RESEARCH ARTICLE

Lycopene attenuates LPS-induced TNF-α secretion in macrophages and inflammatory markers in adipocytes exposed to macrophage-conditioned media

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Scope: Adipose tissue is infiltrated by an increasing number of macrophages during the development of obesity. These immune cells are suspected to be a major source of TNF-α that interferes with adipocyte function. Because lycopene possesses anti-inflammatory properties, we hypothesize that lycopene could reduce the production of TNF-α by macrophages and thus interfere in the cross-talk between macrophages and adipocytes.

Methods and results: We demonstrated that physiological concentrations of lycopene were able to attenuate the lipopolysaccharide (LPS)-mediated induction of TNF-α in RAW 264.7 macrophages, at both the mRNA and protein levels. The molecular mechanism was studied. It appeared that the LPS-activation of both JNK and NF-κB signaling pathways was modulated by lycopene. The anti-inflammatory effects of lycopene on macrophages were accompanied by a decrease in LPS-stimulated macrophage migration in the presence of lycopene. Furthermore, lycopene decreased macrophage conditioned medium-induced proinflammatory cytokine, acute phase protein, and chemokine mRNA expression in 3T3-L1 adipocytes.

Conclusion: These data indicate that lycopene displayed an anti-inflammatory effect on macrophages that beneficially impacted adipocyte function. Thus, these results suggest that lycopene could block the vicious cycle that occurs between adipocytes and macrophages in adipose tissue during obesity.

Keywords:
Adipocyte / Cytokines / Inflammation / Lycopene / Macrophage

1 Introduction

Lycopene is a non-provitamin A carotenoid present in tomatoes and tomato products. Other dietary sources of lycopene also include dried apricots, guava, watermelon, papaya, and pink grapefruit [1]. Like other carotenoids, lycopene is considered to be beneficial in the prevention of several diseases, including certain cancers, cardiovascular diseases, and eye diseases [2]. The effects of lycopene can be related, at least in part, to its anti-inflammatory effects. These effects have been demonstrated in several models of diseases linked to inflammation [3–9].

In humans, lycopene is mainly stored in adipose tissue, where it represents more than half of the total carotenoid concentration [10]. The uptake of lycopene by adipocytes and adipose tissue is mediated at least in part by CD36 [11]. In adipocytes, lycopene is not only stored in lipid droplets, it also can be detected in membranes [12]. The presence of lycopene in membranes suggests that lycopene could actively impact adipocyte physiology, as has been documented for β-carotene [13–15]. Moreover, the effect of lycopene on adipose tissue is also strongly suggested by the association found between high lycopene intake and low waist circumferences, as well as low visceral and subcutaneous fat masses as documented by Sluijs et al. [16]. Recently, we reported that lycopene and its metabolites also impact adipose tissue inflammatory status [7, 17]. Indeed, we demonstrated that lycopene was able to reduce the expression of several genes involved in the

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Abbreviations: IL, interleukins; **JNK**, c-Jun N-terminal kinase; **LPS**, lipopolysaccharide; **MCM**, macrophage-conditioned medium; **NF-kB**, nuclear factor-kappa B; **TNF-alpha**, Tumoral necrosis factor alpha

inflammation of adipose tissue. The NF-κB signaling pathway was demonstrated to be modulated by lycopene. These results provide a mechanistic explanation for the anti-inflammatory effects of lycopene.

It is now apparent that obesity is associated with 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 [18]. Recent studies have placed adipose tissue as a central contributor in the generation of this inflammatory state because of its role in secreting mediators such as inflammatory cytokines, chemokines, and acute-phase proteins. Adipose tissue is also a key site for the interaction of adipocytes with other inflammatory effectors of the immune system, including macrophages, T cells, and dendritic cells [18, 19]. During the development of obesity, adipose tissue is infiltrated by an increasing number of macrophages that are suspected of being a major source of inflammatory mediators, such as TNF-α, that interferes with adipocyte function [18, 20–22].

In this context, we evaluated the impact of lycopene on macrophage TNF-α expression, the molecular mechanisms involved, and the consequences in terms of macrophage migration as well as the effects of macrophage-conditioned media on the expression of inflammatory markers by adipocytes.

2 Materials and methods

2.1 Chemicals

(All-E)-lycopene extracted from tomatoes was kindly provided by Catherine Caris-Veyrat (INRA Avignon, France). Dexamethasone, isobutylmethylxanthine (IBMX), insulin, and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis, MO). The antibodies for cell signaling were purchased from Cell Signaling Technology (Ozyme, France).

