



## Article

# Evaluation of the Effects of Consumption of Portuguese Walnuts (*Juglans regia* L.) on the Risk Factors Related to Cardiovascular Diseases

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**Abstract:** (1) Background: Walnut consumption has been associated with having a positive effect on controlling and/or reducing the co-morbidities associated with cardiovascular disease (CVD). The effects of consuming walnuts of Portuguese origin on risk factors related to CVD were evaluated by measuring glucose, urea, TC, HDL-C, LDL-C, TG, AST, and ALT levels, anthropometric profiles, and blood pressure. (2) Methods: This trial study involved 24 volunteers, both female ( $n = 15$ ) and male ( $n = 9$ ), from Fernando Pessoa University, Porto. It consisted of a daily intake of 25 g of walnut kernels over a period of 45 days. Before and after intake, biochemical parameters, BMI and BP were measured. (3) Results: Despite the intake of nuts revealing a reduction in mean values of most of the parameters assessed, a significant drop was only observed in AST ( $p = 0.04$ ). There was also a significant reduction in the mean values for Glu ( $p = 0.01$ ), UR ( $p = 0.01$ ) and HDL-C ( $p = 0.02$ ) for women but not for men. (4) Conclusions: The dose and the period of intake were not effective in lowering the lipid profile but may have had a protective effect on liver function. The benefits were greater in women than in men.

**Keywords:** walnuts; anthropometric measurements; atherosclerosis; cardiovascular disease; diet; dyslipidaemia; hypertension



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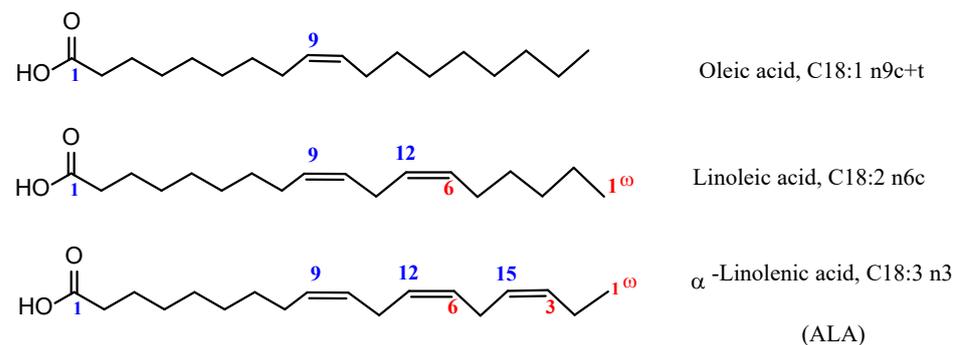
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## 1. Introduction

Cardiovascular disease (CVD), namely coronary heart disease and stroke, continues to be the main cause of mortality and morbidity in the world, affecting around 17.7 million people in 2015 [1]. In Portugal, CVD represents 25.9% of total deaths [2], with one of the main risk factors being dietary patterns [3,4]. The most common pathophysiological process underlying CVD is atherosclerosis, which is associated with multiple risk factors including high blood pressure, diabetes *mellitus*, dyslipidaemia, and pre-obesity or obesity. Most of those risk factors are related to lifestyle and habits, such as inadequate diet [5–7]; therefore, changing eating habits can contribute to a reduction in the risks associated with CVD.

It is well known that nuts, including walnuts, can play a relevant role in controlling and/or reducing co-morbidities associated with CVD, as shown by several studies. Thus, frequent consumption of nuts correlates inversely with CVD risk [8–10].

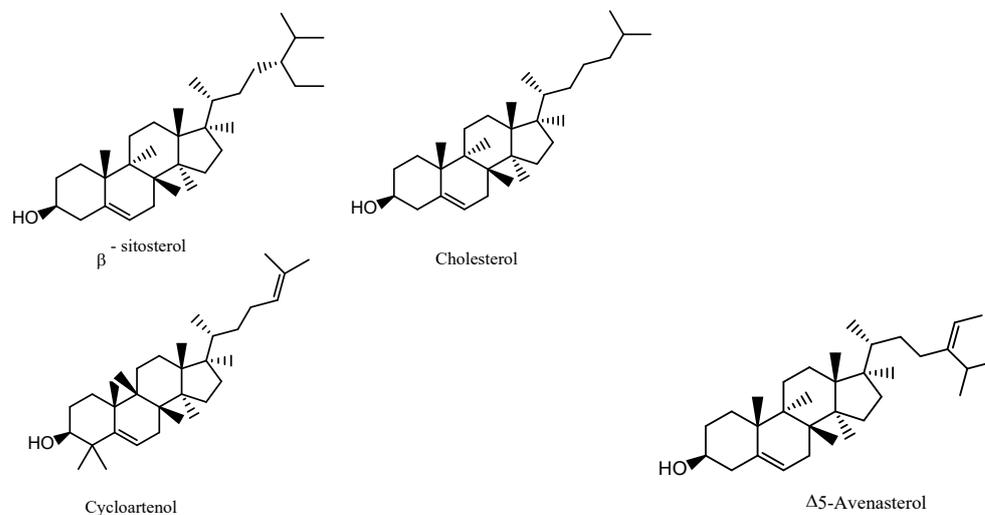
The macronutrients contained in walnuts, which are beneficial in CVD prevention, are (i) mono-unsaturated and polyunsaturated fatty acids (MUFAs and PUFAs), see Figure 1; (ii) fibre; (iii) phytosterols, see Figure 2; and (vi) polyphenols [11–13], see Figure 3.  $\alpha$ -linolenic acid (ALA), see Figure 1, seems to be metabolized into bioactive oxylipins by the action of cyclooxygenase, lipoxygenase, and cytochromeP450 epoxygenase, providing metabolites that can protect microglial cells from inflammation [9] and showing a beneficial effect in the improvement of the endothelial function in conjunction with other polyphenols, magnesium, and *L*-arginine [14]. Phytosterols [15], see Figure 2, are related to cholesterol-lowering effects. Being more hydrophobic than cholesterol, they are able to displace cholesterol from intestinal micelles, thereby interfering with cholesterol absorption, which leads to a reduction in low-density lipoprotein cholesterol LDL-C. Abundant polyphenols like ellagitannins, see Figure 3, undergo hydrolysis, releasing ellagic acid [16]. This may be metabolized by bacteria producing urolithins that are easily absorbed and target several organs, reducing adiposity, LDL-C, and blood glucose [17]. Other micronutrients are vitamins (E, B6 and folic acid) and minerals (magnesium, potassium, calcium, copper, and phosphorus). Vitamin E is a powerful free radical scavenger and reduces pro-inflammatory eicosanoids and the inflammatory response [18]. Vitamin B6 can strengthen the immune system and maintain normal nerve function as well as normal glucose levels [19]. Finally, folic acid plays an important role in the detoxification of homocysteine, an amino acid with atherothrombotic properties [20]. In relation to minerals, a high intake of calcium, magnesium, and potassium together with a low intake of sodium is associated with protection against bone demineralisation, hypertension, insulin resistance, and overall cardiovascular risk [21].



