Lycopene supplementation modulates plasma concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats

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Abstract

Obesity is characterised by chronic low-grade inflammation, and lycopene has been reported to display anti-inflammatory effects. However, it is not clear whether lycopene supplementation modulates adipokine levels in vivo in obesity. To determine whether lycopene supplementation can regulate adipokine expression in obesity, male Wistar rats were randomly assigned to receive a control diet (C, n 6) or a hyperenergetic diet (DIO, n 12) for 6 weeks. After this period, the DIO animals were randomised into two groups: DIO (n 6) and DIO supplemented with lycopene (DIO + L, n 6). The animals received maize oil (C and DIO) or lycopene (DIO + L, 10 mg/kg body weight (BW) per d) by oral administration for a 6-week period. The animals were then killed by decapitation, and blood samples and epididymal adipose tissue were collected for hormonal determination and gene expression evaluation (IL-6, monocyte chemoattractant protein-1 (MCP-1), TNF-α, leptin and resistin). There was no detectable lycopene in the plasma of the C and DIO groups. However, the mean lycopene plasma concentration was 24 nmol in the DIO + L group. Although lycopene supplementation did not affect BW or adiposity, it significantly decreased leptin, resistin and IL-6 gene expression in epididymal adipose tissue and plasma concentrations. Also, it significantly reduced the gene expression of MCP-1 in epididymal adipose tissue. Lycopene affects adipokines by reducing leptin, resistin and plasma IL-6 levels. These data suggest that lycopene may be an effective strategy in reducing inflammation in obesity.

Key words: Lycopene; Obesity; Adipokines

Obesity is characterised by chronic low-grade inflammation(1). Its aetiology is multifactorial, and the current epidemic is partially due to the increased availability and consumption of highly palatable diets and reduced energy expenditure2), which leads to increased adipose tissue. It is recognised as one of the major risk factors for the development of chronic and disabling diseases3). White adipose tissue is a dynamic endocrine organ that releases several adipokines and pro-inflammatory factors4). It has been shown that high levels of pro-inflammatory adipokines in obesity may contribute to the reduction in lipid oxidation in insulin-sensitive organs, leading to lipotoxicity and insulin resistance5). IL-6 is an important acute-phase mediator with both pro- and anti-inflammatory properties6), and exhibits many biological functions. In addition to its role as the main acute-phase protein synthesis regulator, it is induced with other cytokines, such as TNF-α7). TNF-α is a key modulator of adipocyte metabolism, with a direct role in several insulin-mediated processes, including glucose homeostasis and lipid metabolism. High levels of TNF-α are a major contributor to the development of adipose tissue insulin resistance8). Also, it is associated with significant tissue damage from reactive oxygen species and the promotion of angiogenesis9). Moreover, elevated TNF-α concentrations and IL-6 have been linked to insulin resistance in obesity10). In addition, IL-6 and TNF-α promote leptin production by the adipose tissue, but leptin enhances inflammatory cytokine production as well11). Hyperleptinaemia is correlated with pro-inflammatory responses and with the chronic sub-inflammatory state observed in obesity12). Moreover, leptin

Abbreviations: BW, body weight; C, control; DIO, animals subjected to diet-induced obesity; DIO + L, DIO supplemented with lycopene for 6 weeks; MCP-1, monocyte chemoattractant protein-1.

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induces cholesterol uptake by macrophages, angiogenesis and platelet aggregation, and stimulates oxidative stress in endothelial cells, inhibiting vasorelaxation and increasing the risk of atherosclerosis\(^{(12)}\). Resistin, another pro-inflammatory adipokine, appears to act through binding to Toll-like receptor 4, a cell-surface receptor and a key component of the inflammatory response to bacterial lipopolysaccharide\(^{(13)}\). Located at the site of inflammation, resistin is a molecule that shows a strong correlation with other inflammatory markers, such as IL-6 and TNF-\(\alpha\)\(^{(14)}\). Also, resistin is associated with decreased insulin sensitivity\(^{(15)}\) and seems to be correlated with the development of obesity-related diseases, such as non-alcoholic fatty liver disease\(^{(15,16)}\). Studies have suggested that circulating concentrations of pro-inflammatory molecules reflect excess body fat and predispose an individual to a higher risk of developing metabolic diseases\(^{(17,18)}\). In addition, the adipose tissue hypersecretion of pro-inflammatory adipokines, such as IL-6, TNF-\(\alpha\), leptin and resistin, may play an important role in the pathophysiology of obesity-related complications\(^{(19)}\).