2.2 Cell culture

Macrophage and adipocyte cells were grown at 37°C in a 5% CO_2 humidified atmosphere.

RAW 264.7 macrophages (ECACC, Salisbury, GB) were seeded in 12-well plates at a density of 3×10^4 cells/well and grown overnight in DMEM supplemented with 10% FBS, 2% HEPES, and 1% antibiotics. For the preparation of conditioned medium (CM), the cells were incubated for 24 h with 0.5, 1, or 2 μM of lycopene dissolved in tetrahydrofuran (THF) at 0.01%, as previously reported [12]. Thereafter, the cells were stimulated with LPS (1 ng/mL) for 1 h. The supernatants were then collected and stored at −80°C until use.

In addition, 3T3-L1 preadipocytes (ATCC, Manassas, VA) were seeded in 12-well plates at a density of 3×10^4 cells/well and grown in DMEM supplemented with 10% FBS as previously reported [23]. To induce differentiation, 2 days post-confluence, 3T3-L1 preadipocytes (day 0) were stimulated with 0.5 mM IBMX, 0.25 μM dexamethasone and 1 μg/mL insulin in DMEM supplemented with 10% FBS for 48 h. Thereafter, the cells were maintained in DMEM supplemented with 10% FBS and 1 μg/mL insulin. The media was replaced with fresh media every 2 days. All treatments were performed on day 8. The differentiated adipocytes were incubated in the macrophage-conditioned medium (MCM) for 24 h. The data are the mean of three independent experiments. Each experiment was performed in triplicate.

2.3 RNA isolation and qPCR

The total RNA was extracted from RAW 264.7 and 3T3-L1 cells using Trizol reagent according to the manufacturer's instructions. The first strand of cDNA was synthesized from 1 μg of total RNA using random primers and MMLV reverse transcriptase (Invitrogen, France). Real-time quantitative polymerase chain reaction analyses were performed using the Mx3005P Real-Time PCR System (Stratagene, La Jolla, CA) as previously described [24, 25]. For each condition, the expression was quantified in duplicate, and 18S ribosomal RNA was used as the endogenous control in the comparative cycle threshold (C_T) method.

2.4 Cytokine quantification

TNF-α and IL-6 in MCM were quantified with ELISA, using Ready-SET-Go reagents (e-Bioscience, Hatfield, UK).

2.5 Western blot

RAW 264.7 macrophages were pretreated with lycopene for 24 h and then stimulated with 1 ng/mL LPS for 15 min. The cells were washed twice with cold PBS, scraped, and collected in 100 μL of lysis buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 100 mM NaF, 1% Triton X-100, protease inhibitor cocktail; Boehringer Manheim, Germany). The whole cell lysates were incubated at 4°C for 45 min, followed by centrifugation (12 000 $g \times 15$ min, 4°C). The lysate protein concentrations were determined using a Bradford assay (Bio-Rad, France). The protein extracts (25 μg) were separated by SDS-PAGE, transferred to a PVDF membrane, and probed with specific antibodies against phosphorylated p38, JNK, and ERK. The proteins were visualized and quantified by fluorescence detection using the Odyssey infrared imaging system (LI-COR).

2.6 NF-κB transactivation assay

The NF-κB-dependent luciferase reporter plasmid containing the gene for luciferase under the control of four copies of the NF-κB response element (TGGGGATTCCCCA) [26] was

transfected in RAW 264.7 cells seeded in 6-well plates at a density of 7×10^4 cells/well. The transfection was performed using JetPrime (Invitrogen). After overnight incubation with the transfection mixes, the medium was replaced by DMEM supplemented with 10% FBS and 2 μM lycopene. The cells were treated for 24 h followed by 1-h incubation with LPS. The cells were lysed and assayed for luciferase activity using a luciferase assay system (Promega, Madison, WI), which was normalized to β-galactosidase activity as previously described [27]. The transfection experiments were performed in triplicate and repeated at least two times independently.

2.7 Macrophage migration assay

Migration assays were performed using cell culture inserts for 12-well plates with a 3 μm membrane pore size (BD Falcon, BD Biosciences, France). The RAW 264.7 macrophages were preincubated with or without lycopene for 24 h before chemotaxis experiments were performed using LPS (100 ng/mL). For the chemotaxis experiments, the cells were applied to each upper chamber. The lower chambers were filled with media without FBS in the presence or absence of LPS, and the plates were then incubated at 37°C for 4 h to initiate migration. Any non-migrated cells were wiped off with a cotton swab, and the cells adhering to the underside of the membrane were fixed with 2.5% glyceraldehyde for 15 min. The membranes were then washed with PBS and stained with 0.1% crystal-violet for 45 min. The number of migrated cells in five random microscopy fields per membrane was counted at 400× magnification.

