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**Lycopene and tomato powder supplementation similarly inhibit high-fat diet induced obesity, inflammatory response and associated metabolic disorders.**

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**Keywords: lycopene, tomato powder, inflammation, obesity, metabolic disease, adipose tissue, carotenoids.**

## **Abstract**

**Scope:** Several studies have linked the high intake of lycopene or tomatoes products with lower risk for metabolic diseases. The aim of the present study was to evaluate and to compare the effect of lycopene and tomato powder on obesity associated disorders.

**Methods and results:** Male C57BL/J6 mice were assigned into 4 groups to receive: control diet (CD), high fat diet (HFD), high fat diet supplemented with lycopene or with tomato powder (TP) for 12 weeks. In HFD condition, lycopene and TP supplementation significantly reduced adiposity index, organ and relative organ weights, serum triglycerides, free fatty acids, 8-iso-prostaglandin GF2 $\alpha$  and improved glucose homeostasis, but did not affect total body weight. Lycopene and TP supplementation prevented HFD-induced hepatosteatosis and hypertrophy of adipocytes. Lycopene and TP decreased HFD-induced pro-inflammatory cytokine mRNA expression in the liver and in the epididymal adipose tissue. The anti-inflammatory effect of lycopene and TP was related to a reduction in the phosphorylation levels of I $\kappa$ B, and p65, and resulted in a decrease of inflammatory proteins in adipose tissue

**Conclusion:** These results suggest that lycopene or TP supplementation display similar beneficial health effects that could be particularly relevant in the context of nutritional approaches to fight obesity-associated pathologies.

## **Introduction**

Obesity is characterized by a state of chronic low grade inflammation that is closely associated with the pathogenesis of obesity-related diseases, such as atherosclerosis, hypertension, and insulin resistance leading to type 2 diabetes [1]. This low-grade inflammatory state is notably related to the activation of inflammatory pathways (JNK and NF- $\kappa$ B) in adipose tissue [1] and increased production of cytokines such as interleukin 6 (IL-6), interleukin 1beta (IL-1 $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), or various chemokines [2], including monocyte chemotactic protein 1 (MCP-1) and other mediators of the immune response [1].

Lycopene is a lipophilic non-provitamin A carotenoid which is responsible for the red color in various fruits and vegetables such as tomato, water melon, guava, grapefruits [3]. This carotenoid is well known for its antioxidant properties [4]. In humans, lycopene is mainly stored in adipose tissue, where it represents more than half of total carotenoid concentration [5, 6]. The uptake of lycopene by adipocytes and adipose tissue is mediated at least in part by CD36 [7] and is not related to its physicochemical properties [8]. Moreover, high intake of lycopene is associated with low waist circumferences, as well as low visceral and subcutaneous fat masses as reported by Sluijs et al, suggesting that lycopene could actively impact adipocyte physiology [9]. Several studies showed that lycopene have been reported to display anti-inflammatory effects in adipocytes and adipose tissue; [10-12] [13] and liver [14, 15]. Among others, these effects could be responsible of the numerous health effect attributed to lycopene [16, 17] in the field of CVD [18], adiposity and obesity [19].

Tomatoes are a valuable source of many micronutrients including lycopene, which is classically associated to the health benefits of tomato consumption [20]. However, in the field of obesity and associated disorders, despite the beneficial effect of lycopene has already been described [12], the effect of TP consumption and the comparison between purified lycopene

and TP has never been reported. Thus, the aim of present study was to investigate and to compare the effects of purified lycopene and or tomato powder (TP) to exhibit prevention/reduction of high fat diet (HFD) induced global changes.

## Materials and methods

**Animals and dietary supplementation** - The care and use of mice were in accordance with the French guidelines and approved by the experimental animal ethic local committee. Eight weeks old male C57BL/6J mice were obtained from Janvier (Le Genest Saint Isle, France) and were acclimatized to the experimental facility for 1 week. The mice were kept in a temperature and humidity controlled room and fed with water and food ad libitum. The mice were randomly divided into four groups, as follows: 1. CD: n=10, control group given a control diet, 2. HFD: n=10, given a high-fat diet (DIO Rodent Purified Diet with 45 % Energy from Fat, 58V8, TestDiet). 3. HFD+lycopene: n=10, mice given a high fat diet supplemented with lycopene and (4) HFD+TP: n =10 given a high fat diet supplemented with tomato powder for 12 weeks. Purified lycopene (Oversal, 10% lycopene beadlets, Naturex, Avignon, France) and TP (Tomato 404, Naturex, Avignon, France; [lycopene concentration: 214 mg/kg of dry tomato powder](#)) were generously provided by NATUREX. The dose used for lycopene was 10 mg/kg food per day. The quantity of TP was calculated to provide the same amount of lycopene (10mg/kg food/day). For all protocols, diets were made once a week, blended to ensure adequate distribution of the supplement and stored in opaque bottles at 4°C. During the experiment, animal growth was monitored via weekly weighing and dietary intake was assessed daily. At the end of treatment period, mice were fasted overnight and blood was collected by retro-orbital puncture under anesthesia. After sacrificing the animals, liver, muscle, epididymal, and perirenal adipose depots were collected then weighted and snap frozen in liquid nitrogen and stored at -80°C until use.

