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Brazilian *Curcuma longa L.* attenuates comorbidities by modulating adipose tissue dysfunction in obese rats

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Abstract

Background: Adipose tissue dysfunction is a condition characterized by inflammation and oxidative stress able to lead metabolic disorders. *Curcuma longa* L. (*Cl*) is a rhizome commonly used in Indian culinary which presents anti-inflammatory and antioxidant compounds. The aim of this study was to evaluate the effect of *in natura Curcuma longa* L. on adipose tissue dysfunction and comorbidities in obese rats.

Methods: Male Wistar rats (8 weeks old, $n = 16$) received standard chow + fructose in drinking water (30%) ad libitum for 16 weeks. After this period, animals were randomly divided to receive placebo treatment (fructose, $n = 8$) or *Curcuma longa* L. treatment (fructose + *Cl*, $n = 8$) for more 8 weeks, totalizing 24 weeks of experiment. *Curcuma longa* L. was mixed in water and gave to the animals by gavage in a dose of 80 mg/kg of body weight. Body composition, systolic blood pressure, metabolic, hormonal, inflammatory, and oxidative stress analysis were performed in plasma and adipose tissue.

Results: *Curcuma longa* L. reduced adiposity index and adipocyte hypertrophy, improved insulin resistance and systolic blood pressure, and reduced inflammation and oxidative stress in adipose tissue.

Conclusion: *Curcuma longa* L. *in natura* is able to modulate adipose tissue dysfunction, avoiding the development of comorbidities. It can be considered a phytochemical treatment strategy against obesity-related chronic diseases.

Keywords: Adipose dysfunction, Comorbidities, Obesity

Background

White adipose tissue (WAT) is the primary site for energy storage and is also responsible by thermal isolation and mechanical protection. Moreover, it is considered an important endocrine organ which secrets a large number of adipokines responsible by the whole body metabolism maintenance [1, 2]. However, obese individuals are susceptible to adipose tissue dysfunction, which is characterized by altered adipokine secretion, increased reactive oxygen species, and inability to store triacylglycerol [2–4].

This condition is able to lead to metabolic syndrome and cardiovascular diseases [5–7].

Studies show that the excessive fructose intake is one cause for the current epidemics of metabolic syndrome and obesity [8–11]. Fructose is a sugar commonly found in fruits. However, the industry has used corn syrup, which is rich in fructose, to sweet beverages and foods, increasing the intake of this sugar by the population [12]. Although glucose and fructose present similar molecular structures, their metabolism is different. Fructose has a lower glycemic index and does not generate an insulin response, but present a higher sweetener power [13]. Moreover, fructose is quickly absorbed by the liver and converted into glucose, glycogen, lactate, and fat [14]. For this reason, fructose is considered a potent

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lipogenic and adipogenic nutrient, able to promote hypertrophy in adipocyte precursor cells (APCs), other condition related to adipose tissue dysfunction, and metabolic disorders [15].

Considering this condition, functional foods have presented many health benefits, protecting against several diseases such as hypertension, diabetes, and cancer [16]. *Curcuma longa* L. (*Cl*) is a cultivated and appreciated spice since antiquity in the Mediterranean region, commonly used as coloring, flavoring, and seasonings [17]. This rhizome is composed mainly by curcumin (1,7-bis (4-hidroxi-3-metoxifenil)-1,6-heptadieno-3,5-diona) but also by bis-demethoxycurcumin and demethoxycurcumin. Curcumin is a polyphenol very studied in the isolated form and presents anti-inflammatory and antioxidant activity and is able to attenuate obesity-related disorders [17–21]. *Curcuma longa* L. extract is another administration form with health benefit results [17]. However, most of population ingests this food *in natura* in the diet, added in the preparations. Considering that studies with *Curcuma longa* L. *in natura* in the literature are scarce, it is important to evaluate the action of this food on inflammation and oxidative due adipose tissue dysfunction. So, the aim of this study was to evaluate the *in natura* effect of *Curcuma longa* L. on adipose tissue dysfunction and comorbidities in obese rats.