2.8 Statistical analysis

Three separate cultures per treatment were utilized for analysis in each experiment. The data are expressed as means ± SEM. Statistical significance was determined by one-way ANOVA, followed by Fisher's PLSD test. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Lycopene inhibits expression of TNF-α in LPS-stimulated RAW 264.7 macrophages

To evaluate the effect of lycopene at physiological concentrations on TNF-α in macrophages, RAW 264.7 cells were preincubated with lycopene (from 0.5 to 2 μM, note that the incubation of macrophages with lycopene resulted in detectable quantities of lycopene within macrophages, data not shown) for 24 h and then incubated with LPS (1 ng/mL) for 1 h. These LPS-treatment conditions were established from dose-response effect experiments (data not shown) that clearly indicated that this concentration was sufficient to mimic the metabolic inflammation found during obesity [28]. Indeed, in RAW 264.7 cells, LPS increased the mRNA expression of TNF-α (approximately 8-fold), but also the expression of other proinflammatory cytokines such as IL-6 (approximately 4-fold) and IL-1β (approximately 30-fold). Preincubation with various concentrations of lycopene reduced the expression of these cytokines approximately 30–40% (Fig. 1). In the case of TNF-α, a tendency toward a dose-response effect was observed, whereas in the case of interleukins, no dose-response effect was observed.

3.2 Lycopene inhibits secretion of TNF-α in LPS-stimulated RAW 264.7 macrophages

As expected, LPS induced the secretion of TNF-α in macrophage culture medium. The preincubation of macrophages with lycopene significantly reduced the secretion of TNF-α as compared with LPS treatment alone (Fig. 2). The lycopene effect appeared to be dose-dependent. Surprisingly, LPS and lycopene preincubation had no effect on IL-6 secretion in our experimental conditions (data not shown).

3.3 Lycopene reduces the LPS-mediated activation of the JNK and NF-κB pathways in RAW 264.7 macrophages

To examine the involvement of MAP kinases (p38, JNK, and ERK1/2) in the regulation of the TNF-α, RAW 264.7 cells were preincubated with lycopene and then incubated for 15 min with LPS. The phosphorylation levels of p38, JNK, and ERK1/2 were evaluated by Western blot. No increase in the phosphorylation of ERK1/2 in response to LPS was observed (data not shown). In contrast, p38 and JNK displayed a strong increase in phosphorylation (Fig. 3A). The lycopene pretreatment did not modify the ERK1/2 and p38 phosphorylation level, whereas a slight but significant decrease of JNK phosphorylation was observed.

The involvement of the NF-κB signaling pathway was also studied in RAW 264.7 macrophages. For this purpose, cells were transiently transfected with a reporter plasmid containing the luciferase gene under the control of four NF-κB response elements. As expected, LPS (1 ng/mL, 1 h) induced an increase of luciferase gene expression, which was reversed by lycopene preincubation (2 μM, 24 h) (Fig. 3B). Altogether, these data suggest that lycopene is able to reduce the LPS-mediated activation of the JNK and NF-κB pathways in macrophages.

3.4 Lycopene limits the LPS-stimulated migration of RAW 264.7 macrophages

To examine the potential of lycopene to limit the motility of macrophages, RAW 264.7 cells were preincubated with

