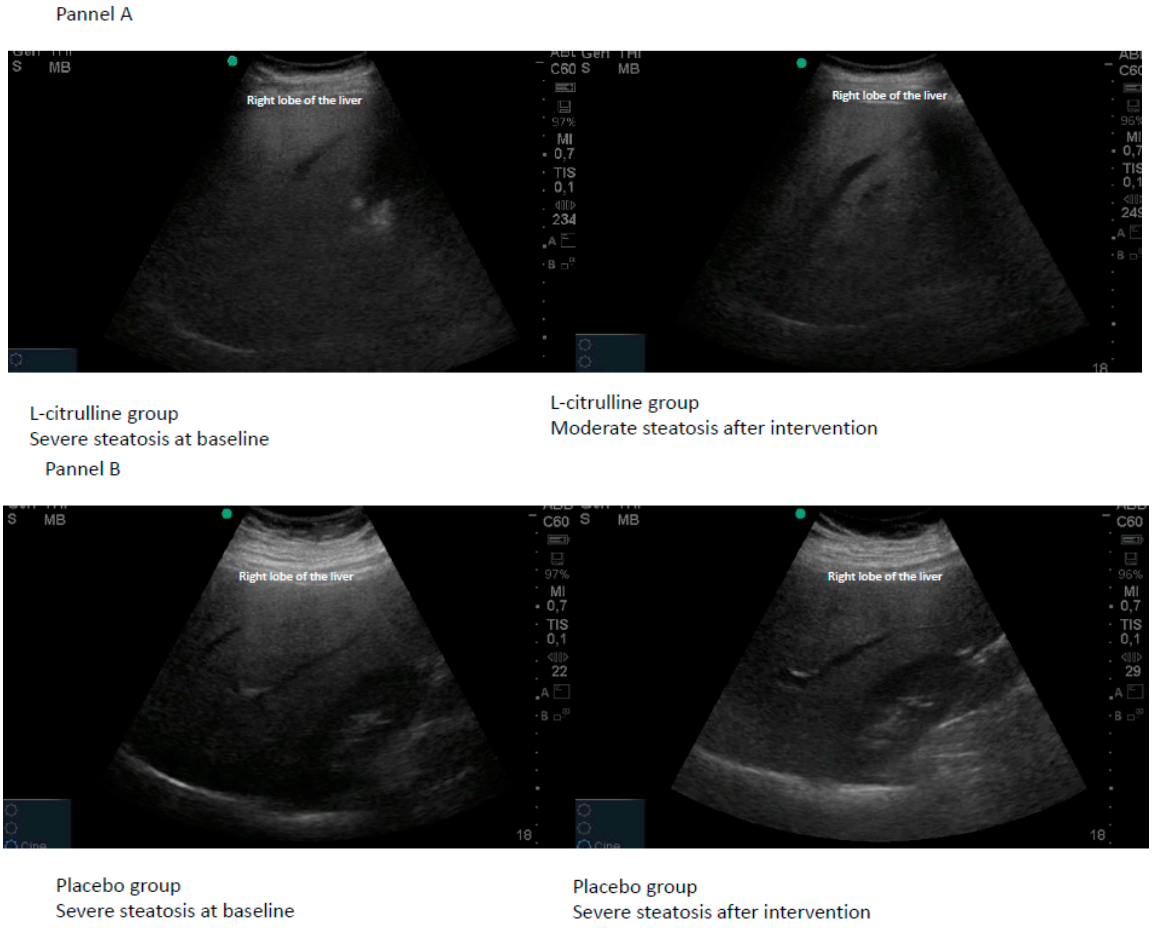

Figure 4. Liver ultrasound images for L-citrulline group (**A**) and placebo group (**B**) at baseline and after interventions.

4. Discussion

This is the first study evaluating the effects of oral L-citrulline supplementation on liver function and steatosis in adolescents with abdominal obesity and MAFLD. In the present study, 100% of the sample had undiagnosed MAFLD, with the majority in a moderate degree of steatosis (55%). This prevalence is worrisome given the young age of the participants (16 years), who may be at risk of developing liver fibrosis and cirrhosis in the short term if lifestyle changes are not made. In addition, other cardiometabolic risk factors, such as increased waist circumference, dyslipidemia, insulin resistance, and high blood pressure, could contribute to early development of chronic degenerative diseases such as type 2 diabetes and cardiovascular diseases [38].

We hypothesized that L-citrulline supplementation would decrease liver steatosis in adolescents with obesity. Our results show that L-citrulline supplementation can positively modify the degree of liver steatosis. This suggests that the effect of L-citrulline on the degree of steatosis is more evident in adolescents with mild and severe stages of MAFLD at baseline. In contrast, L-citrulline is less effective in the intermediate and moderate degrees. In agreement with our results, Darabi et al. [25] reported that L-citrulline (2 g/day) for 3 months decreased liver steatosis measured by ultrasound in adults with MAFLD. Unlike our study, their results were significant within the group but not between groups. These differences on the responsiveness of liver steatosis to citrulline supplementation can be explained by the greater dose of L-citrulline used in the present study, a dose that has been shown to be safe and effective for improving arterial function [30,31].

Obesity-induced oxidative stress impairs the nitric oxide (NO)–guanylyl cyclase pathway, and reduced NO synthesis aggravates liver steatosis and fat metabolism [39,40]. NO is produced from L-arginine via the L-arginine–NO synthase pathway.