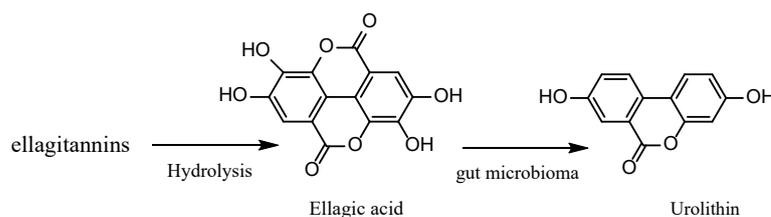
**Figure 1.** Main fatty acids found in walnuts: oleic acid, a type of monounsaturated fatty acid (MUFA), and linoleic and linolenic acids, which are examples of polyunsaturated fatty acids (PUFAs). Linoleic acid is an example of a  $\omega$ -6 polyunsaturated acid and a  $\omega$ -3 polyunsaturated acid. In blue: atom numbering according to IUPAC; in red atom numbering for unsaturated fatty acids.

The intake of walnuts has been recognized as a protective and preventive measure against CVD. It is in this context that the EAT-LANCET Commission, after review, has recommended increasing the daily consumption of nuts to 25 g/day [22], although the Global Burden of Disease study states that an intake of 21 g/day of nuts is already sufficient for a lower risk of mortality [23]. However, the probability of these recommendations being followed by consumers depends on the socio-economic characteristics of the population, particularly the availability and cost of walnuts. In addition, consumers may not be willing to consume the recommended dose. As such, the aim of this study was to test the feasibility of introducing a dose of 25 g of walnuts into the diet of a current “Portuguese city population” and to ascertain to what extent the consumption of locally produced walnuts is effective in terms of cardiovascular disease risk parameters. Thus, in the present work, we evaluated biochemical parameters [glucose (Glc), urea (UR), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)], anthropometric variables [Body Mass Index (*BMI*)], and blood pressure (BP) profile

in a group of 24 volunteers before and after the intake of 25 g/day of locally produced walnuts for a period of 45 days.



**Figure 2.** Chemical formulas for three main phytosterols present in walnuts:  $\beta$ -sitosterol,  $\Delta^5$ -Avenasterol, and cycloartenol, highlighting the similarity between compounds. According to previous research [15], phytosterols may compete with cholesterol in biochemical pathways.



**Figure 3.** Important polyphenols: ellagitannins undergo hydrolysis, releasing ellagic acid that may be metabolized by bacteria producing urolithins that are easily absorbed and target several organs, reducing adiposity [16].

## 2. Materials and Methods

The study was carried out at Fernando Pessoa University, involving 24 volunteers (62.5% female and 37.5% male) with different academic qualifications who were aged between 20 and 65 (with an average age of 36.8). The sample size is small but in line with samples used in several similar works [24–26].

None of the volunteers were on antihypertensive or in anti-dyslipidaemia therapies. All participants were subjected to previous nut allergy testing.

The study was approved by the UFP Ethics Committee and each participant signed the informed consent. Initial information about anthropometric data, biochemical analysis, and blood pressure were obtained. After the daily consumption of 25 g walnut kernel for 45 days, the same evaluations were carried out. The participants were informed that they should maintain their regular dietary habits.

The nuts were produced biologically and supplied by the company Terra-Noz de Pereira de Selão, Chaves, situated in the Northeast of Portugal.

### 2.1. Anthropometric Profile Assessment

The anthropometric assessment was carried out according to the methodology proposed by ISAK's kinanthropometry [27]. The weight was determined using a body analysis scale (BF 551 Tanita). The height was measured using a portable wall stadiometer (SECA

213) according to the Frankfurt plan. The Body Mass Index (*BMI*) was calculated using the standard Formula (1) with weight (*w*) in kg and height (*h*) in meters [28].

$$BMI(\text{kg}\cdot\text{m}^2) = \frac{w}{h^2} \quad (1)$$

## 2.2. Biochemical Assessments

Blood tests were carried out on the participants before and after the period of nut consumption. Fasting blood samples were collected, and serum and levels of glucose (Glc), urea (UR), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analysed. The analyses were carried out in the Unilabs laboratory at the Hospital of Medical School of Fernando Pessoa University.

## 2.3. Blood Pressure Measurement

Blood pressure (BP) was measured using a digital arm monitor TA meter (OMRON-HEM-7130, Omron, Kyoto, Japan). The measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP) was carried out immediately before and after the 45-day period of nut consumption. Two consecutive measurements were taken, with only the second measurement recorded. The results and methodologies were congruent with those presented by the European Society of Cardiology [29].