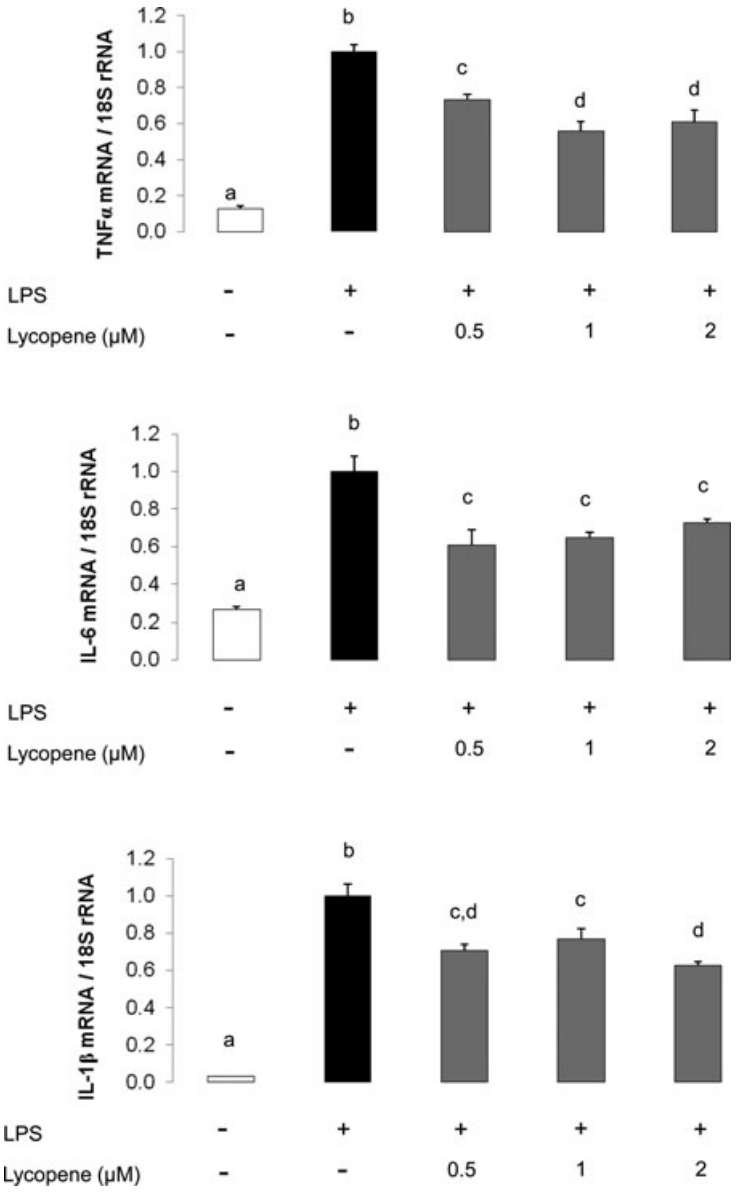

Figure 1. Lycopene decreases proinflammatory cytokine expression in LPS-stimulated macrophages. RAW 264.7 macrophages were incubated with lycopene (from 0.5 to 2 μM) for 24 h, and a 1h incubation with LPS (1 ng/mL) was then performed. RNA was extracted and reverse transcribed with MMLV. Real-time PCR was performed using specific primers. 18S rRNA was used as the endogenous control. Data are expressed as the relative expression ratio mean ± SEM. Bars not sharing the same letter are significantly different, $p < 0.05$.

lycopene prior to LPS incubation and compared with cells incubated with LPS alone. As expected, LPS induced a significant migration of macrophages (approximately 2-fold, Fig. 4). This process was inhibited by preincubation with lycopene (approximately 20%).

3.5 Lycopene decreases macrophage conditioned medium-induced proinflammatory cytokine and chemokine mRNA expression in 3T3-L1 adipocytes

To evaluate the impact of lycopene treatment of macrophages on adipocytes, 3T3-L1 cells were incubated for 24 h with MCM. MCM was obtained after incubation of macrophages with LPS (1 ng/mL, 1 h) alone or after lycopene pretreatment (0.5–2 μM, 24 h). The mRNA levels of several inflammatory genes (cytokines, chemokines, and acute-phase proteins), adiponectin, and matrix metalloproteinases (MMP3 and MMP9) were measured. The adiponectin mRNA levels decreased following LPS-MCM incubation (Table 1), whereas inflammatory gene mRNA expression (IL-6, MCP-1, IL-1β, RANTES, CXCL1, CXCL10, SAA3, and haptoglobin) and remodeling gene (MMP3 and MMP9) mRNA expression increased. Interestingly, lycopene preincubation reversed the proinflammatory character of the LPS-MCM. Indeed, adiponectin was induced, whereas the inflammatory and remodeling genes were significantly downregulated, as compared with LPS-MCM, and in accordance with a dose-response effect.