Material and methods
Experimental protocol
All the experiments and procedures were approved by the Animal Ethics Committee of Botucatu Medical School (1065/2013) and performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Male Wistar rats (8 weeks old) were housed in individual cages in an environmental controlled room (22 °C ± 3 °C; 12-h light-dark cycle and relative humidity of $60 \pm 5\%$). During 16 weeks, animals ($n = 16$) received standard chow + fructose in drinking water (30%) ad libitum. After this period, animals were randomly divided to receive placebo treatment (fructose, $n = 8$) or *Curcuma longa* L. treatment (fructose + *Cl*, $n = 8$) for more 8 weeks, totalizing 24 weeks of experiment. Both groups (fructose and fructose + *Cl)* continued receiving standard chow + fructose in drinking water (30%) ad libitum. In order to confirm obesity in the fructose group, additional rats of the same age, fed with a chow diet and water, were used as control group ($n = 8$).

Curcuma longa L. preparation and treatment
Curcuma longa L. was harvested in São Manuel city, São Paulo, Brazil, in the Experimental Farm of Teaching, Research and Production, belonging to the Agronomic Sciences Faculty (FCA) - UNESP/Botucatu-SP. After the harvest, the rhizomes were washed and sent to the Department of Chemistry and Biochemistry of the Institute of Biosciences (IB) - UNESP/Botucatu-SP, where they were chopped and dried in a forced air circulation oven at 65 °C until stabilization of pasta. The rhizomes were ground in a knife mill and stored in amber glass bottles at room temperature, protected from light and moisture. After this, animals received both placebo or *Curcuma longa* L. (*Cl*) by gavagem. *Curcuma longa* L. was mixed in water and administered to the animals in a dose of 80 mg/kg of body weight [22]. It was also added black pepper (1%) to increase *Curcuma longa* L. absorption [23]. Placebo was only water.

Body composition
Body composition was evaluated by initial and final body weight and adiposity index. Adiposity index (AI), considered an estimative of body fat, was calculated according to the formula: [(epididymal + retroperitoneal + visceral)/body weight] × 100 [24, 25].

Systolic blood pressure
Systolic blood pressure (SBP) evaluation was assessed in conscious rats by the non-invasive tail-cuff method with a NarcoBioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA). The animals were kept in a wooden box (50 × 40 cm) between 38 and 40 °C for 4–5 min to stimulate arterial vasodilation [26]. After this procedure, a cuff with a pneumatic pulse sensor was attached to the tail of each animal. The cuff was inflated to 200 mmHg pressure and subsequently deflated. The blood pressure values were recorded on a Gould RS 3200 polygraph (Gould Instrumental Valley View, OH, USA). The average of three pressure readings was recorded for each animal.

Plasma metabolic and hormonal analysis
After 12 h of fasting, blood was collected and plasma was used for the following analysis. An enzymatic-colorimetric kit was used to measure glucose and triglycerides (Bioclin®; Belo Horizonte) using an automatic enzymatic analyzer system (Chemistry Analyzer BS-200, Mindray Medical International Limited, Shenzhen, China). The insulin level was measured using the enzyme-linked immunosorbent assay (ELISA) method using commercial kits (EMD Millipore Corporation, Billerica, MA, USA). The homeostatic model of insulin resistance (HOMA-IR) was used as an insulin resistance index, calculated according to the formula: HOMA-IR = (fasting glucose (mmol/L) × fasting insulin (µU/mL))/22.5 [27].

Plasma inflammatory cytokines
Plasma levels of tumoral necrose factor-alpha (TNF-α) and interleukin-6 (IL-6) were measured by ELISA kits (R&D System, Minneapolis, USA). Reading was made in a microplate spectrophotometer reader (SpectraMax 190; Molecular Devices).

Plasma extraction and identification of curcuminoids by HPLC-DAD-UV

The extraction was made according to Asai and Miyazawa (2000) [28]. Aliquots (20 μL) were injected into a UHPLC Thermo Scientific Dionex UltiMate 3000 system (Thermo Fisher Scientific Inc., MA, USA), coupled to a quaternary pump an Ultimate 3000RS auto sampler and a diode array detector (DAD-3000RS). The reading was performed at a wavelength of 245 nm using an isocratic method composed of ethanol: methanol (60:40) as the mobile phase under a flow of 1.0 mL per minute. The column used was C18, 5 μm, 150 × 4.6. The results were obtained through a curve performed with the sigma standards (98% purity) of curcumin (C08511), bisdemethoxycurcumin (B6938), and demethoxycurcumin (D7696).