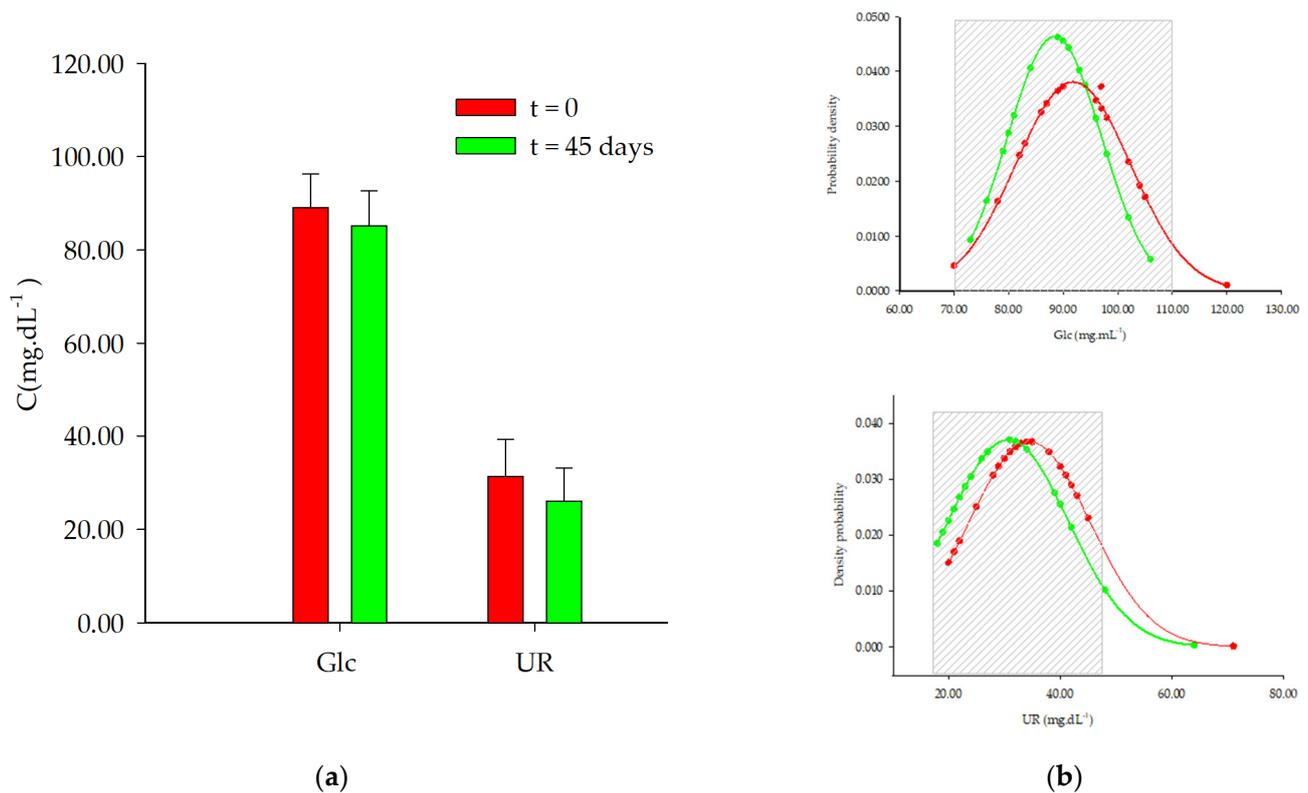
## 2.4. Statistical Analysis

Data were analysed with the IBM SPSS Statistics version 27.0 software [30]. The comparison between the two time points in the study (before and after eating the nuts) was analysed using the Student's *t*-test. The results are presented as mean values  $\pm$  standard deviation. Differences of  $p < 0.05$  (95% of confidence level) are considered significant enough to accept or reject the hypothesis as to whether or not consumption of walnuts has an impact.

## 3. Results

*BMI* varied from an initial mean value of  $26.9 \pm 5.5$  to a final mean value of  $26.8 \pm 5.4 \text{ kg/m}^2$ ; thus, the mean value remained the same. DBP values ranged from  $7.5 \pm 1.2$  to  $7.6 \pm 0.9 \text{ mmHg}$  before and after intake, and systolic blood pressure did not vary significantly: the mean value was  $11.8 \pm 1.4 \text{ mmHg}$  at the beginning and  $11.3 \pm 1.5 \text{ mmHg}$  at the end.