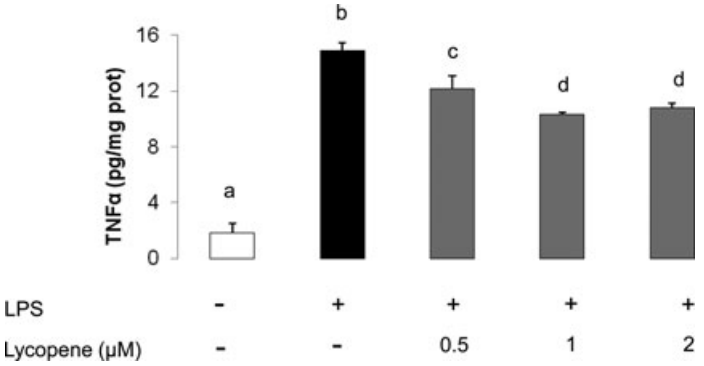

Figure 2. Lycopene decreases proinflammatory cytokine secretion in LPS-stimulated macrophages. RAW 264.7 macrophages were incubated with lycopene (from 0.5 to 2 μM) for 24 h, and a 1-h incubation with LPS (1 ng/mL) was then performed. The quantification of secreted TNF-α in the medium was performed using dedicated ELISA. Mean ± SEM. Bars not sharing the same letter are significantly different, $p < 0.05$.

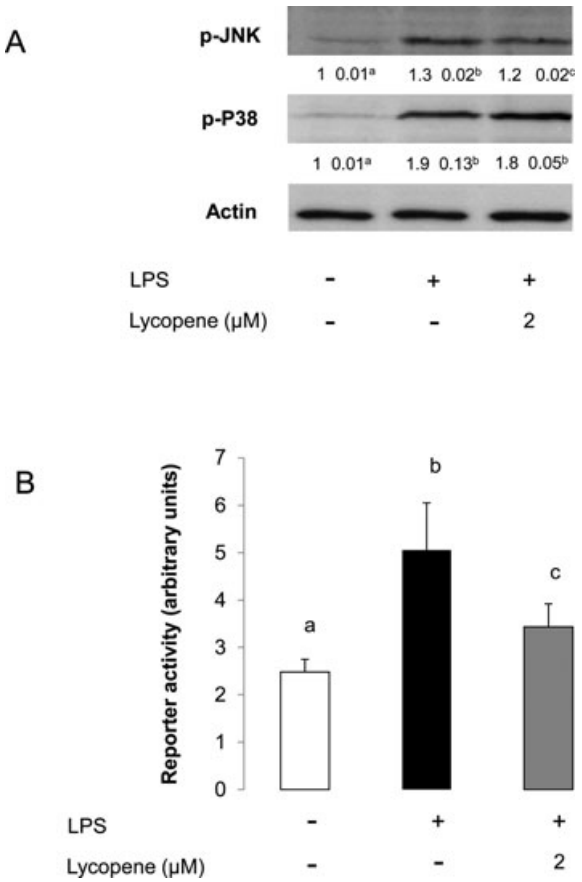

Figure 3. Lycopene modulates the LPS-mediated activation of the JNK and NF-κB pathways. (A) After a 24-h preincubation with lycopene (2 μM), RAW 264.7 cells were treated with LPS (1 ng/mL) for 15 min. The phosphorylation level of JNK and p38 were revealed by Western blot. (B) RAW 264.7 macrophages were transiently transfected with a reporter plasmid containing the luciferase gene under the control of four NF-κB response elements. Cells were incubated for 24 h with or without lycopene (2 μM), before incubation with LPS (1 ng/mL) for 1 h. The β-galactosidase doses and luciferase amounts are described in the Materials and methods section. Mean ± SEM. Bars not sharing the same letter are significantly different, $p < 0.05$.

4 Discussion

In the present study, we reported an anti-inflammatory effect of lycopene on macrophages characterized by a reduction of LPS-stimulated expression of TNF-α. The molecular mechanism seems to involve both the JNK and NF-κB signaling pathways. The anti-inflammatory effect of lycopene on macrophages is associated with a decrease of macrophage migration and modulation of adipocyte gene expression for adipocytes incubated in MCM.