Epididymal adipose tissue analysis
Tissue preparation

Epididymal adipose tissue (400 mg) was homogenized with 2 ml of PBS (pH 7.4) and then centrifuged at 3000 rpm, 4 °C, 10 min. The supernatant was used to evaluate inflammatory cytokines, malondialdehyde, and carbonylation. The results were corrected by the protein amounts of each sample, quantified by the Bradford method [29].

Inflammatory cytokines in adipose tissue

Tumoral necrosis factor-alpha (TNF-α) and interleukin – 6 (IL-6) were measured by ELISA kits (R&D System, Minneapolis, USA) according to the manufacturer's instructions. Reading was made in a microplate spectrophotometer reader (SpectraMax 190; Molecular Devices).

Malondialdehyde (MDA) in adipose tissue

For MDA quantification, 250 μL of epididymal adipose tissue supernatant was mixed with 750 μL of 10% trichloroacetic acid for protein precipitation. After centrifugation (3000 rpm, 5 min; Eppendorf® Centrifuge 5804-R, Hamburg, Germany), the supernatant was removed. Thiobarbituric acid (TBA, 0.67%) was added in ratio (1: 1), and the samples were heated for 15 min at 100 °C. MDA reacts with TBA in the ratio 1: 2 MDA-TBA, absorbed at 535 nm. After cooling, the reading at 535 nm was performed on Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA). The MDA concentration was obtained by the molar extinction coefficient (1.56×105 M^{-1} cm^{-1}) and the sample absorbance and the final result expressed in nmol/g protein [30].

Protein carbonylation in adipose tissue

Epididymal adipose tissue supernatant was used to measure protein carbonylation (PC) by an unspecific method based on the photometric detection of DNPH (2,4-dinitrophenyl hydrazine) derivatizing agent [31]. Briefly, 10 μL of diluted (1:10) supernatant was incubated in an acid DNPH solution for 10 min. After this, NaOH 1 M was added and the absorbance was checked. Results were obtained according to the molecular extinction coefficient of DNPH and adjusted according to the total tissue proteins amount (mg).

Histological analysis

Adipose tissue was fixed in 4% formaldehyde and embedded in paraffin. Two consecutive sections from each sample were cut (4 μm) and stained with hematoxylin/eosin. The entire slide was scanned using a 3DHISTECH Panoramic MIDI System attached to a Hitachi HV-F22 color camera and ten fields/slide were analyzed under × 100 magnification in a blinded manner. The inflammatory reactions are reported as present/absent. Using the same slides, adipocyte mean area was calculated using a method previously described by Osman et al. in 2013 [32].

Statistical analysis

Results are expressed as mean + standard deviation (SD). The significance of differences was calculated by the Student's *t* test, using SigmaStat version 3.5 for Windows (Systat Software, Inc., San Jose, CA, USA). A *p* value of 0.05 was considered as statistically significant.

Results
Effect of *Curcuma longa* L. on body weight and adiposity index

There were no differences in BW among the groups in the beginning of this study. At the end of the experiment, the mean BWs of the fructose group was significantly higher than control group (control 489 + 58 g vs. fructose 579 + 71 g, *p* < 0.05). Regarding the effect of *Curcuma longa* L. on body weight, the treated group (fructose + *Cl*) did not present difference compared to fructose group. The adiposity index was also significantly higher in fructose group (control 5.3 + 1.2% vs. fructose 9.5 + 1.7%, *p* < 0.001) compared to control group, which confirms obesity in fructose group. However, fructose + *Cl* group presented after the treatment, lower adiposity index compared to fructose group, demonstrating the positive effect of *Curcuma longa* L. against obesity. In order to confirm the presence of *Curcuma longa* L. in the treated group, plasma detection of curcumin and two isoforms, bis-demethoxycurcumin and demethoxycurcumin, were analyzed. It is possible to verify the presence of curcuminoids only in fructose + *Cl* group. All the results are presented in Table 1.