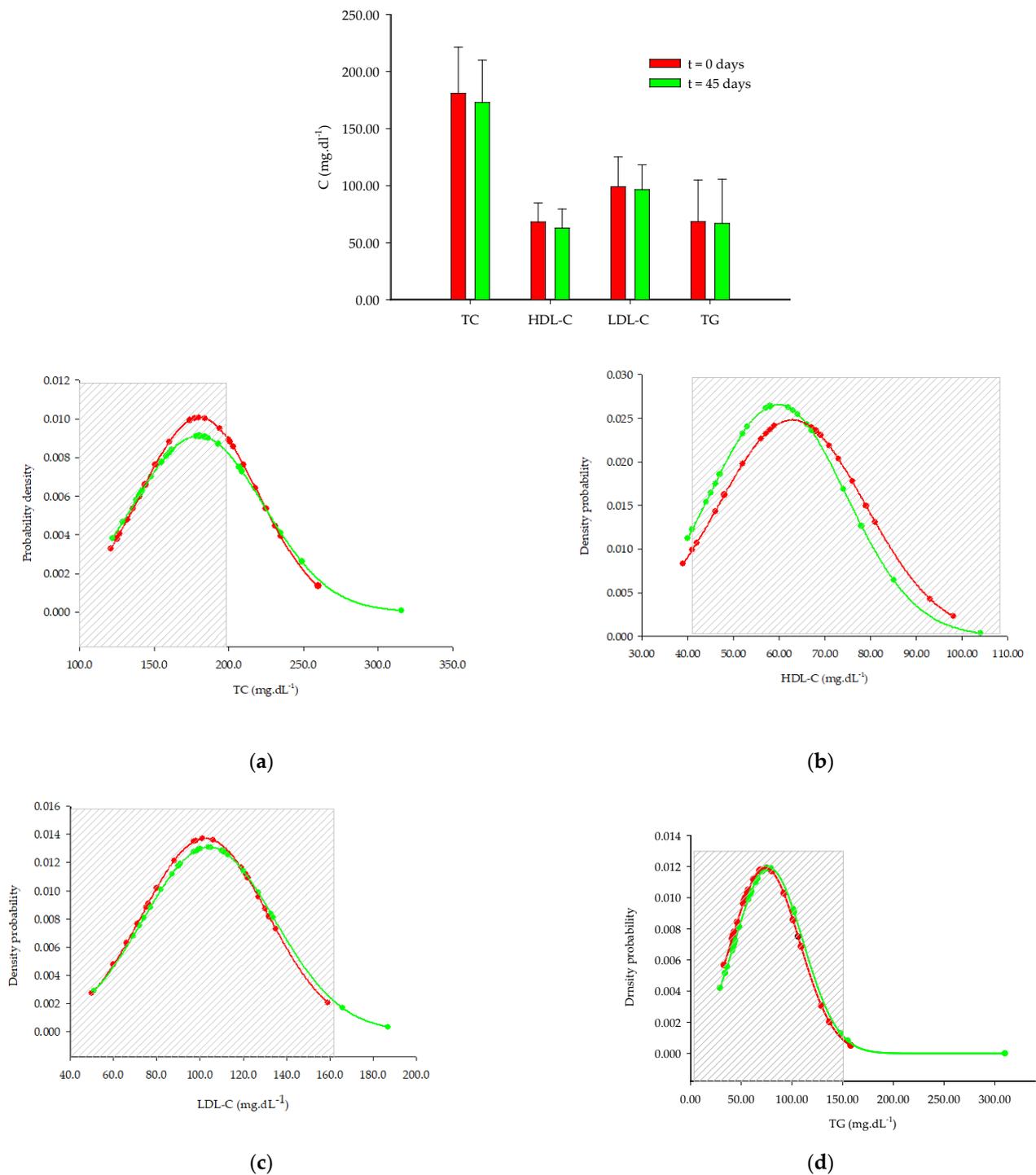
Major variations were observed in the biochemical parameters. Figures 4–6 show the graphic representations of the mean variation obtained for those variables, grouped as follows: Glc/UR (Figure 4), TC/HDL-C/ LDL-C/TG (Figure 5) and AST/ALT (Figure 6). In all figures, red bars/lines correspond to values obtained before the intake, and green bars/lines after the consumption of the walnuts. The mean values and their SD are given in the figure captions for the reader's information. The accompanying Gaussian representations were calculated based on the probability density of the normal distribution, taking the values of mean and SD for the samples. These provide a better interpretation of the variance in results before and after intake. In grey shading are the normal values for each biochemical parameter according to the official values from the Portuguese Health Bureau [31].



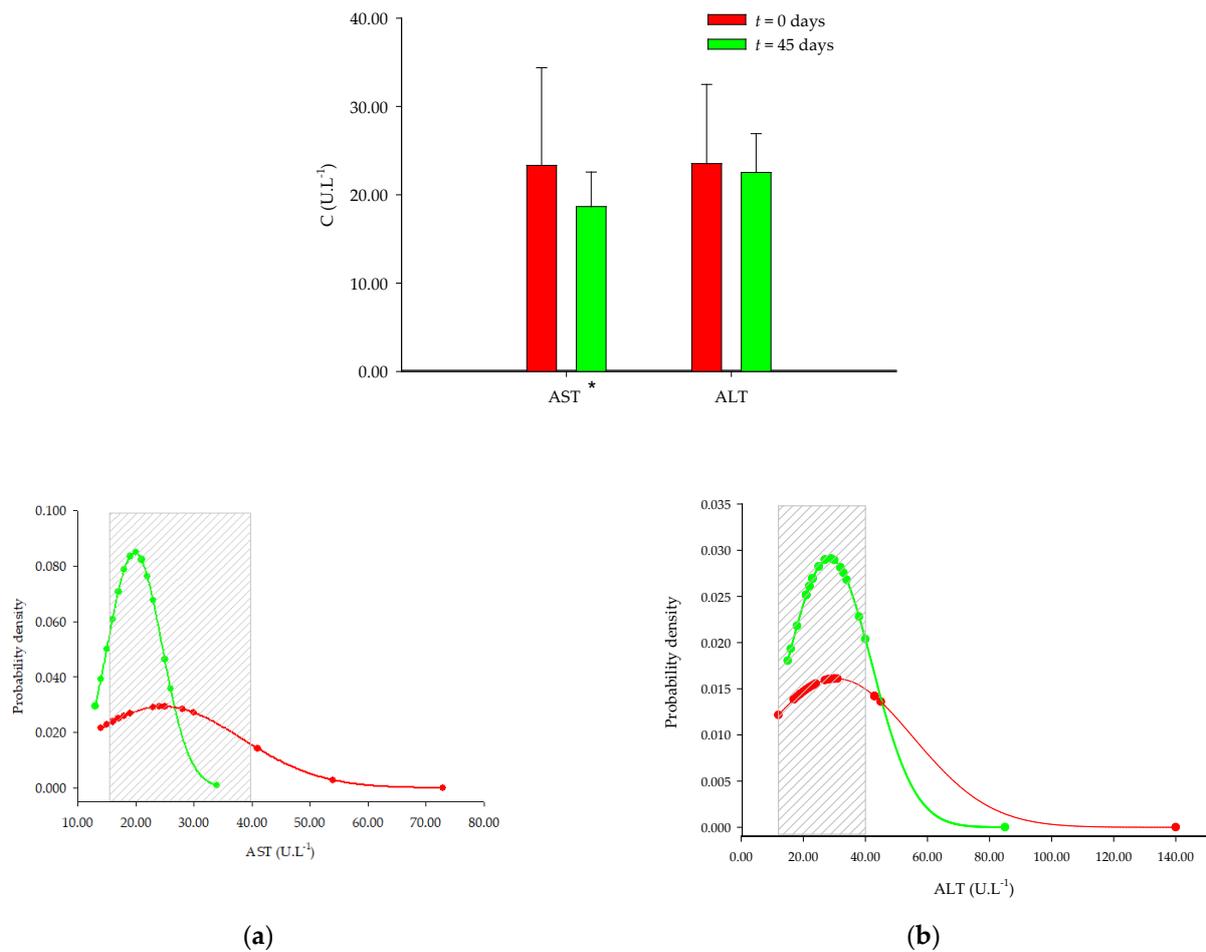
**Figure 4.** (a) Variation in glucose (Glc) and urea (UR) before and after walnut consumption. Glc ( $\text{mg.dL}^{-1}$ ) =  $93.5 \pm 17.2$  before and  $88.4 \pm 8.6$  after ( $p = 0.09$ ); UR ( $\text{mg.dL}^{-1}$ ) =  $34.5 \pm 10.9$  before and  $30.7 \pm 10.8$  after ( $p = 0.09$ ). (b) Gaussian representations for the data were calculated by the probability density based on normal distribution, taking the values of mean and SD for the samples. Red line: before the intake; green line: after intake. In grey shading are the normal values for each biochemical parameter.