In order to demonstrate the effect of lycopene on macrophage TNF-α expression and secretion, we used a cellular model based on the incubation of macrophages (RAW 264.7) with LPS. The metabolic concentration of plasma LPS used in our experiments is sufficient in vivo to trigger high-fat diet-induced metabolic diseases [28] such as macrophage infiltration and inflammation [29]. Thus, the LPS stimulation of macrophages mimics the inflammatory state of macrophages observed during obesity and could be considered a good model of obesity-associated inflammation. Using this model, we applied lycopene concentrations ranging from 0.5 to 2 μM. These concentrations are considered basal lycopene plasma concentrations or otherwise easily achievable by nutritional supplementations [30]. In these conditions, we were able to show that lycopene pretreatment reduced LPS-stimulated expression of TNF-α but also other proinflammatory cytokines including IL-6 and IL-1β (at mRNA level; Fig. 1). Since we reported an impact on IL-6 and IL-1β mRNA levels which was not associated to a protein secretion modification, thus we considered these data as not relevant as putative mediators of adipocytes-macrophages dialog. Thus, we focused on TNF-α in this study since it is known to be upregulated in obese patients [18, 31], it has been indicated as being particularly relevant in the context of adipose tissue inflammation and insulin resistance [32–34], and cross-talk between adipocytes and macrophages [22]. This inflammatory factor mostly originates from the stromavascular fraction of adipose tissue, which includes infiltrated macrophages. Indeed, obesity is a chronic low inflammatory disease and is characterized by an enhanced infiltration of macrophages in adipose tissue [35, 36]. These recruited macrophages release

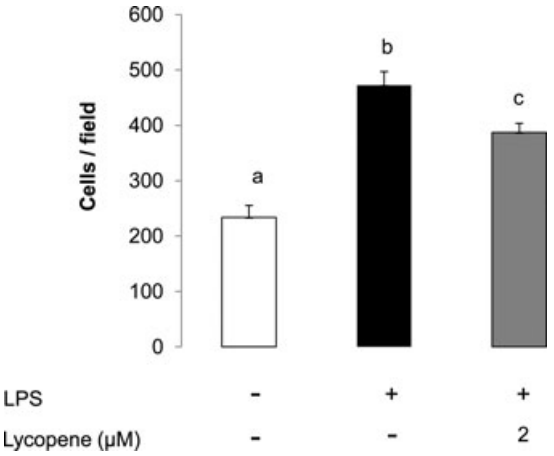

Figure 4. Lycopene decreases LPS-mediated macrophages migration. RAW 264.7 macrophages were preincubated with lycopene (2 μM) for 24 h and then incubated with LPS (100 ng/mL) for 4 h to initiate migration across membrane pores. The number of migrated cells was counted in five random microscopy fields per membrane. Mean ± SEM. Bars not sharing the same letter are significantly different, $p < 0.05$.

proinflammatory cytokines, including TNF-α, known to induce inflammatory status and insulin resistance at a local level in adipocytes [34] but also at a systemic level. It is noteworthy that a similar anti-inflammatory effect of lycopene on macrophages has already been suggested notably via a decrease of iNos, Cox-2, and IL-6 [6, 37], however, for the first time we report here an impact of lycopene on TNF-α expression which is of particular interest in the context of adipose tissue metabolism.

In addition to the effect of lycopene on TNF-α expression and secretion, we also reported for the first time an effect of lycopene on macrophage migration. Lycopene was able to counteract the LPS-mediated migration of macrophages in cell culture. This point is of particular interest as this migratory process is suspected to have a crucial role in the infiltration of macrophages that occurs within adipose tissue during obesity [20, 21, 35, 36].

From a molecular point of view, both the JNK and NF-κB signaling pathways were impacted by lycopene treatment. These two pathways play a central role in the pathogenesis of obesity-linked inflammation [18]. Our observations are consistent with a role of these two pathways in LPS-mediated inflammation. Moreover, our data are also strongly consistent with several other studies that have reported an effect of lycopene on the NF-κB signaling pathway [6–9]. As previously reported [7], a lycopene-linked inhibition of NF-κB activation could represent a major gene expression regulatory mechanism in various cell types.

Finally, we reported the impact of MCM on adipocytes. This MCM was notably characterized by an increased concentration of TNF-α under an LPS effect that had been decreased by lycopene incubation (Fig. 2). Interestingly, TNF-α has been indicated as the major actor in the deleterious paracrine loop between adipocytes and macrophages [22]. Thus, we speculated that lycopene could be a modulator of the TNF-α-dependent vicious cycle observed between adipocytes and macrophages. To test this hypothesis, we used a coculture model of differentiated adipocytes and macrophages. MCM was recovered after preincubation with or without lycopene before being stimulated with LPS (LPS-MCM). Adipocytes were then incubated in the media. The media strongly modified the expression of highly relevant adipocytes genes. Indeed, the downregulation of adiponectin by LPS-MCM was counteracted by a preincubation of macrophages with lycopene, allowing a normalization of adiponectin, a well-known insulin-sensitizing protein [38]. In contrast, several inflammatory gene markers (IL-6, IL-1β), acute-phase

Table 1. Lycopene decreases macrophage conditioned medium-induced proinflammatory cytokine, acute-phase proteins, and chemokine mRNA expression in 3T3-L1 adipocytes