Effect of *Curcuma longa* L. on the adipose tissue dysfunction

Adipose tissue histological analysis showed inflammatory cells in both groups that received fructose. About the

Table 1 BW, adiposity index, and plasma levels of curcuminoids

Variables	Groups	
	Fructose	Fructose + Cl
Initial BW (g)	257 ± 15	258 ± 14
Final BW (g)	538 ± 15	467 ± 42
Adiposity index (%)	6.59 ± 0.69	4.92 ± 1.05*
Caloric intake (Kcal)	66.9 ± 8.3	70.2 ± 9.3
Feed efficiency (%)	2.64 ± 0.23	2.37 ± 0.57
Plasm total curcumin (nmol)	ND	0.08 ± 0.04
Bisdemetoxicurcumin (µg/dL)	ND	0.09 ± 0.03
Demetoxicurcumin (µg/dL)	ND	0.066 ± 0.002

* indicates $p<0.05$

effect of *Curcuma longa* L. the treated group (fructose + Cl) presented reduction in adipocyte area compared to fructose group, confirming the positive result of this food to reduce hypertrophy (Fig. 1).

Inflammatory cytokine levels are presented in Fig. 2. Fructose + Cl group also presented reduced plasma and adipose tissue IL-6 levels as well as reduced TNF-α concentration in adipose tissue compared to fructose group.

Figure 3 presents oxidative stress parameters in adipose tissue. Fructose + Cl group showed lower levels of MDA compared to fructose group. No effect on carbonylation was observed.

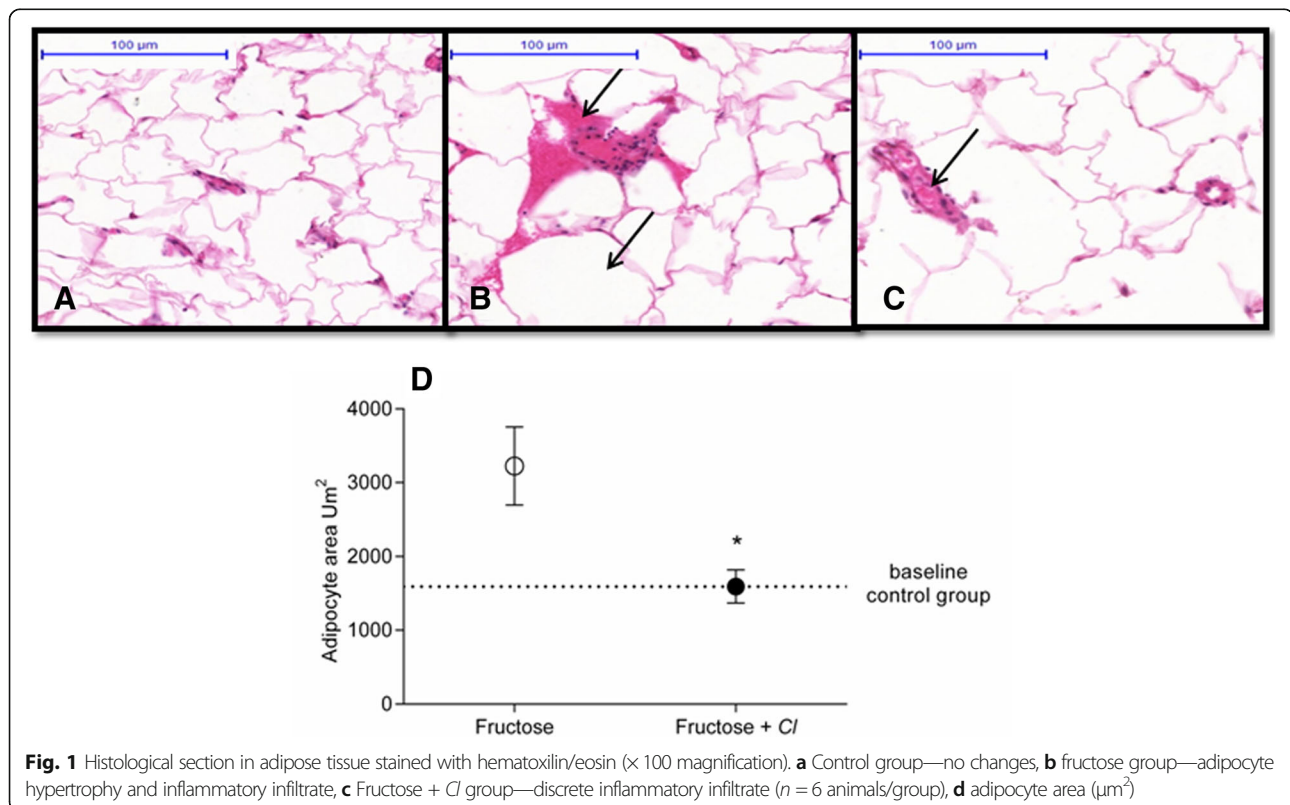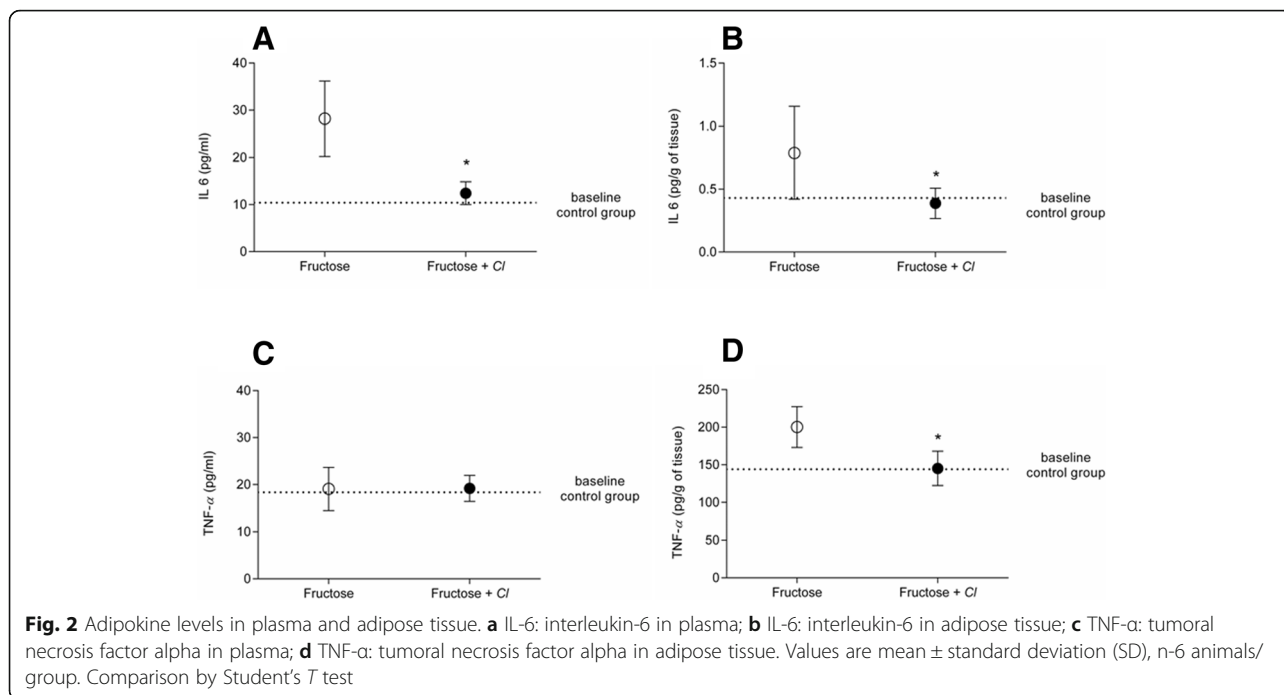### Effect of *Curcuma longa* L. on comorbidities