**Biochemical analysis** - Blood samples were obtained after 12h of overnight fasting, then centrifuged (3500 rpm, for 15min, 4°C), serum total cholesterol(TC), triglyceride (TG) and glucose were determined by enzymatic methods (Spinreact, Esteve De Bas, Spain), non

esterified fatty acids (NEFA) were measured by colorimetric method (RANDOX, Crumlin, Co. Antrim, United Kingdom). Insulin was measured using an enzyme-linked immunosorbent assay ELISA (ALPCO Diagnostics, New Hampshire, United States) and 8-iso-prostaglandin  $F_{2\alpha}$  (8-iso-PGF $_{2\alpha}$ ) known as an oxidative stress marker was quantified using the ELISA kit (ENZO Life Sciences, Farmingdale, USA) according to the manufacturer's instructions. The HOMA-IR index was calculated according to the following formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5.

**Histological analysis** - Liver and epididymal adipose tissue samples were fixed in 10% buffered formalin, embedded in paraffin and sliced to prepare 5  $\mu\text{m}$  tissue sections whose were stained with hematoxylin and eosin (H&E) [21] the images were captured by a light microscope (EZAD, Leica, Germany). Steatosis was quantified as a grading system based on the percentage of liver section that is occupied by fat vacuoles was used. The grading system is defined as follows: grade 0 < 6%; grade 1 = 6 – 33%; grade 2 = 33 – 66%, and grade 3 > 66% as reported [22]. The number of lipid droplet in liver and adipocyte area ( $\mu\text{m}^2$ ) were determined using (Image J) software.

**RNA isolation and qPCR** - Total cellular RNA from liver and epididymal adipose tissue was extracted using TRIzol reagent according to the manufacturer's instructions. The cDNA was synthesized from 1  $\mu\text{g}$  of total RNA in 20  $\mu\text{l}$  using random primers and Moloney murine leukemia virus reverse transcriptase. Real Time Quantitative RT-PCR analyses were performed using the Mx3005P Real-Time PCR System (Stratagene, La Jolla, CA) as previously described [23]. For each condition, expression was quantified in duplicate, and 18S rRNA was used as the endogenous control in the comparative cycle threshold (CT) method [24].

**Cytokines and Chemokines quantification in adipose tissue** - Tissue homogenates were prepared from 25 mg of epididymal adipose tissue homogenized in 250  $\mu\text{L}$  of buffer

( $\text{KH}_2\text{PO}_4$ , 100 mM,  $\text{NaH}_2\text{PO}_4$ , 50 mM, pH 7). Protein degradation is inhibited by adding protease inhibitors (Complete cocktail, Roche, Basel, Swiss) to the buffer (9:1, V: V). After centrifugation at 13000 g at 4 ° C for 30 minutes the aqueous phase was separated from the pellet and the lipid phase. The secretion of CCL2, CCL5,  $\text{TNF}\alpha$ , and IL-6 were quantified in the aqueous phase by ELISA, using Ready-SET-Go mouse kits ( $\text{TNF}\alpha$  and IL-6 eBiosciences SAS, Paris, France) and (CCL2, CCL5 DuoSet ELISA, R&D systems, Paris, France).

**NF- $\kappa$ B activation** - To examine the involvement of NF- $\kappa$ B signaling pathway The levels of p65 (Ser) and I $\kappa$ B $\alpha$  (Ser32/36) phosphorylation were quantified in adipose tissue homogenates using the ELISA Instant One Kit according to the manufacturer's instructions (eBiosciences SAS, Paris, France), as previously described [25]

**Statistical analysis** - Data are expressed as the mean  $\pm$  SEM. Significant differences between control and treated groups were determined using ANOVA, followed by the PLSD Fischer post hoc test using Statview software. Values of  $p < 0.05$  were considered significant.