Gene name	Experimental conditions				
	Control	LPS	LPS + 0.5 μM	LPS + 1 μM	LPS + 2 μM
Adiponectin	1.65 ± 0.16^{a}	1 ± 0.07^{b}	0.97 ± 0.09^{b}	1.34 ± 0.04^{c}	$1.47 \pm 0.07^{c,a}$
IL-6	0.10 ± 0.01^{a}	1 ± 0.01^{b}	0.85 ± 0.05^{b}	0.44 ± 0.06^{c}	0.44 ± 0.08^{c}
IL-1β	0.46 ± 0.02^{a}	1 ± 0.08^{b}	0.37 ± 0.03^{c}	$0.44 \pm 0.07^{c,d}$	0.50 ± 0.06^{d}
MCP-1	0.35 ± 0.01^{a}	1 ± 0.03^{b}	0.69 ± 0.02^{c}	0.54 ± 0.05^{d}	$0.51 \pm 0.04^{d,a}$
Rantes	0.10 ± 0.01^{a}	1 ± 0.14^{b}	0.65 ± 0.04^{c}	0.42 ± 0.04^{c}	0.60 ± 0.1^{c}
CXCL1	0.14 ± 0.03^{a}	1 ± 0.13^{b}	1.05 ± 0.06^{b}	0.59 ± 0.02^{c}	0.60 ± 0.06^{c}
CXCL10	0.53 ± 0.07^{a}	1 ± 0.1^{b}	0.50 ± 0.07^{a}	0.66 ± 0.13^{a}	0.7 ± 0.12^{a}
MMP3	0.06 ± 0.01^{a}	1 ± 0.23^{b}	0.64 ± 0.09^{c}	$0.39 \pm 0.05^{c,d}$	$0.27 \pm 0.04^{d,a}$
MMP9	0.48 ± 0.04^{a}	1 ± 0.05^{b}	0.91 ± 0.08^{b}	0.71 ± 0.06^{c}	0.59 ± 0.06^{c}
SAA3	0.02 ± 0.005^{a}	1 ± 0.2^{b}	1.08 ± 0.37^{b}	0.71 ± 0.12^{c}	0.47 ± 0.11^{c}
Haptoglobin	0.15 ± 0.03^{a}	1 ± 0.12^{b}	1.06 ± 0.1^{b}	0.77 ± 0.09^{c}	0.64 ± 0.06^{c}

After a 24-h preincubation with various concentrations of lycopene (from 0.5 to 2 μM), RAW 264.7 macrophages were treated with LPS (1 ng/mL) for 1 h. Supernatants were collected and incubated for 24 h with differentiated 3T3-L1. Total RNA was extracted and reverse transcribed with MMLV. Real-time PCR was performed using specific primers. 18S rRNA was used as the endogenous control. Data are expressed as the relative expression ratio mean ± SEM. Values not sharing the same letter were significantly different, $p < 0.05$.

proteins (SAA3, haptoglobin) and chemokines (RANTES, MCP1, CXCL1, CXCL10) induced by LPS-MCM were downregulated by LPS-MCM collected from lycopene-treated macrophage cultures. These downregulations of IL-6, IL-1β, and SAA3 could limit the local inflammatory status as well as the genesis of insulin resistance in adipocytes [32, 33, 39]. A downregulation of the chemokines (MCP-1, RANTES, CXCL1, and CXCL10), as well as haptoglobin could result in a decrease in macrophage recruitment [40–42] [43]. Finally, the reduction of matrix metalloproteinases (MMP3 and MMP9) may provide a benefit by limiting the adipose tissue remodeling normally observed during obesity [44, 45]. Altogether, these global effects on secretion of TNF-α associated to a decrease of macrophage migratory ability in the presence of lycopene and the consequent impact on adipocyte gene expression could be associated with a global decrease of macrophage infiltration, inflammation, and insulin resistance in adipocytes in the presence of lycopene.

These data, which are in accordance with the previously reported anti-inflammatory effect of lycopene on adipocytes and preadipocytes [7], strongly suggest that lycopene could be a modulator of inflammatory status in obesity-associated adipose tissue. Our present data also highlight the possibility that lycopene interfered in the cross-talk between macrophages and adipocytes resulting in an attenuation of the deleterious cycle that occurs in adipose tissue [22]. These results support and reinforce the hypothesis that lycopene can fight the inflammatory process in the context of obesity. The putative anti-inflammatory effects of lycopene could provide major health benefits, especially for obesity-associated pathologies.