Figure 4 shows the effect of Cl on metabolic parameters. It is possible to verify a positive effect to reduce plasma triglycerides, HOMA-IR, and systolic blood pressure in fructose + Cl group compared to fructose group.

Discussion

The aim of this study was to evaluate the *in natura* effect of *Curcuma longa* L. on adipose tissue dysfunction and comorbidities in obese rats. Adipose tissue dysfunction is characterized by an imbalanced production and release of pro- and anti-inflammatory adipokines, increased production of reactive oxygen species (ROS), and increased inflammatory cell infiltrate [33]. This condition is associated with metabolic systemic consequences such as systemic low-grade inflammation, hypercoagulability, hypertension, dyslipidemia, and insulin resistance [12, 15, 34–36]. Therefore, it is extremely important to prevent or to treat the adipose tissue dysfunction in order to avoid the development of diabetes and cardiovascular and renal diseases. The consumption of bioactive compounds present in many foods can be a treatment option for these conditions. In this way, our results showed a positive effect of *Curcuma longa* L. on attenuation of adipose tissue dysfunction and comorbidities.
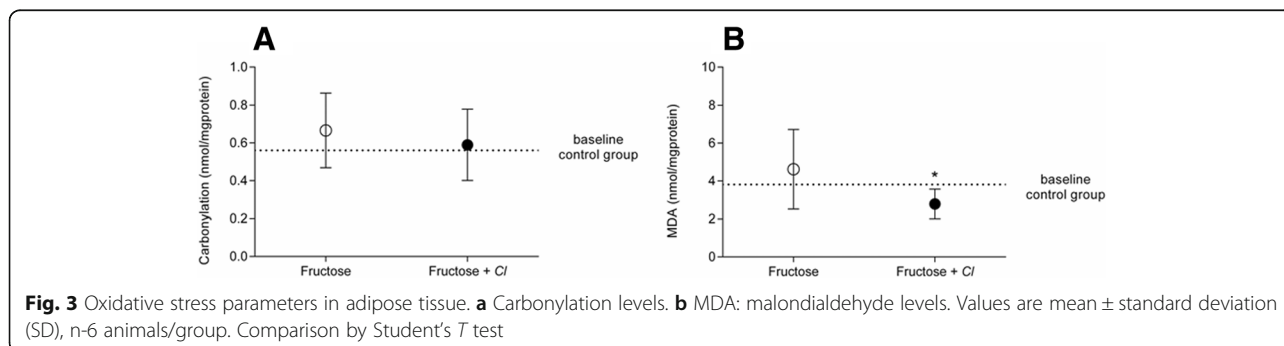
Curcumin is the major active component of turmeric, a yellow compound originally isolated from the plant

Fig. 1 Histological section in adipose tissue stained with hematoxilin/eosin (× 100 magnification). **a** Control group—no changes, **b** fructose group—adipocyte hypertrophy and inflammatory infiltrate, **c** Fructose + Cl group—discrete inflammatory infiltrate (n = 6 animals/group), **d** adipocyte area (µm²)

Fig. 2 Adipokine levels in plasma and adipose tissue. **a** IL-6: interleukin-6 in plasma; **b** IL-6: interleukin-6 in adipose tissue; **c** TNF-α: tumoral necrosis factor alpha in plasma; **d** TNF-α: tumoral necrosis factor alpha in adipose tissue. Values are mean ± standard deviation (SD), n-6 animals/group. Comparison by Student's *T* test
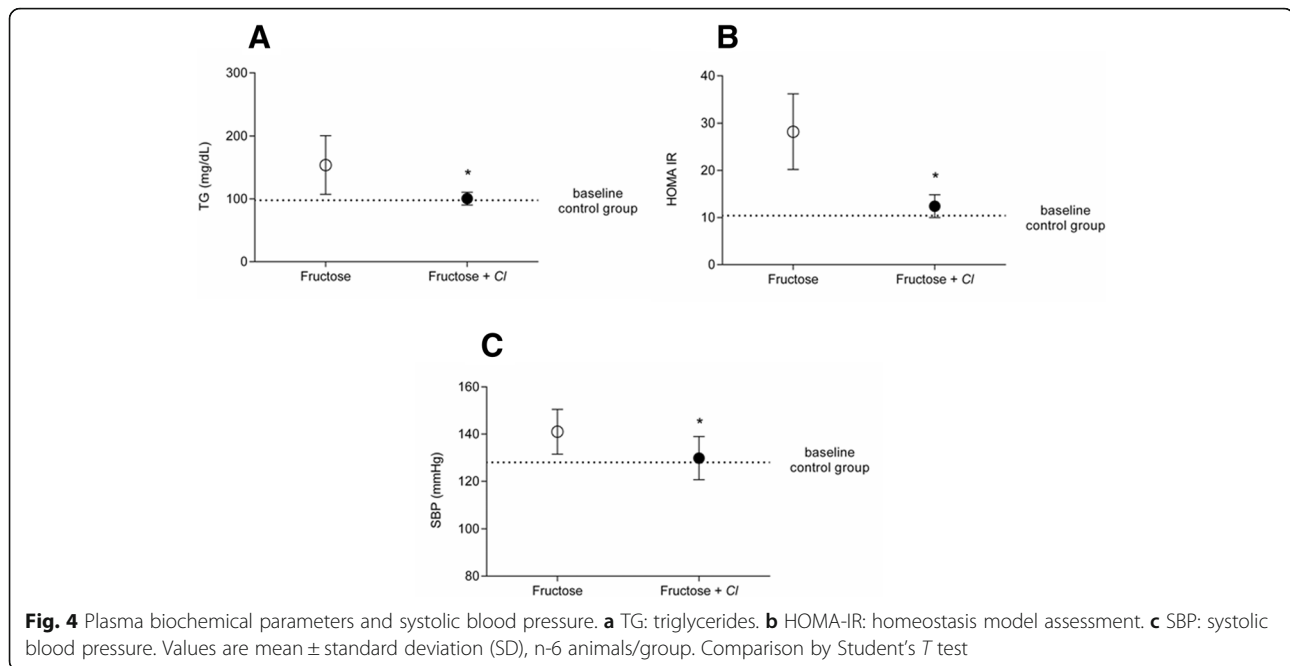
Curcuma longa. It is a member of the curcuminoid family and has been used for centuries in traditional medicines. As a spice, it provides curry with its distinctive color and flavor. Furthermore, traditional Indian medicine has considered curcumin a drug effective for many disorders including asthma and hepatic diseases. However, evidence from numerous literatures revealed that the major challenge about curcumin is to increase the absorption and bioavailability [37]. Uptake and distribution of curcumin in body tissues is obviously important for its biological activity. Most of curcumin get metabolized in the liver and intestine; however, a small quantity still remains detectable in the organs [37]. In order to increase the absorption, piperine, a constituent of pepper, is an inhibitor of hepatic and intestinal glucuronidation. Thus, the ingestion of piperine contributes to increase the serum concentration of curcumin and thereby its bioavailability [38]. In our study, we used *in natura Curcuma longa* associated with black pepper to improve the absorption and the result was the