## Results

### **Lycopene or tomato powder supplementation prevents alterations in body composition mediated by HFD and improves metabolic parameters.**

To evaluate the phenotypic effect of lycopene or TP supplementation, C57BL/6J mice were fed a CD, HFD, supplemented with lycopene or TP for 12 weeks. As shown in Figure 1A, mice consuming HFD have significant progressive body weight increase, compared to mice in the CD group ( $p < 0.05$ ), and supplementation with lycopene or TP has no effect on the mice body mass compared to HFD fed mice. It is noteworthy that energy intake in HFD fed mice was higher compared to CD fed mice where no significant difference of energy intake was observed between HFD fed mice and HFD supplemented with lycopene or TP (Figure 1B). Our results also showed that HFD consumption significantly increased liver, epididymal and peri-renal adipose tissue (both absolute and relative mass) but had no effect on peri-renal adipose tissue weight (Figure 1C). Compared to HFD group, the supplementation with lycopene decreased significantly the weight and relative weight of liver, epididymal adipose tissue, whereas the supplementation with TP decreased significantly the weight and relative weight of epididymal adipose tissue ( $p < 0.05$ ) and has no effect on liver and peri-renal adipose tissue (Figure 1D and 1E). The calculated adiposity index (sum of epididymal, peri-renal adipose tissue mass relative to total body mass) increased by 85% in the HFD group compared to the CD group, and it was significantly reduced by supplementation with lycopene or TP (27% and 34%, respectively; Figure 1F).

In order to study more deeply the effect of the different diets, several biochemical and antioxidant parameters were measured. As reported in Table 1, HFD fed mice displayed an increase in serum of total cholesterol (TC), triglycerides (TG), non-esterified fatty acids (NEFA) and 8-iso-PGF<sub>2</sub> $\alpha$  concentrations in blood (23%, 14%, 45% and 54% respectively)

compared to CD fed mice. Supplementation with lycopene or TP strongly decreased the TG concentration ( $p < 0.01$  and  $p < 0.05$  respectively) as well as 8-iso-PGF $_{2\alpha}$  concentration ( $p < 0.01$  and  $p < 0.001$  respectively) but had no effect on TC levels. The NEFA concentrations were significantly decreased by supplementation with lycopene ( $p < 0.01$ ) and didn't change with TP supplementation. We next evaluated the effect of lycopene and TP supplementation on glucose homeostasis by quantifying fasting glycemia and insulinemia. As expected, these parameters were strongly increased in HFD fed mice compared to CD fed mice (35% for glycemia and 26% for insulinemia), and significantly decreased in lycopene ( $p < 0.0001$ ) and TP ( $p < 0.01$ ) supplemented mice. Consequently, HOMA-IR index was increased in HFD fed mice ( $5.42 \pm 0.39$ ) compared to CD fed mice ( $2.13 \pm 0.03$ ) and decreased with lycopene and TP supplemented mice ( $3.27 \pm 0.19$  and  $3.47 \pm 0.43$  respectively; Table 1).

### **Lycopene or tomato powder supplementation improves hepatic steatosis and pro-inflammatory genes expression.**

The effects of lycopene and TP supplementation on hepatic steatosis in C57BL/6J mice were examined at histological level by (H&E) staining. Our results showed that HFD fed mice exhibited hepatic steatosis grade 2 compared to CD fed mice (Figure 2A). Lycopene or TP supplementation effectively ameliorated the hepatic steatosis from grade 2 to grade 1, (number of lipid droplet: 6 - 33%) in a significant way ( $p < 0.001$ ) (Figure 2B). To examine the effect of lycopene or TP supplementation on hepatic lipid metabolism, hepatic genes expression analysis was undertaken (Figure 2C). Genes coding for enzymes (acetyl-CoA carboxylase 1 (Acaca), fatty acid synthase (Fasn) and the transcriptional regulator (sterol-regulatory element binding protein 1c (Srebp-1c)) involved in hepatic de novo lipogenesis were quantified. The mRNA levels of Acaca, Fasn and Srebp-1c were significantly increased in HFD fed mice, whereas the supplementation with lycopene or TP led to a significant