It is well known that there are non-modifiable risk factors associated with CVD such as age and gender. Thus, the data were analysed by subgroups, according to gender, ( $n = 15$  for female and  $n = 9$  for male) and age (20–39 years ( $n = 13$ ) and 40–65 years ( $n = 11$ )). The resulting values are presented in Tables 1–3, in accordance with the previously mentioned graphic representations 4 to 6. It would be interesting to simulate the Gaussian profile for gender/age results, but the sample is small; thus, the number of experimental points hinders the task.

As can be seen in Figure 4, mean values for Glc decreased from  $93.5 \pm 17.2$  to  $88.4 \pm 8.6$ , but this variation was not statistically significant ( $p = 0.09$ ). Despite that, 75% of the individuals showed a decrease in their blood Glc concentration after the intake of the walnuts. That percentage was higher (85%) in the younger population when compared with the older population (64%); thus, the drop in Glc was statistically significant for the younger group ( $p = 0.02$ ) in comparison with the older one ( $p = 0.09$ ), see Table 1. The mean value for UR also dropped from  $34.5 \pm 10.9$  to  $30.7 \pm 10.8 \text{ mg.dL}^{-1}$  ( $p = 0.09$ ). The women showed a statistically significant difference in Glc and UR levels (both decreased), see Table 1.



**Figure 5.** Variations in total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) before and after walnut consumption. Gaussian representations were calculated from the probability density based on normal distribution, taking the values of mean and SD for the samples, Red line: before the intake; green line: after the intake. In grey shading are the normal values for each biochemical parameter. **(a)** TC (mg.dL<sup>-1</sup>): initial mean value  $180.5 \pm 39.7$ ; final mean value  $179.7 \pm 43.8$  ( $p = 0.87$ ); **(b)** HDL-C (mg.dL<sup>-1</sup>): initial mean value  $62.8 \pm 16.1$ ; final mean value  $59.7 \pm 15.0$  ( $p = 0.05$ ); **(c)** LDL-C (mg.dL<sup>-1</sup>): initial mean value  $102.4 \pm 29.1$ ; final mean value  $104.0 \pm 30.5$  ( $p = 0.61$ ); **(d)** TG (mg.dL<sup>-1</sup>): initial mean value  $73.8 \pm 33.5$ ; final mean value  $77.3 \pm 59.2$  ( $p = 0.71$ ).



**Figure 6.** Variation in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) before and after walnut consumption. Gaussian representations were calculated from the probability density based on normal distribution, taking the values of mean and SD for the samples. Red line: before the intake; green line: after the intake. In grey shading are the normal values for each biochemical parameter. **(a)** AST ( $\text{U.L}^{-1}$ ): initial mean value  $24.7 \pm 13.6$ ; final mean value  $19.8 \pm 4.7$  ( $p = 0.03^*$ ); **(b)** ALT ( $\text{U.L}^{-1}$ ): initial mean value  $30.6 \pm 24.8$ ; final mean value  $28.4 \pm 13.7$  ( $p = 0.42$ ).

**Table 1.** Results of the statistical analysis for glucose (Glc) and urea (UR) accessed before and after eating walnuts for the 20–39 age group ( $n = 13$ ) and the 40–65 age group ( $n = 11$ ) as well as for women ( $n = 15$ ) and men ( $n = 9$ ). Results are expressed as mean  $\pm$  standard deviation. Concentrations given in  $\text{mg.dL}^{-1}$ .

	Variables	$t = 0$ Days	$t = 45$ Days	$p$ -Value
<b>20–39 y</b>	Glc	$86.7 \pm 6.8$	$83.8 \pm 6.7$	0.02 *
	UR	$29.3 \pm 6.4$	$26.7 \pm 8.4$	0.16
<b>40–65 y</b>	Glc	$96.6 \pm 6.6$	$93.9 \pm 6.8$	0.09
	UR	$37.0 \pm 6.4$	$35.5 \pm 10.8$	0.61
<b>Women</b>	Glc	$89.1 \pm 7.0$	$85.2 \pm 7.2$	0.01 *
	UR	$31.3 \pm 7.7$	$26.0 \pm 6.9$	0.01 *
<b>Men</b>	Glc	$100.8 \pm 24.3$	$93.8 \pm 7.6$	0.39
	UR	$39.9 \pm 12.5$	$38.6 \pm 10.9$	0.80

\* Significant differences between variables ( $p < 0.05$ ),  $p$ -values based on  $t$ -student.

**Table 2.** Results of the statistical analysis of lipidic profile assessed before and after eating walnuts for the 20–39 age group ( $n = 13$ ) and the 40–65 age group ( $n = 11$ ) and for females ( $n = 15$ ) and males ( $n = 9$ ). Concentrations given in  $\text{mg}\cdot\text{dL}^{-1}$  (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG)).