three main curcuminoids present in plasma of the treated group. Aiming to increase the bioavailability, longer circulation, better permeability, and resistance to metabolic processes of curcumin several formulations have been prepared which include nanoparticles, liposomes, micelles, and phospholipid complexes. All of them show improvement in the bioavailability of curcuminoids [37]. However, these forms have not gained significant attention in human since most people find and use to cook in natura *Curcuma longa* and studies show health benefits from oral administration. The dietary treatment with curcumin improved insulin sensitivity, inflammatory disorders, or prevented liver fat accumulation in rodents fed with a HF diet. It is worth noting that the beneficial effects observed in those studies were always demonstrated after a long period of administration (up to 8 weeks) [39]. Other in vitro studies show that curcumin treatment for 12 weeks could diminish expansion of adipose tissue and body weight gain probably through inhibition of angiogenesis

Fig. 3 Oxidative stress parameters in adipose tissue. **a** Carbonylation levels. **b** MDA: malondialdehyde levels. Values are mean ± standard deviation (SD), n-6 animals/group. Comparison by Student's *T* test

Fig. 4 Plasma biochemical parameters and systolic blood pressure. **a** TG: triglycerides. **b** HOMA-IR: homeostasis model assessment. **c** SBP: systolic blood pressure. Values are mean ± standard deviation (SD), n-6 animals/group. Comparison by Student's *T* test

and adipogenesis in adipose tissue [40]. So, to investigate the effects of *Curcuma longa* in adipose tissue of animals and humans is very important.

The adipose tissue becomes dysfunctional when the demand of triglycerides is too high, and the adipocyte needs to hypertrophy to store this excessive TG. Our results showed that the group treated with *Curcuma longa* L. presented reduction of triglyceride levels. Regarding this effect, two mechanisms could explain these findings. The first one is that *Curcuma longa* L. could impact on TG synthesis and oxidation in the liver by increasing PPAR-α expression and activation. It has been demonstrated that PPAR-α regulates liver enzymes related to lipid synthesis as well as beta-oxidation enzymes [41, 42]. The second is that *Curcuma longa* L. can upregulate fatty acid oxidation in the skeletal muscle and/or adipose tissue in association to a greater expression and activation of UCP-1 [43, 44]. Independent of which mechanism happened in our animals, the reduced plasma TG concentrations reflected in lower adipocyte fat deposition with consequent reduction in adiposity index, in adipocyte area, and in IL-6, TNF-alpha, and MDA levels in the treated group.