decrease in the expression of *Acaca*, *Fasn* and *Srebp-1c*. Furthermore, the expression of genes involved in the oxidation of fatty acids (Carnitine palmitoyl transferase (*Cpt-1*), Acetyl CoA oxidase (*Acox*) and the transcriptional regulator peroxisome proliferator activated protein  $\alpha$  (*Ppara*) was quantified. *Cpt-1*, *Acox* and *Ppara* mRNA levels were upregulated in HFD-fed animals as compared with CD, and were further upregulated by supplementation with lycopene and TP (Figure 2C). To evaluate the effect of lycopene and TP supplementation on inflammation of liver, we analyzed the mRNA expression of different markers of inflammation such as tumoral necrosis factor alpha (*Tnfa*), monocyte chemoattractant protein 1 (*Mcp-1*) and interleukin 6 (*Il-6*). As expected, HFD induced a strong increase of *Tnfa* and *Mcp-1* mRNA levels (50 % and 51 % respectively) compared to the CD. In addition, supplementation with lycopene decreased significantly the expression of *Tnfa* ( $p < 0.002$ ) but had no effect on *Mcp-1* mRNA expression, whereas TP supplementation decreased significantly mRNA levels of *Mcp-1* ( $p < 0.05$ ) but had no effect on *Tnfa* expression (Figure 2C). No impact of lycopene or TP supplementation was observed on *Il-6* expression (data not shown).

**Lycopene or tomato powder supplementation modifies lipid metabolism and decreases HFD induced proinflammatory cytokine and chemokine expression in adipose tissue.**

To evaluate the effect of lycopene or TP supplementation on the phenotype of adipose tissue, epididymal adipose tissue was used for histological analysis. HFD promoted adipocyte hypertrophy with a significant increase of adipocyte area (Figure 3A and 3B) compared to CD group. Interestingly, this effect was prevented by lycopene or TP supplementation (Figure 3A and 3B). Furthermore, *Acaca* and *Fasn* mRNA levels were increased in HFD-fed group (52 % and 78 % respectively) as compared with CD group (Figure 3C). Lycopene supplementation significantly prevented these upregulations, however only TP improved significantly the

Acaca increase but had no effect on FAS mRNA expression. The effects of lycopene or TP supplementation was also evaluated by measuring the mRNA level of peroxisome proliferator-activated receptor- $\gamma$  (Pparg), a major transcription factor involved in lipid metabolism. Our results indicated that the expression of Pparg was significantly increased by 85 % in mice receiving HFD relative to the CD group whilst lycopene or TP supplementation led to a significant decrease of Pparg mRNA levels (Figure 3C). The expression of several Pparg target genes were evaluated, including cluster of differentiation (Cd36), adipocyte fatty acid-binding protein (aP2) and lipoprotein lipase (Lpl). Their expression was significantly induced by HFD (85 %, 83 % and 89 % respectively) and reduced by lycopene or TP supplementation. Expression of Acox was significantly increased in HFD fed group but was not modified by lycopene or TP supplementation (Figure 3C)

To evaluate the effect of lycopene and TP on inflammation in adipose tissue, the expression of different markers of inflammation such as cytokines (Il-6, Tnf $\alpha$ , Mcp-1), chemokines (Chemokine C-C motif ligand 5 (Ccl5), chemokine C-X-C ligand motif 10 (Cxcl10)) acute-phase proteins (haptoglobin, serum amyloid A 3(Saa3)), adipokines (resistin, visfatin, leptin) and matrix metalloproteinases 3 (Mmp3) and 9 (Mmp9) was measured. As expected, the HFD induced a strong increase of Il-6, Tnf $\alpha$ , Mcp1, Ccl5, Cxcl10, Saa3, leptin, haptoglobin, resistin, visfatin, Mmp3 and Mmp9 mRNA compared to the CD. (Table2). Interestingly, lycopene or TP supplementation decreased the expression of most inflammatory markers, however the expression of Tnf $\alpha$  and Cxcl10 was not affected (Table2). The expression of Ccl5 decreased significantly with TP supplementation but was not modified with lycopene. In contrast mRNA levels of visfatin decreased strongly with lycopene supplementation and was not modified by TP.

The expression of anti-inflammatory genes such as Il-10 and transforming growth factor  $\beta$  (Tgf- $\beta$ ) was also evaluated. Interestingly, lycopene and TP supplementation strongly increased

the expression of Il-10 (64 % and 78% respectively) and Tgf- $\beta$  (56 %, 30 %) respectively (Table 2).

To confirm the anti-inflammatory effect of lycopene or TP supplementation at the protein levels, epididymal adipose tissue homogenates were prepared to quantify pro-inflammatory cytokines. An increase of IL-6, TNF $\alpha$ , CCL2 and CCL5 protein levels was observed in the tissue homogenates from HFD mice compared to CD. As reported at the mRNA level, lycopene or TP supplementation decreased the amount of TNF $\alpha$ , IL-6, CCL2 and CCL5 proteins in adipose tissue ( $p < 0.001$ ) (Figure. 4C, 4D, 4E, 4F).