	Variables	$t = 0$ Days	$t = 45$ Days	$p$ -Value
20–39 y	TC	164.7 ± 40.5	164.2 ± 30.7	0.90
	HDL-C	63.9 ± 15.4	61.0 ± 12.5	0.19
	LDL-C	86.0 ± 24.3	88.2 ± 18.5	0.47
	TG	74.1 ± 36.5	74.7 ± 37.1	0.74
40–65 y	TC	199.8 ± 28.5	198.1 ± 47.7	0.84
	HDL-C	61.6 ± 16.1	58.5 ± 17.2	0.20
	LDL-C	121.7 ± 19.6	123.5 ± 29.4	0.78
	TG	82.6 ± 47.4	80.5 ± 75.4	0.87
Women	TC	189.9 ± 38.9	172.7 ± 35.7	0.10
	HDL-C	68.2 ± 16.2	62.7 ± 16.1	0.02 *
	LDL-C	99.0 ± 25.2	96.3 ± 20.9	0.46
	TG	68.5 ± 35.2	66.9 ± 37.5	0.49
Men	TC	179.7 ± 38.7	191.0 ± 50.8	0.20
	HDL-C	53.9 ± 9.9	54.4 ± 11.3	0.74
	LDL-C	107.8 ± 32.7	116.7 ± 37.3	0.14
	TG	82.7 ± 26.1	94.8 ± 78.3	0.64

\* Significant differences between variables ( $p < 0.05$ ),  $p$ -values based on  $t$ -student.

**Table 3.** Results of the statistical analysis of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assessed before and after eating walnuts for the 20–39 age group ( $n = 13$ ) and the 40–65 age group ( $n = 11$ ). Results are expressed as mean ± standard deviation;  $p$ -values based on  $t$ -student. AST and ALT are given in  $\text{U}\cdot\text{L}^{-1}$ .

	Variables	$t = 0$ Days	$t = 45$ Days	$p$ -Value
20–39 y	AST	21.8 ± 7.4	18.5 ± 3.4	0.09
	ALT	24.1 ± 9.2	23.5 ± 5.9	0.75
	AST/ALT ratio	0.9 ± 0.7	0.8 ± 0.4	
40–65 y	AST	28.2 ± 17.4	21.5 ± 5.2	0.13
	ALT	38.3 ± 32.8	34.3 ± 17.0	0.47
	AST/ALT ratio	0.7 ± 1.4	0.6 ± 0.7	
Women	AST	23.3 ± 10.7	18.7 ± 3.8	0.06
	ALT	23.5 ± 8.7	22.3 ± 4.1	0.50
	AST/ALT ratio	1.0 ± 0.8	0.8 ± 0.4	
Men	AST	27.0 ± 16.7	21.8 ± 5.1	0.26
	ALT	42.3 ± 35.0	38.2 ± 17.2	0.55
	AST/ALT ratio	0.6 ± 1.4	0.6 ± 0.6	

As mentioned, Figure 5 shows the results obtained for the lipid profile and Table 2 depicts the mean values for the variables depending on subgroups (gender and age). The change in mean values for TC, HDLC, and LDL-C was not significant when all the individuals were subjected to statistical analysis, but when the values were analysed by subgroups, a significant decrease was observed in HDL-C for women, from  $68.2 \pm 16.2$  to  $62.7 \pm 16.1$   $\text{mg}\cdot\text{dL}^{-1}$ ,  $p = 0.02$ , see Table 2. The profiles of the bell curves were similar concerning LDL-C (Figure 5c) and TG (Figure 5d); the mean value for TC decreased slightly, but the variance did increase (Figure 5a).

Figure 6 refers to the mean values variations for AST and ALT presented in a similar way as before. Table 3 depicts the obtained mean values depending on gender and age. In this table, the ratio of AST to ALT was calculated because this ratio pattern was used to evaluate the degree of liver damage. The drop in AST was significant; it varied from

an initial mean value of  $24.7 \pm 13.6$  to a final mean value of  $19.8 \pm 4.7$  ( $p = 0.03$ ). This variation can best be viewed by differences in mean values and variances of the Gaussian curves in Figure 6a, which decreased significantly before and after consumption. For ALT levels, as shown in Figure 6b, a decrease in the variance and an approach of the individual values to the mean can also be observed. In addition, we found that despite having no statistical meaning, the percentage of young individuals whose ALT decreased was 39%, while the percentage in the older group was 18%. Contrary to what happened with the other biochemical variables, there was no significant variation in the AST and ALT when the analysis was made by age or gender, see Table 3.

## 4. Discussion

### 4.1. Caloric Intake and BMI

The walnuts used in this study were organic and came from the north-eastern region of Portugal. According to Pereira et al., 2008 [32], walnuts from this region contain between 68.8 to 72.1 g/100 g of fat, 14.4 to 18.0 g/100 g of crude protein, and 3.8 to 7.2 g/100 g of carbohydrates, of which about 50% is fibre. The mean value for total energy of the walnut cultivars grown in Portugal is 721 kcal/100 g. Thus, it is estimated that the participants were subjected to a daily caloric intake of about 180 kcal from nut intake over a period of 45 days. Despite this, the results obtained suggest that there was no significant change in body weight or BMI due to the ingestion of the walnuts, the initial mean being  $26.9 \pm 5.5$  kg/m<sup>2</sup> and the final mean being  $26.8 \pm 5.4$  kg/m<sup>2</sup>.

Those observations are in line with many other studies that have shown no association between walnut consumption and changes in body weight/BMI despite walnuts being a highly energetic food. This could be a consequence of the feeling of satiety that nuts cause after consumption and/or inefficient absorption [33].

### 4.2. Mean Values for Glc and UR

In this section, we opted to discuss Glc in tandem with UR. Some of the latest research has provided experimental evidence that higher levels of urea may increase insulin resistance and suppress insulin secretion [34]. In addition, a study carried out in the USA involving a national cohort of 1,337,452 individuals (without diabetes) showed that a concentration of blood urea nitrogen over 25 mg.dL<sup>-1</sup> correlated positively with the risk of developing diabetes [35]. Interestingly, while without statistical significance, both mean values for Glc and UR decreased, and similar  $p$ -values were obtained ( $p < 0.09$ ). In addition, when the analyses compared groups (younger and older/women and men), the drop in mean values, before and after the intake, acquired statistical significance at  $p < 0.05$  (see Tables 1 and 2 for younger people and women).