This improvement in adipose tissue dysfunction avoided the manifestation of some comorbidities, among them insulin resistance and type 2 diabetes, since these diseases are closely associated with chronic inflammation. Regarding the association of adipokines and diseases, the literature reports that TNF-α was the first link among obesity, diabetes, and chronic inflammation in adipose tissue [45, 46]. Later, IL-6 was discovered to be also increased in obese individuals. In this study, both TNF-α and IL-6 were increased in fructose group and these animals also presented some

comorbidities, such as insulin resistance and hypertension. TNF-α is a pro-inflammatory cytokine able to activate signal transduction cascades, including insulin action inhibition pathways. In physiological conditions, insulin stimulates tyrosine phosphorylation by insulin-receptor substrate (IRS), which is a crucial event in mediating insulin action. However, TNF-α also targets this element of insulin-receptor signaling through inhibitory serine phosphorylation of IRS-1, which interferes with the ability of this protein to engage in insulin-receptor signaling and results in alterations in insulin action [45]. Hypertension in fructose group can be explained by increased IL-6, a cytokine that acts under the renin-angiotensin system (RAS) activity leading to angiotensin II (ANGII)-mediated hypertension [47]. On the other hand, fructose + *Cl* group presented a reduction of TNF-α and IL-6 levels which can explain insulin resistance improvement and the reduction in systolic blood pressure.

Together with the anti-inflammatory results above described, other studies have already showed the anti-inflammatory and also the antioxidant effect of *Curcuma longa* L. [48–50]. The treated group presented reduction in MDA levels compared to untreated group, and no difference in carbonylation was found. Protein carbonylation is an irreversible protein oxidation promoted by reactive oxygen species, which leads to the loss of protein function and considered a marker of severe oxidative damage. This reaction can happens via the addition of aldehydes such as those generated from lipid peroxidation. Oxidative decomposition of polyunsaturated fatty acids initiates chain reactions that lead to the formation of a variety of carbonyl species, among them

malondialdehyde [51]. Although fructose + *Cl* group presented reduction in MDA levels, protein oxidation did not present difference from fructose group. Since the group started the treatment with *Curcuma longa* L. after 16 weeks receiving fructose, protein carbonylation have already happened, even with a reduction in MDA levels. This reduction in MDA levels corroborates the antioxidant effect of *Curcuma longa* L.; however, a preventive consumption of this food should have more interest to avoid protein oxidation.

Conclusion

In summary, in obese condition, *Curcuma longa* L. reduced adiposity index and adipocyte hypertrophy, improved insulin resistance and systolic blood pressure, and reduced inflammation and oxidative stress in the adipose tissue. These results can be attributed to the modulation of adipose tissue dysfunction after treatment with this functional food. So, it is possible to conclude that *Curcuma longa* L. *in natura* is able to modulate adipose tissue dysfunction, avoiding the development of comorbidities. It can be considered a phytochemical treatment strategy against obesity-related chronic diseases.

Abbreviations
AI: Adiposity index; ANGll: Angiotensin II; APCs: Adipocyte precursor cells; *Cl*: *Curcuma longa* L.; DNPH : 2,4-Dinitrophenyl hydrazine; ELISA : Enzyme-linked immunosorbent assay; HOMA-IR: Homeostatic model of insulin resistance; IL-6: Interleukin-6; IRS: Insulin receptor substrate; MDA: Malondialdehyde; PC: Protein carbonylation; PPAR-α: Peroxisome proliferator-activated receptor alpha; RAS : Renin-angiotensin system; ROS: Reactive oxygen species; SBP: Systolic blood pressure; SD: Standard deviation; TBA: Thiobarbituric acid; TG: Triglycerides; TNF-α: Tumoral necrose factor-alpha; UCP1: Uncoupling protein 1; WAT: White adipose tissue

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
ATCL contributed to the experimental design and data analysis. FVF contributed to the experimental design and data analysis, and wrote and reviewed the manuscript. FKH contributed to the experimental design and data analysis. APCRF contributed to the experimental design. IOM contributed to the data analysis and wrote and reviewed the manuscript. JLG and KCS contributed to the data analysis of the manuscript. PHRA contributed to the experimental design and data analysis. GPPL, FM and AJTF wrote and reviewed the manuscript. CRC contributed to the experimental design and data analysis, and wrote and reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval
This study was approved by the Animal Ethics Committee of Botucatu Medical School (1065/2013).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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