L-citrulline produces its effects indirectly through the production of L-arginine in the kidneys without producing side effects. In our study, a significant reduction in the degree of steatosis was found after L-citrulline supplementation. Some studies in animal models suggest that L-citrulline may prevent the development of hepatic steatosis even at lower doses [21,23] by promoting an L-arginine lipolytic effect and protecting against fat accumulation. Oral L-citrulline, but not L-arginine, for 4 weeks was effective to attenuate liver fat accumulation in rats with NAFLD, suggesting improved liver lipid metabolism [18]. L-citrulline can reduce liver steatosis via inhibition of sterol regulator element binding protein 1, a factor involved in triglyceride synthesis, leading to decreased lipogenesis in hepatocytes [41,42]. Moreover, the increase in NO by L-citrulline may reduce glyceroneogenesis and thereby triglyceride synthesis [43] and a reduction in liver steatosis via stimulation of hepatic AMP-activated protein kinase, which stimulates fatty acid β-oxidation in rats [41]. In rats with NAFLD, L-citrulline reduced hepatic triglycerides by enhancing hormone-sensitive lipase activity [42], leading to a greater release of free fatty acids [23]. The attenuation of liver steatosis may be associated with the lipolytic effect of L-citrulline observed on visceral fat in rats [44]. Thus, increased NO production would reduce liver steatosis by improving fat metabolism [39,40].

In the current study, both groups had insulin resistance at baseline, and no changes were observed after the supplementation with L -citrulline; a systematic review in patients with diabetes mellitus and the potential role of citrulline on metabolic and inflammatory variables showed that citrulline intake caused a significant reduction in HOMA-IR in one study in an animal model [45], and inconsistent results were revealed on the effects of citrulline on insulin levels [44]. On the other hand, a protective effect of L-citrulline on HOMA-IR was found in rats with NAFLD [21]. However, further studies are required to shed light on the underlying mechanisms.

As expected, LDL cholesterol values decreased after L-citrulline administration for eight weeks. Similarly, other authors have shown that L-citrulline improves dyslipidemias [21–23,25,44]. The mechanism by which L-citrulline improves blood lipids is by attenuating de novo lipogenesis and increasing beta-oxidation [28]. The hepatic synthesis of lipoproteins occurs through the interaction of different fats, including triglycerides and apolipoproteins, mainly apoB100. Insulin participates in the regulation of lipoprotein synthesis and in response to insulin apoB100 being degraded [46]. In MAFLD, there is increased hepatic expression of apoB100 and overproduction of lipoproteins, which may result in increased lipid availability from de novo lipolysis and synthesis and failure of insulin to suppress VLDL production [46]. Jegatheesan proposed that L-citrulline increases the elimination of LDL cholesterol as a consequence of the increase in insulin sensitivity and decreased plasma triglycerides [21]. L-citrulline also decreases fat accumulation in the liver, leading to a greater release of free fatty acids in adipose tissue [23].

In our study, L-citrulline supplementation did not increase serum levels of L-arginine and L-citrulline. Similarly, oral L-citrulline did not modify plasma citrulline and arginine in rats with NAFLD [21,23]. This could be explained by the high arginine metabolic rate in disease states [47] due to increased arginase activity. Given that arginase activity and expression are increased in obesity and metabolic abnormalities [45], a greater catabolism of circulating arginine to ornithine by arginase may explain the lack of change in arginine levels. Unfortunately, we did not measure serum ornithine levels to support our hypothesis.

On the other hand, it is worth emphasizing some unexpected results, such as the significant weight gain in the citrulline group; despite the small increase (1.3 kg) in body weight, the change was not significantly different between groups. Some potential scenarios would explain the positive energy balance that led to weight gain. According to the dietary and physical activity evaluations (not shown), an increase in energy consumption and a decrease in physical activity could explain these results [48]. In fact, a non-significant decrease in METS expended by physical activity and exercise was found, added to a habitual consumption of products rich in trans and saturated fats, which could lead to a decrease in HDL-C in this same group of participants.

During the preparation of this study, the COVID-19 pandemic appeared, which complicated the recruitment and follow-up of the adolescents; however, it was possible to conclude and obtain these results. Even so, these results are promising and promote further investigation on the effects of L-citrulline in liver and metabolic profiles.

Some of the limitations of our work are listed below. The main limitation of this study is the sample size, which was relatively small. Although our calculation was considered an adequate number to find statistical significance, in practice it seems not to be enough since there were decreases in within-group measures (TC and LDL) that did not reach between-group differences. Another limitation could be that there was no healthy control group to compare the results to and the inclusion of adolescents with a mild degree of steatosis. Therefore, it would be essential to include healthy adolescents to compare with the case-control group in future studies. A third limitation is the use of ultrasound for the detection of liver steatosis, which is not the gold standard test for diagnosis; however, in the pediatric population, it is a reliable, low-risk technique that allows establishing an accurate diagnosis. It is important to highlight that the results show a possible effect of L-citrulline on the accumulation of fat in the liver and improvement in the lipid profile in adolescents with obesity and MAFLD. However, more research is necessary to confirm these findings, especially in individuals with MAFLD and different doses of L-citrulline.

This is the first study that evaluated the effects of oral L-citrulline supplementation on liver steatosis and function in adolescents with abdominal obesity. Our results show that adolescents with obesity may have MAFLD without knowing it. L-citrulline is an amino acid that could decrease hepatic fat accumulation and serum LDL-C levels after eight weeks of supplementation in adolescents with abdominal obesity and MAFLD.

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Informed Consent Statement: The participants and their guardians signed an informed consent form.

Data Availability Statement: Data from the present study are available upon request.

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