#### **Lycopene and tomato powder limit NF- $\kappa$ B activation in epididymal adipose tissue.**

NF- $\kappa$ B is a well-known transcription factor involved in the regulation of proinflammatory cytokines and chemokines [26]. Thus, we examined the involvement of NF- $\kappa$ B signaling pathway in our adipose tissue inflammatory model and the ability of lycopene and TP to modulate them. To this purpose, the phosphorylation of p65 and I $\kappa$ B were quantified through ELISA. Expectedly, the phosphorylation levels of p65 and I $\kappa$ B were significantly increased with HFD compared to CD whereas HFD + lycopene and HFD + TP strongly limited the phosphorylation of p65 and I $\kappa$ B, suggesting that lycopene and TP reduced NF- $\kappa$ B activation in epididymal adipose tissue (Figure 4A and 4B).

## Discussion

In the present study, we used a model of obesity induced by a HFD to examine the metabolic effect of lycopene or TP supplementation. In addition, we aimed at comparing the effect of purified lycopene vs. TP, which contained lycopene included in a natural matrix (TP) on metabolic parameters. As expected, the HFD diet led to a significant increase in the total mass and adiposity index of mice, modified lipidic and glucidic serum parameters, as well as cell morphology and gene expression in adipose tissue and in the liver. Interestingly, lycopene or TP supplementation improved most of these phenotypical perturbations except total body weight, which was not modified. Similar lack of effect of lycopene supplementation on body weight has already been reported in rats [13], contrarily to the recently reported data in mice [12]. The origin of this discrepancy is presently unresolved and could be due to species or to genetic discrepancies between animal models used.

Even if total body mass was not affected by lycopene or TP supplementation, HFD mediated adiposity was clearly improved, as reported by Singh et al. [12], as well as several related parameters including lipidic (TG and NEFA) and glucidic parameters (glycemia, insulinemia and HOMA-IR). Most of these results are in agreement with the literature that suggests a role of lycopene or tomato product on various biochemical parameters such as lipemia [27], or glucose homeostasis [28]. In addition, we also reported a decrease of circulating 8-iso-PGF 2 $\alpha$ , a well-established marker of oxidative stress, which is in agreement with the antioxidant effect of lycopene or TP supplementation [29] and the subsequent protective effect against cell oxidative damages associated to obesity [30].

We next investigated the effect of lycopene or TP supplementation on hepatosteatosis and lipid metabolism in liver. Hepatosteatosis is defined as the presence of diffuse infiltration in

the liver and is characterized by ballooning hepatocytes injury and inflammation in the hepatocytes [31, 32]. Our results showed that supplementation with lycopene or TP for 12 weeks ameliorated the hepatic steatosis induced by HFD, which is consistent with several studies that demonstrated the amelioration of hepatosteatosis under lycopene or tomato products effect [14, 15, 33-36] [37]. The protective effects of lycopene and TP in hepatosteatosis may be related to multiple mechanisms [15, 34], including anti-oxidant/anti-inflammatory effects [38, 39] and lipid metabolism modulation [34, 40-42]. In agreement, we reported an anti-inflammatory effect of lycopene or TP in the liver but also an impact on lipid metabolism. Based on our gene expression analysis, we postulated that lycopene or TP supplementation affected both lipogenesis and  $\beta$ -oxidation. Indeed, a strong down-regulation of the Srebp-1c expression was observed under lycopene or TP supplementation, as well as a reduction of Fasn expression compared to HFD condition. SREBP-1c is a membrane-bound transcription factor that regulates key genes involved in the regulation of lipid metabolism [43]. The role of SREBP-1c in lipogenesis has been demonstrated in mice overexpressing the active form of this factor in the liver. These mice developed massive hepatic steatosis due to activation the expression of the lipogenic genes [44], including Fasn gene which encode for a multi-enzyme that catalyses fatty acid synthesis [45]. In addition, lycopene or TP supplementation upregulated genes involved in fatty acids (FA)  $\beta$ -oxidation. Indeed, Cpt-1 and Acox were induced as well as Ppara. It is noteworthy that these 3 genes encode for major actors of the beta-oxidation pathway [43]. PPAR $\alpha$  is considered as the master regulator of hepatic FA oxidation [46], CPT-1 facilitates FA translocation into mitochondria [43] and ACOX catalyses the peroxisomal FA oxidation [47]. Altogether, our results support the idea that hepatosteatosis was reduced in our condition via a limitation of lipogenesis associated to an improvement of FA  $\beta$ -oxidation resulting in a limitation of lipid accumulation in the liver as suggested by histological data. Similar results have already been reported with apo-10-

lycopenoic acid, a lycopene metabolite that counteracts the hepatic steatosis elicited by a HFD in C57BL/6J mice via a Sirt1-mediated suppression lipogenesis and stimulation of lipid catabolism [34].