Lycopene is a lipophilic carotenoid which is responsible for the red colour in various fruits and vegetables\(^{(20)}\) and is commonly found in tomatoes\(^{(21)}\). This carotenoid is well known for its antioxidant properties\(^{(22–24)}\), and has been reported to display anti-inflammatory effects in adipocytes\(^{(25)}\) and liver\(^{(26)}\), along with preventing CVD\(^{(27)}\). Evidence is increasing that lycopene or tomato preparations can decrease inflammatory markers\(^{(25–26)}\), and may improve diseases with chronic inflammatory backgrounds such as obesity\(^{(28)}\). However, the effect of lycopene on pro-inflammatory adipokines, especially leptin and resistin, in obesity has not yet been evaluated.

Since pro-inflammatory adipokines, such as IL-6, TNF-\(\alpha\), leptin and resistin, have been linked to adiposity, and lycopene presents anti-inflammatory effects, we hypothesise that lycopene supplementation can modulate epididymal adipose tissue \textit{in vivo}, reducing the expression of pro-inflammatory cytokines in obesity. The decreased production of these adipokines by lycopene could have a major impact on obesity and the prevalence of obesity-related diseases.

### Methods

#### Animals and experimental protocol

Male Wistar rats (10 weeks old, weighing approximately 350 g), from the Animal Center of Botucatu Medical School, São Paulo State University, UNESP (Botucatu, SP, Brazil), were initially divided to receive either a commercial chow diet (C; \(n=6\); 12% energy from fat) or a high-fat diet (49.7% energy from fat) and sugar in the drinking water (300 g/l) (DIO; \(n=12\)), for 6 weeks. The high-fat diet was designed in our laboratory to contain a powdered commercial chow diet – NUVILAB CR-1 (Nuvital\(^{8}\); Sagobi Indústria e Comércio Ltda), a wafer biscuit, condensed milk, palm oil, vitamins and minerals. The diet-induced obesity model was adapted from our previous study\(^{(28)}\), which was used to mimic obesity from Western occidental dietary habits. The nutritional composition of the diets is presented in Table 1. After 6 weeks under a nutritional overload, DIO rats were randomly assigned into two groups: DIO \((n=6)\) and DIO supplemented with lycopene-rich tomato oleoresin (DIO + L, \(n=6\)). Tomato oleoresin was mixed with maize oil equivalent to 10 mg lycopene/kg body weight (BW) per \(d\)\(^{31,32}\) and given orally every morning for a 6-week period\(^{(33,34)}\). To avoid differences in the energy provided, all groups received the same maize oil volume (approximately 2 ml/kg BW per \(d\)). Rats were housed in individual cages in an animal facility at the Internal Medicine Experimental Laboratory, Botucatu Medical School, UNESP, under a controlled ambient temperature (22–26°C) and lighting (12 h light–12 h dark cycle) condition. Dietary consumption was measured daily, and BW was assessed weekly. The animals were killed by decapitation under deep sodium pentobarbital anaesthesia (50 mg/kg, intraperitoneal injection). Plasma and epididymal adipose tissues were collected at 12 weeks and stored at –80°C until ready for analysis. Epididymal adipose tissue was selected because of its similar inflammatory patterns in visceral fat\(^{(35)}\). The experiment was conducted in accordance with the Guidelines for the Care and Use of Experimental Animals and the diets followed the specifications from Nutrient Requirements of the Laboratory Rats. The protocol was approved by the local Ethical Committee for Animal Research (protocol no. 920-2012).

#### Lycopene preparation

Tomato oleoresin (Lyc-O-Mato 6% dewaxed; LycoRed Natural Products Industries) was mixed with maize oil and stored at 4°C in the dark until used as described previously\(^{(24)}\). The tomato oleoresin–maize oil mixture was stirred for 20 min in a water-bath at 54°C before being fed to the animals. Each millilitre of the solution contained 5 mg of total lycopene. Stability of lycopene was monitored at 450 nm, and confirmed by diode-array spectra, as described previously\(^{(26)}\). Lycopene