Systematic [36] and narrative reviews [37] conducted between 2014 and 2017 suggest that eating walnuts has a favourable effect on fasting blood glucose. In the present study, although a very significant percentage of participants (75%) experienced a decrease in fasting blood glucose levels after ingesting 25 g/day of walnuts for 45 days, the decrease was not statistically significant (Figure 4). Concerning our sample, it is important to note that none of the participants had co-morbidities and glycaemia levels were within the normal range for all participants except for one individual (before the experiment). After the intake of walnuts, all participants have levels within the normal range. Our data are insufficient to draw conclusions concerning the benefit of walnuts alleviating the risk of diabetes, as shown by other studies [37–42], although the number of participants that shift their values to the mean is higher after walnut consumption, as can be seen in the decrease in the variability in Glu values after the intake.

It is known that nuts, given their lipid profile, increase the concentration of circulating PUFAs, particularly in CLA and ALA. These acids may improve insulin sensitivity [39] by promoting an increase in the uptake of glucose and preventing fasting glucose concentrations. Arab L. and co-workers have recently [38] discussed the association between walnut consumption and diabetes risk. They examined the available data from the National Health

and Nutrition Examination Survey, which represents the US population, and concluded that walnut consumers showed a lower risk of developing diabetes when compared with non-nut consumers. The evaluated data pointed to an increase in fasting plasma glucose (FPG) and in glycohemoglobin, HbA1c. According to those authors, for each standard deviation of increase in walnut intake, prevalence of diabetes dropped by 47%, and they point out that the effect may be more potent among women than men (dose response  $p = 0.061$ ). In addition, our results point out that the Glc reduction was more significant in women when compared to men.

Regarding non-insulin-mediated mechanisms, evidence suggests that nuts reduce postprandial glucose since they delay gastric emptying, which may also explain improvements in fasting blood glucose, especially when nuts are consumed as part of a meal [39]. Despite this, not all studies have clearly demonstrated the beneficial effect of glycaemia from eating walnuts. In fact, according to Njike et al. [42], daily consumption of nuts for a period of 6 months in adults at risk of developing diabetes did not improve fasting blood glucose or other biochemical/anthropometric parameters. Whilst our results indicate a decrease in glucose levels, nothing can be stated concerning diabetes risks; in fact, as aforementioned, most of the participants in our sample had normal levels of glycaemia. Furthermore, our results point out that the Glu reduction was more significant in younger participants when compared to older participants.

Only a few studies have investigated the possible relationship between walnut intake and uraemia or blood urea nitrogen changes. Concerning our results, this study revealed that there was a decrease in the mean values after walnut consumption, but these variations were not statistically significant. The UR mean value dropped from  $34.5 \pm 10.9$  to  $30.7 \pm 10.8$  mg.dL<sup>-1</sup>, see Figure 4, but the variance in UR increased after walnut intake. However, if the analysis is made by subgroups, e.g., male and female, Table 2, one can find a significant decrease in the values of UR in women. The mean variation for UR was  $31.3 \pm 7.7$  mg.dL<sup>-1</sup> and dropped to  $26.0 \pm 6.9$ mg.dL<sup>-1</sup>. Those observations contrast with what happened for men, suggesting that the intake could have an effect on preventing uraemia in women but not in men. In fact, UR dropped by 80% in women compared to 55.6% in men.

#### 4.3. Lipid Profile

Walnuts are considered inexpensive and safe to use for treating dyslipidaemia. It has been shown that they can significantly reduce TC, LDL-C, and triglycerides [43–46], but our results do not follow that line; the graphic representations in Figure 5 and the values in Table 2 show that the variation in mean values for TC, HDL-C, LDL-C, and TG before and after walnut consumption had no statistical significance except for HDL-C in women. HDL-C, LDL-C, TC, and TG decreased in women but increased in men.

The literature points out that the benefits of walnut consumption for lipid blood markers depend on (i) hypercholesterolemia, (ii) period of intake, and (iii) daily quantity [43–46]. The effects are more pronounced in individuals with hypercholesterolemia [45]. TC profiles show that nearly 40% of the participants present higher values than those recommended by Portuguese health authorities. Walnut intake did not show efficacy in reducing the number of individuals with higher values than those recommended or in reducing the mean values for TC. A significant decrease in HDL-C was observed for the female population, which presented an initial mean value of  $68.2 \pm 16.2$  then dropped to  $62.7 \pm 16.1$  after 45 days of intake (Table 2); however, despite the negative effect that a drop in HDL-C may have had in the feminine group, it should be noted that this decrease did not alter the degree of probability of intermediate risk of cardiovascular disease as stipulated by the Portuguese Standard [47]. Concerning LDL-C, nut intake did not change the mean values or the variance of the concentrations, although most of the individuals had values within the normal range before the experiment.

The effect of walnuts on dyslipidaemia blood markers seemed to follow a dose and time response pattern as discussed by Ashraf et al. [43], which showed that the higher the

daily dose and period of ingestion, the greater the observed benefits for the lipid profile. Alshahrani et al. [45] showed that there is a dose–response effect for cholesterol reduction in individuals subject to nut diets: an average daily intake of 67 g, gives an estimated average reduction in TC and LDL of  $11 \text{ mg.dL}^{-1}$  (5%) and  $10 \text{ mg.dL}^{-1}$  (7%), respectively, while Sabaté et al. [46] found that for a daily intake of 84 g of walnuts/d for 30 days, there was a 16% decrease in LDL-C. In our study, the daily intake was 25 g, which is far lower than the dosages used in the referred studies. Alshahrani et al. [45] also demonstrated that the influence of nuts on the lipid profile was more advantageous when participants had hypercholesterolemia and followed a diet enriched with nuts (26–64 g/d) for one year. Thus, the observations in our study of the inefficacy of walnut intake in altering lipid profiles could be due to the small dose of walnuts or the short period of ingestion.