We investigated the effect of lycopene or TP supplementation on epididymal adipose tissue. First, we observed that the HFD-mediated adipocyte hypertrophy (quantified from histological analysis) was prevented by supplementation with lycopene or TP. Concerning the effect of lycopene, this observation is in agreement with Singh et al. who demonstrated that oral supplementation with lycopene prevents adipocyte hypertrophy in mice [12]. In addition, we demonstrated that the impact on adipose tissue cellularity was associated with modifications of expression of genes coding for protein involved in lipogenesis and lipid metabolism. Indeed, lycopene or TP supplementation reduced the *Fasn* and *Acaca* compared to HFD condition. These supplementations also reduced *Pparg* mRNA expression as well as many target genes of PPAR $\gamma$  such as *aP2*, *Cd36*, *Lpl*. Interestingly, since PPAR $\gamma$  is considered as the master regulator of adipogenesis, its reduced expression and the down-regulation of its target genes likely explain the reduced adiposity of mice upon lycopene or TP supplementation.

We also investigated the impact of lycopene and TP supplementation on adipose tissue inflammatory status, and we reported an overall down-regulation of genes encoding pro-inflammatory markers such as cytokines (IL-6, Tnf $\alpha$ ), adipokines (resistin, visfatin, leptin), acute phase proteins (*Saa3*, haptoglobin) and chemokines (*Ccl5*, *Mcp-1*, *Cxcl10*), a down-regulation of genes encoding metalloproteinases (*Mmp3* and *Mmp9*), and an up-regulation of genes encoding anti-inflammatory proteins such as *Il10* and Tgf- $\beta$ . In addition, we confirmed at protein level that some of these pro-inflammatory markers (IL-6, TNF $\alpha$ , MCP-1, CCL5) were reduced compared to HFD conditions. Most of these genes are deeply involved in the



genesis of obesity associated pathologies, such as insulin resistance and type II diabetes. Indeed, IL-6 and TNF $\alpha$  are major obesity-related pro-inflammatory cytokines [48, 49], and chemokines (MCP-1, CCL5 or CXCL10) induce macrophage infiltration, which largely participates in the low-grade inflammation of adipose tissue [2, 50-52]. Anti-inflammatory cytokines are largely up-regulated in adipose tissue during obesity to compensate the massive production of pro-inflammatory mediators [53]. Acute phase proteins (SAA3 and haptoglobin) are also strongly related to metabolic inflammation [54]. Adipokines (leptin, resistin and visfatin) are known to be deregulated in obesity and have systemic impact on insulin sensitivity [55] and metalloproteinases participate to adipose tissue remodeling observed during obesity [56]. Altogether, the regulations depicted in the present article are clearly in favor of an improvement of inflammatory tone in adipose tissue and an improvement of adipose tissue function under lycopene or TP supplementation. Our results are fully consistent with previously reported anti-inflammatory effect of lycopene on adipose tissue, preadipocytes and adipocytes [10-13]. Interestingly an anti-inflammatory effect of TP on adipose tissue has never been reported yet, but similar results, i.e. decrease of pro-inflammatory adipokines, have already been reported after consumption of tomato juice in healthy women [57].

To identify the molecular mechanisms involved in this reduction of the inflammatory tone in adipose tissue, we evaluated the effect of lycopene and TP on the phosphorylation of p65 and I $\kappa$ B, two actors of the NF- $\kappa$ B signaling pathway [1, 58]. We observed that lycopene and TP strongly reduced the phosphorylation levels of p65 and I $\kappa$ B. These results are highly consistent with several other studies suggesting the potent effect of lycopene on inhibition of NF- $\kappa$ B signaling through multiple mechanisms [59, 60], and suggest that the anti-inflammatory effect of lycopene and TP on adipose tissue results from their ability to inhibit NF- $\kappa$ B signaling in adipose tissue. Despite this clear impact of lycopene or TP on NF- $\kappa$ B

signaling pathway, we cannot exclude that this effect is strictly due to the reduced adiposity, which could result in a deactivation of this signaling pathway. However, it is noteworthy that we already reported the *per se* anti-inflammatory effect of lycopene in adipocytes, preadipocytes [10] and macrophages [11], suggesting that both direct and indirect effects are probably responsible of the overall anti-inflammatory effect reported.