<table>
<thead>
<tr>
<th>Table 1. Nutritional composition of the diets</th>
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<tbody>
<tr>
<td>Components</td>
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<tr>
<td>Protein (%)</td>
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<tr>
<td>Carbohydrate (%)</td>
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<tr>
<td>Fat (%)</td>
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<tr>
<td>% Energy from protein</td>
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<tr>
<td>% Energy from carbohydrate</td>
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<td>% Energy from fat</td>
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<tr>
<td>% Energy from saturated fat</td>
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<tr>
<td>% Energy from unsaturated fat</td>
</tr>
<tr>
<td>Energy (kcal/g)</td>
</tr>
<tr>
<td>Energy (kJ/g)</td>
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<tr>
<td>Fatty acid composition (%)</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
</tr>
<tr>
<td>Oleic (18:1n-9c)</td>
</tr>
<tr>
<td>Linoleic (18:2n-6)</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Vitamin/mineral mixture†</td>
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</tbody>
</table>

* Energy from sugar in the drinking water (300 g/l) was not included.
† Based on the vitamin/mineral amounts of the chow diet, for each kg of the hyperenergetic diet, the following nutrients were added: Fe, 25·2 mg; K, 104·8 mg; Se, 7·31 μg; molybdenum sulphate, 150·0 μg; vitamin B12, 3·45 μg; vitamin B6, 6·8 mg; biotin, 0·12 mg; vitamin E, 32·6 mg; vitamin D, 61·2 μg; vitamin A, 4·6 mg.
was stable in the tomato oleoresin–maize oil mixture for 9 weeks at −20°C.

**Total body fat**

Total body fat was measured as the sum of epididymal, retroperitoneal and visceral fat deposits, and was used to calculate the adiposity index<sup>37,38</sup> to confirm obesity in the animals.

**Plasma lycopene analysis**

A 400 μl aliquot of plasma was used for lycopene analysis, as described previously<sup>24</sup>. Briefly, plasma samples were extracted with 3 ml chloroform–methanol (2:1) followed by 3 ml hexane. The samples were dried under N₂ and resuspended in 100 μl ethanol, of which 25 μl were injected into the HPLC. The results were adjusted by an internal standard containing echinenone. The inter- (<sup>n</sup> 3) and intra-assay (<sup>n</sup> 8) CV was 9%. The recovery of the added internal standard was consistently >90%. All sample processing and analyses were performed under red light.

**Biochemical measurements**

Glucose concentration was assayed by using a glucometer (Accu-Chek Go Kit; Roche Diagnostic Brazil Limited). Hormonal concentrations of insulin, leptin, adiponectin (Millipore), resistin (Immuno-Biological Laboratories, Inc.), TNF-α and IL-6 (R&D Systems, Inc.) were measured by an immunoassay, using a microplate reader (Spectra Max 190; Molecular Devices). The glucose/insulin ratio was used for insulin sensitivity assessment<sup>39</sup>.

**Gene expression**

Total RNA was extracted from epididymal adipose tissue using the reagent TRIzol (Invitrogen). The SuperScript II First-Strand Synthesis System for RT-PCR (Invitrogen) kit was utilised for the synthesis of 20 μl of complementary DNA from 1000 ng of total RNA. The mRNA levels of leptin (assay Rn 00565158_m1; Applied Biosystems), resistin (assay Rn 00595224_m1; Applied Biosystems), TNF-α (assay Rn 00562055_m1; Applied Biosystems), IL-6 (assay Rn 01410330_m1; Applied Biosystems) and monocyte chemotactic protein-1 (MCP-1, assay Rn 00580555_m1; Applied Biosystems) were determined by real-time PCR. Quantitative measurements were made with a commercial kit (TaqMan qPCR; Applied Biosystems) in a detection system (StepOne Plus; Applied Biosystems). Cycling conditions were as follows: enzyme activation at 50°C for 2 min, denaturation at 95°C for 10 min, complementary DNA products were amplified for forty cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Gene expression was quantified in relation to the values of the C group after normalisation by an internal control (cyclophilin: assay Rn 00690933_m1; Applied Biosystems) by the method 2^-ΔΔC<sub>T</sub>, as described previously<sup>39</sup>.

**Statistical analysis**

Results are expressed as means and standard deviations, and significance of differences were calculated by one-way ANOVA followed by Tukey’s post hoc test, using SigmaStat version 3.5 for Windows (Systat Software, Inc.). Differences were considered significant at P<0·05. Power calculations for the main outcome variables were above 80%.

**Results**

**Body weight and body fat**

Food intake was reduced in the DIO groups; however, energy intake was similar among the groups. The animals showed the same BW at baseline. At the end of the experiment, the hyperenergetic-fed animals showed a significant BW and adiposity index when compared with control rats. In comparison with the DIO group (Table 2), consumption of the lycopene-containing maize oil mixture (DIO + L) did not interfere with BW and the adiposity index (Table 2).