In addition, and according to the literature [5,8,48,49], beyond the doses and the period of ingestion, the origin of the cultivar of the nuts may also influence the capacity of nuts to alter the lipid profile of the studied population. Depending on the variety of the nuts, the composition of phytosterols (see Figure 2) and of  $\alpha$ -linolenic acid (18:3n – 3) (ALA) (see Figure 1) can contribute to lower cholesterol blood concentrations. Phytosterols are also considered to interfere with absorption of cholesterol [15], and the amount of ALA seems to facilitate the uptake of cholesterol by LDL particles [9]. The total percentage of ALA in Portuguese walnut cultivars (oil extract) ranges from 13 to 17% [32], contrasting with the mean value of Californian walnuts, which is estimated to be about 9 g per 100 g [8]. Therefore, it would be expected that the Portuguese walnuts would show higher efficacy in reducing the TC, but this was not observable in our results.

According to the meta-analysis and systematic review of controlled trials related to the effects of walnut consumption on blood lipids and other cardiovascular disease risk factors, as carried out by M. Guasch-Ferré et al. [44] and Banel et al. [48], the consumption of walnuts had more pronounced effects on lowering TC and LDL cholesterol concentrations when walnuts accounted for 10–25% of total energy compared with a lower intervention dose of <10% of total energy. In addition, walnut diets were less effective in improving blood lipids in individuals that followed Mediterranean (which is the case for our study) or low-fat background diets when compared with Western dietary patterns. Thus, adhering to a Mediterranean diet supplemented with walnuts could be a good approach to improving lipid profiles.

#### 4.4. Liver Enzymes

The liver enzymes AST and ALT are important biomarkers of liver diseases and may reflect liver damage and non-alcoholic fatty liver disease (NAFLD), whose main trigger is inflammation [50,51]. An elevation of alkaline phosphatase and the AST to ALT ratio to more than 2 may indicate hepatocellular or cholestatic disease, while late-stage acute liver damage often results in an AST/ALT ratio < 1 [52]. Graphic representations of Figure 5 show that the variation in the mean levels for ALT were not significant, whereas the mean values for AST decreased from  $24.71 \pm 13.65 \text{ U.L}^{-1}$  to  $19.83 \pm 4.66 \text{ U.L}^{-1}$ ,  $p = 0.03$  after walnut consumption. The analysis of bell graphics also showed that after the consumption, the variability in the data did decrease suggesting that walnuts may have some effect on controlling enzyme levels. Moreover, the mean AST/ALT ratio was <1 and, after the intake, the ratio decreased. This could indicate that walnut constituents may increase the time taken to clear AST.

In the period of 2005–2018, more than 25,000 adults who did not engage in excessive alcohol consumption were treated with a moderate daily intake of nuts [53]. At the end of the study, the authors were able to find a negative correlation between nut consumption (15–20 g/day) and the prevalence of NAFLD, especially in females where the reduction in risk was 15%. Despite this, studies concerning the influence of walnuts in AST and ALT are scarce. Barrera et al. [54] reported a study involving 150 participants and concluded that walnut intake correlated with reduced ALT ( $r = -0.31$ ) and AST ( $r = -0.21$ ,  $p < 0.05$ ) at the end of 3 months.

Some authors hypothesize that the richness of substances with anti-inflammatory action such as tocotrienols, ellagic acid, and ALA [55] could have an effect on lowering the quantities of ALT and AST. According to Leung et al. [56], the ALA present in dried fruits can reduce the production of pro-inflammatory mediators in the liver, demonstrating the regulation of anti-inflammatory lipid mediators. In addition, Gu et al. [57], showed that ellagic acid can reduce the content of malondialdehyde in the liver and tumour necrosis factor alpha because the levels of ALT and AST in the serum have anti-inflammatory effects. Some studies suggest that eating walnuts can reduce the concentration of liver enzymes and reduce the risk of NAFLD, inhibiting the occurrence of hepatitis [58]. More recently, Liu et al. [59] described a study on the effect of walnuts on alcohol-induced acute liver injury. The study was performed in rats, highlighting the importance of walnut oligopeptides in the reduction of ALT and AST levels, indicating that those substances had a protective effect on damage to hepatocytes induced by alcohol.

#### 4.5. Blood Pressure

Results regarding the effect of nut intake on BP remain controversial. Some studies report that nut intake does not reduce BP [50,60,61]; however, most studies have shown that nut intake has a beneficial effect on SBP, DBP, or both [62,63]. Our results showed that the consumption within the 45 day period did not alter the SBP and DBP significantly. However, when we analyse these parameters in percentages, there were a significant number of individuals whose SBP, DBP, or both dropped. The relative quantity of constituents that may influence the drop in BP, such as Mg, MUFAS, PUFAS and *L*-arginine, may have been lower in our walnuts due to edaphic climatic factors, although other factors cannot be ruled out.

### 5. Conclusions

Despite the low number of participants, the present work provided data concerning some effects of walnut consumption produced locally in a sample of a Portuguese population living in a city; these data can be used for other systematic studies. The study was also based on feasible premises in terms of consumption period (45 days) and quantities (no more than 25 g/day); thus, the results obtained reveal a realistic order as to the extent of the benefits that eating nuts can have on a normal dietary pattern. Walnuts can be used as functional food as they have beneficial effects on prevention and control of some cardiovascular risk factors: there was a significant reduction in the AST when all participants were analysed, suggesting better control of liver enzymes.

The results suggest a trend in the sense that the consumption of the nuts seems to be more effective in lowering cardiovascular risk factors in women than in men. It was found that the women benefitted the most from the intake of walnuts. It was observed that Glu, UR, and HDL-C levels dropped significantly in women, which indicates the positive effect of walnuts on glycaemia and kidney and liver function. A possible explanation for this may be that females have a greater capacity for synthesizing docosahexaenoic and arachidonic acids, which are considered the most important long-chain PUFAs, from CLA and ALA. This happens because in females, oestrogen stimulates this production, while in males, testosterone inhibits the transformation of longer-chain essential fatty acids.

Regarding age, the biggest differences observed between the two groups were in blood Glc, LDL-C and TG levels. It could be theorized that these differences could be related to the presence of antioxidant and anti-inflammatory substances in walnuts that act synergistically to reduce age-related oxidative stress and inflammation. Thus, nuts as complete functional food can positively adjust aging processes and play a fundamental role in the relationship between lifespan and health.

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