In the present study, similar effects were observed for lycopene and TP supplementation regarding improvement of metabolic parameters. These observations are noteworthy since in the literature the effect of tomato products (extract or powder) are globally more efficient to drive health effects at both epidemiological and preclinical level. This has notably been highlighted in the field of cancer [61], cardiovascular diseases [62], or hepatosteatosis [22]. Indeed, Stice et al. recently reported the superior protective effects of TP when compared to partial tomato extracts, and purified lycopene against alcohol-induced hepatic injury in rats, where only TP reduced severity of alcohol induced disorders [22]. However, the superior effect of tomato product is not the absolute rule since the effect of lycopene is sometime higher, notably in the management of the blood pressure [62].

As a conclusion, we show for the first time that TP supplementation was able to improve obesity-associated disorders, similarly to the lycopene effect that has already been depicted. Since similar amounts of lycopene were brought by TP and lycopene supplementation and resulted to similar effects, it strongly suggests that the TP supplementation effects were probably related to lycopene content. However, we cannot exclude that other components naturally found in tomatoes ( $\alpha$ - and  $\beta$ -carotene, lutein, zeaxanthin, phytoene, phytofluene, ascorbic acid, folate,  $\alpha$ -tocopherol, quercetin and other polyphenols) could be responsible via isolated, additive or synergic effects. Altogether, our data clearly highlight the potential

beneficial effect of TP as an effective whole food intervention against obesity associated comorbidities.

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#### **Authors contributions**

HH and JFL designed the experiments. SF, JA, LB, EK and FT performed the experiments and analyzed the results. SF and JFL wrote the manuscript.

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#### **Conflict of interest statement**

No conflict of interest has to be disclose.

#### **Legends for figures.**

**Figure1.** Effect of lycopene and TP on body composition. **A.** Body weight evolution curve. **B.** The energy intake was quantified by measuring food intake every day for a period of 12 week. **C.** Before each sacrifice, animal weight was established. **D.** During the sacrifice, organs and adipose tissues were weighed. **E.** Relative organs weight (organ weight / final body weight). **F.** An adiposity index was calculated by calculating the ratio between total fat mass and body

weight of animal. Values are presented as mean  $\pm$ S.E.M. Values not sharing the same letter are significantly different  $<0.05$ .

**Figure 2.** Effect of lycopene and TP supplementation on different parameters in liver. **A.** Representative histological images of liver tissue ~~from all groups of mice after~~ H&E stained, taken at 10X magnification (scale bar represent 200  $\mu$ m). CD: control diet (grade 0 of steatosis), HFD: high fat diet (grade 2 of steatosis), HFD + LYC, high fat diet supplemented with lycopene (grade 1 of steatosis), HFD + TP, high fat diet supplemented with tomato powder (grade 1 of steatosis). ~~Mice fed with HFD developed a high degree of steatosis, supplementation with lycopene and TP resulted in prevention of steatosis.~~ **B.** Number of accumulated lipid droplets in liver per liver area determined by Image J software. **C.** Relative expression of mRNA inflammatory genes and genes related to lipid metabolism measured through qPCR and expressed relative to 18S ribosomal RNA. Values are presented as mean  $\pm$ S.E.M. Values not sharing the same letter are significantly different,  $p < 0.05$ .

**Figure 3.** Effect of lycopene and TP supplementation in mice epididymal adipose tissue. **A.** Representative histological images of epididymal fat pads H&E stained, taken at 10X magnification (scale bar represents 200  $\mu$ m). CD: control diet, HFD: high fat diet, HFD+ LYC: high fat diet supplemented with lycopene, HFD+ TP: high fat diet supplemented with tomato powder. **B.** Adipocyte area, determined using Image J software. **C.** Relative expression of mRNA inflammatory genes and genes related to lipid metabolism measured through qPCR and expressed relative to 18S ribosomal RNA. Values are presented as mean  $\pm$ S.E.M. Values not sharing the same letter are significantly different  $<0.05$ .

**Figure 4.** Effects of LYC and TP on the phosphorylation levels of the NF- $\kappa$ B subunits (p65 and I $\kappa$ B) evaluated using ELISA. CCL2, CCL5, TNF $\alpha$ , and IL-6 secretion in epididymal adipose tissue was quantified using ELISA. Values are presented as mean  $\pm$ S.E.M. Values not sharing the same letter are significantly different,  $p < 0.05$ .