**Lycopene uptake and absorption**

In the present study, lycopene was analysed as the total of both cis and all-trans isomers in plasma. Due to the lack of

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>DIO</th>
<th>DIO + L</th>
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<tbody>
<tr>
<td>Initial BW (g)</td>
<td>341&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27</td>
<td>350&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final BW (g)</td>
<td>489&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58</td>
<td>579&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Food intake (g/100 g BW per d)</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water intake (ml/100 g BW per d)</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
<td>7.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Energy intake (kJ/100 g BW per d)</td>
<td>23.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2</td>
<td>22.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Energy intake (kJ/100 g BW per d)*</td>
<td>98.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0</td>
<td>94.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adiposity index (%)</td>
<td>5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2</td>
<td>9.5&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

C, control; DIO, animals subjected to diet-induced obesity; DIO + L, DIO supplemented with lycopene for 6 weeks.

<sup>a,b</sup>Mean values with unlike superscript letters were significantly different (P<0·05; one-way ANOVA with Tukey’s post hoc test).

<sup>*</sup>Energy intake includes energy from sugar in the drinking water.
lycopene in the fed diets\(^{(24)}\), there was no detectable lycopene in the plasma of the C or DIO groups. However, after 6 weeks of carotenoid supplementation, lycopene plasma concentrations were evident in the DIO + L group (Table 3).

**Insulin sensitivity**

The hyperenergetic diet was associated with a significant increase in glucose and insulin levels when compared with the C animals, showing reduced insulin sensitivity. Glucose and insulin levels were not modulated by lycopene supplementation. Also, lycopene showed no effect on insulin sensitivity (Table 3).

**Adipokine levels**

The consumption of the hyperenergetic diet was associated with a significant increase in inflammatory marker expression, such as \(\text{IL-6, MCP-1}\) and leptin. However, resistin and TNF-\(\alpha\) expression in epididymal adipose tissue showed no difference between the C and DIO groups. Lycopene supplementation restored the gene expressions of \(\text{IL-6, MCP-1}\) and leptin to the C levels, while a decreased resistin gene expression in epididymal adipose tissue (Table 4). The plasma levels of TNF-\(\alpha\), IL-6, leptin and resistin were found to be significantly elevated in the DIO group. Lycopene supplementation significantly decreased leptin levels, and restored the plasma concentrations of IL-6 and resistin to the C levels. There was no difference between the DIO and DIO + L groups in plasma TNF-\(\alpha\) concentrations (Table 3). As a recent inflammatory biomarker\(^{(40)}\), the leptin:adiponectin ratio was calculated. The DIO group showed an increase in the leptin:adiponectin ratio (C: 0.21 (SD 0.07) v. DIO: 1.11 (SD 0.26), \(P<0.001\)), while the lycopene-supplemented group presented a lower ratio (DIO: 1.11 (SD 0.26) v. DIO + L: 0.56 (SD 0.11), \(P<0.001\)).

**Discussion**

Obesity is usually associated with the consumption of hyperenergetic diets and a decrease in energy expenditure\(^{(2)}\), resulting in the expansion of the adipose tissue mass and inducing a chronic inflammatory state\(^{(59)}\). A high SFA intake from a hyperenergetic diet has been associated with obesity-linked inflammation, and induces inflammation-related gene expression in adipose tissue\(^{(41)}\). In the present study, lycopene displayed anti-inflammatory effects\(^{(25,28)}\) in plasma (0.01–0.04 \(\mu\)M) after 6 weeks of supplementation in concentrations below the range that can be normally seen in human subjects (0.2–0.9 \(\mu\)M)\(^{(42)}\). This can be related mainly to low lycopene bioavailability\(^{(42)}\), and also in part to the animals fasting overnight. A previous study has found that a peak accumulation of lycopene in rat plasma occurs between 4 and 8 h after a single oral administration\(^{(43)}\).

Experimental high-fat diet models are considered appropriate to study obesity and its consequences\(^{(44)}\). In addition to BW gain, the present experimental model induced an increase in body adiposity from the DIO animals (DIO and DIO + L\(^{(45,46)}\)). As with our recent study\(^{(57)}\), animals from the DIO groups consumed smaller dietary amounts and similar energy intakes than those without the treatment (C group; Table 2). Even though the protocol devised for the present study was able to induce obesity, it showed that body adiposity is the best indicator of obesity\(^{(47)}\). The expansion in weight, and especially body fat, occurs because the augmentation of fat consumption is not accompanied by a proportional increase of fat oxidation. This leads to the deposit of fat as TAG\(^{(48,49)}\) in adipose tissue.