**Table 1.** Effect of lycopene and TP on metabolic parameters and glucose homeostasis

	CD	HFD	HFD+LYC	HFD+TP
<b>Total cholesterol (ng/dL)</b>	45.92±2.20 <sup>a</sup>	60.34±2.96 <sup>b</sup>	54.2±4.12 <sup>a,b</sup>	60.8 ±3.93 <sup>b</sup>
<b>Triglyceride (ng/dL)</b>	79.42±3.93 <sup>a</sup>	93.14±3.21 <sup>b</sup>	81.1±3.42 <sup>a</sup>	83.3 ±3.01 <sup>a</sup>
<b>Non esterified fatty acids (mmol/L)</b>	0.83 ±0.05 <sup>a</sup>	1.52±0.32 <sup>b</sup>	0.80±0.12 <sup>a</sup>	1.13±0.08 <sup>b</sup>
<b>8-iso-PGF2α (pg/mL)</b>	231.45±35.85 <sup>a</sup>	510 ±21.99 <sup>b</sup>	303 ±20.23 <sup>c</sup>	412 ±19.49 <sup>d</sup>
<b>Glycemia (mg/dL)</b>	102.9 ±32 <sup>a</sup>	158,1 ±56.08 <sup>b</sup>	129 ±41 <sup>c</sup>	137 ±43.47 <sup>c</sup>
<b>Insulinemia (ng/mL)</b>	0.37±0.01 <sup>a</sup>	0.50±0.03 <sup>b</sup>	0.14±0.02 <sup>a</sup>	0.42±0.04 <sup>a</sup>
<b>HOMA-IR index</b>	2.13±0.03 <sup>a</sup>	5.42±0.39 <sup>b</sup>	3.27±0.19 <sup>c</sup>	3.47±0.43 <sup>c</sup>

CD: control diet, HFD: high fat diet, HFD + LYC, high fat diet supplemented with lycopene, HFD + TP, high fat diet supplemented with tomato powder. HOMA-IR index was calculated according to the following formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5. Values are presented as mean ±S.E.M. Values not sharing the same letter are significantly different,  $P < 0.05$ .

**Table2.** Relative values of mRNA expression in the epididymal adipose tissue.

	CD	HFD	HFD+ LYC	HFD + TP
<b>IL-6</b>	100±18 <sup>a</sup>	394±50 <sup>b</sup>	244±35 <sup>c</sup>	185±22 <sup>d</sup>
<b>TNF<math>\alpha</math></b>	100±28 <sup>a</sup>	344±46 <sup>b</sup>	341±44 <sup>b</sup>	313±30 <sup>b</sup>
<b>MCP-1</b>	100±17 <sup>a</sup>	810±11 <sup>b</sup>	656±75 <sup>c</sup>	582±83 <sup>c</sup>
<b>CCL5</b>	100±30 <sup>a</sup>	530±64 <sup>b</sup>	441±57 <sup>b,c</sup>	382±74 <sup>c</sup>
<b>CXCL10</b>	100±20 <sup>a</sup>	210±42 <sup>b</sup>	176±21 <sup>b</sup>	171±37 <sup>b</sup>
<b>MMP3</b>	100±19 <sup>a</sup>	685±88 <sup>b</sup>	498±118 <sup>c</sup>	607±94 <sup>c</sup>
<b>MMP9</b>	100±12 <sup>a</sup>	185±40 <sup>b</sup>	84±10 <sup>c</sup>	107±18 <sup>c</sup>
<b>SAA3</b>	100±8 <sup>a</sup>	22369±9943 <sup>b</sup>	7449±2497 <sup>c</sup>	8258±2356 <sup>c</sup>
<b>Leptin</b>	100±23 <sup>a</sup>	3175±494 <sup>b</sup>	1765±194 <sup>c</sup>	1628±336 <sup>b</sup>
<b>Haptoglobin</b>	100±17 <sup>a</sup>	1521±316 <sup>b</sup>	594±85 <sup>c</sup>	921±174 <sup>d</sup>
<b>Resistin</b>	100±20 <sup>a</sup>	1273±256 <sup>b</sup>	363±79 <sup>c</sup>	668±119 <sup>d</sup>
<b>Visfatin</b>	100±19 <sup>a</sup>	213±38 <sup>b</sup>	147±14 <sup>c</sup>	203±28 <sup>b</sup>
<b>IL-10</b>	100±24 <sup>a</sup>	83±9 <sup>b</sup>	234±29 <sup>c</sup>	378±67 <sup>d</sup>
<b>TGF<math>\beta</math></b>	100±25 <sup>a</sup>	219±22 <sup>b</sup>	501±62 <sup>c</sup>	302±43 <sup>d</sup>

CD: control diet, HFD: high fat diet, HFD + LYC, high fat diet supplemented with lycopene, HFD + TP, high fat diet supplemented with tomato powder. Values are presented as mean  $\pm$ S.E.M. Values not sharing the same letter are significantly different,  $P < 0.05$ .