Many studies have demonstrated that white adipose tissue represents an important site of inflammation\(^{(1)}\), showing insulin resistance and direct associations between adipose tissue and concentrations of TNF-\(\alpha\), IL-6 and C-reactive protein\(^{(17)}\). Here, we demonstrate, \(\text{in vivo}\), that the DIO-treated group showed an increase in the epididymal adipose tissue gene expression of IL-6 and MCP-1 (Table 4) and in the plasma concentrations of IL-6 and TNF-\(\alpha\) (Table 3). Given that mRNA levels do not always represent the protein content, which exerts functional activity, plasma concentration is more relevant than mRNA levels. The divergent data between mRNA levels and plasma concentrations of TNF-\(\alpha\) could be explained in part by the stability and translational efficiency.

<table>
<thead>
<tr>
<th>Table 3. Plasma measurements of lycopene, adipokines, glucose, insulin and the glucose:insulin ratio (Mean values and standard deviations, (n=6))</th>
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<tbody>
<tr>
<td><strong>Groups</strong></td>
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<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td>Lycopene (nmol)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
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<tr>
<td>Resistin (ng/ml)</td>
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<tr>
<td>IL-6 (pg/ml)</td>
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<td>TNF-(\alpha) (pg/ml)</td>
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<td>Glucose (mg/l)</td>
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<tr>
<td>Insulin (ng/l)</td>
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<td>Glucose:insulin ((\times 10^{-6}))</td>
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\(^{a,b}\text{-Means with unlike superscript letters were significantly different (}\(P<0.05\); one-way ANOVA with Tukey’s post hoc test).\)
Finally, we demonstrated that the increased leptin:adiponectin ratio in the DIO group was significantly decreased by lycopene supplementation. This suggests inflammatory attenuation associated with lycopene treatment in obese rats. Given that obesity is recognised as a chronic and systemic inflammatory disease (56), the present data suggest that lycopene supplementation may attenuate the inflammatory response in obesity, at least in part, by minimising hyperleptinaemia and improving the leptin:adiponectin ratio. Resistant concentration has been reported to be increased in obesity (57,62), and to be a link to obesity and insulin resistance (63). The DIO animals did not show an increase in resistant gene expression in epididymal adipose tissue when compared with the C group (Table 4). However, resistant plasma concentration was greater in the DIO group than in the C group (Table 3). Increased resistant expression has been correlated with inflammatory markers, coronary artery disease (64) and atherosclerosis in patients with the metabolic syndrome (65). Furthermore, resistant itself has been found to induce the expression of cytokines and chemokines in human articular chondrocytes (66). Also, in patients with gestational diabetes, elevations in serum resistant were correlated with serum IL-6 levels, but not with insulin levels. This suggests that changes in insulin sensitivity in these patients were mediated by inflammatory pathways that may involve resistant (67). Both gene expression and plasma resistant levels were decreased with lycopene supplementation, which has been shown to display anti-inflammatory effects (25,26), suggesting less inflammation in adipose tissue. The exact mechanism of lycopene affecting leptin and resistant levels remains to be determined. To the best of our knowledge, this is the first study which shows that lycopene can modulate both leptin and resistant gene expression and plasma concentrations in obese rats.

In summary, it was observed that lycopene has the ability to down-regulate adipokine mRNA levels in epididymal adipose tissue, such as leptin, resistant, IL-6 and MCP-1, along with the ability to restore leptin, resistant and plasma IL-6 concentrations in diet-induced obese rats. Therefore, it is highly probable that lycopene supplementation attenuates inflammation levels in adipose tissue. This could evidence the health effects of this carotenoid. This is the first time that lycopene has been shown to modulate leptin and resistant levels. Therefore, dietary lycopene may be proposed as an effective strategy to reduce inflammation in diet-induced obesity. Although the adopted experimental design did mimic the clinical situation, it gives no information regarding as to whether these findings are applicable to human subjects or not. However, it addresses some important benefits by using additional non-pharmacological therapy that is based on natural compounds in the treatment of human obesity. Moreover, the present study represents a contribution to the role of lycopene on inflammation related to obesity.

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