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The authors have declared no conflict of interest.

5 References

[1] Rao, A. V., Ray, M. R., Rao, L. G., Lycopene. *Adv. Food Nutr. Res.* 2006, *51*, 99–164.

[2] Krinsky, N. I., Johnson, E. J., Carotenoid actions and their relation to health and disease. *Mol. Aspects Med.* 2005, *26*, 459–516.

[3] Joo, Y. E., Karrasch, T., Muhlbauer, M., Allard, B., et al., Tomato lycopene extract prevents lipopolysaccharide-induced NF-kappaB signaling but worsens dextran sulfate sodium-induced colitis in NF-kappaBEGFP mice. *PLoS One* 2009, *4*, e4562.

[4] Bignotto, L., Rocha, J., Sepodes, B., Eduardo-Figueira, M., et al., Anti-inflammatory effect of lycopene on carrageenan-induced paw oedema and hepatic ischaemia-reperfusion in the rat. *Br. J. Nutr.* 2009, *102*, 126–133.

[5] Hung, C. F., Huang, T. F., Chen, B. H., Shieh, J. M., et al., Lycopene inhibits TNF-alpha-induced endothelial ICAM-1 expression and monocyte-endothelial adhesion. *Eur. J. Pharmacol.* 2008, *586*, 275–282.

[6] Feng, D., Ling, W. H., Duan, R. D., Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK, p38MAPK, and NF-kappaB in macrophages. *Inflamm. Res.* 2010, *59*, 115–121.

[7] Gouranton, E., Thabuis, C., Riollet, C., Malezet-Desmoulins, C., et al., Lycopene inhibits proinflammatory cytokine and chemokine expression in adipose tissue. *J. Nutr. Biochem.* 2011, *22*, 642–648.

[8] Palozza, P., Parrone, N., Catalano, A., Simone, R., Tomato lycopene and inflammatory cascade: basic interactions and clinical implications. *Curr. Med. Chem.* 2010, *17*, 2547–2563.

[9] Simone, R. E., Russo, M., Catalano, A., Monego, G., et al., Lycopene inhibits NF-kB-mediated IL-8 expression and changes Redox and PPARgamma signaling in cigarette smoke-stimulated macrophages. *PLoS One* 2011, *6*, e19652.

[10] Chung, H. Y., Ferreira, A. L., Epstein, S., Paiva, S. A., et al., Site-specific concentrations of carotenoids in adipose tissue: relations with dietary and serum carotenoid concentrations in healthy adults. *Am. J. Clin. Nutr.* 2009, *90*, 533–539.

[11] Moussa, M., Gouranton, E., Gleize, B., Yazidi, C. E., et al., CD36 is involved in lycopene and lutein uptake by adipocytes and adipose tissue cultures. *Mol. Nutr. Food Res.* 2011, *55*, 578–584.

[12] Gouranton, E., Yazidi, C. E., Cardinault, N., Amiot, M. J., et al., Purified low-density lipoprotein and bovine serum albumin efficiency to internalise lycopene into adipocytes. *Food Chem. Toxicol.* 2008, *46*, 3832–3836.

[13] Tourniaire, F., Gouranton, E., von Lintig, J., Keijer, J., et al., Beta-carotene conversion products and their effects on adipose tissue. *Genes. Nutr.* 2009, *4*, 179–187.

[14] Lobo, G. P., Amengual, J., Li, H. N., Golczak, M., et al., Beta,beta-carotene decreases peroxisome proliferator receptor gamma activity and reduces lipid storage capacity of adipocytes in a beta,beta-carotene oxygenase 1-dependent manner. *J. Biol. Chem.* 2010, *285*, 27891–27899.

[15] Amengual, J., Gouranton, E., van Helden, Y. G., Hessel, S., et al., Beta-carotene reduces body adiposity of mice via BCMO1. *PLoS One* 2011, *6*, e20644.

[16] Sluijs, I., Beulens, J. W., Grobbee, D. E., van der Schouw, Y. T., Dietary carotenoid intake is associated with lower prevalence of metabolic syndrome in middle-aged and elderly men. *J. Nutr.* 2009, *139*, 987–992.

[17] Gouranton, E., Aydin, G., Reynault, E., Malezet, C., et al., Apo-10′-lycopenoic acid is an active metabolite of lycopene in adipose tissue. *H. Biochim. Biophys. Act.* 2011, *1811*, 1105–1114.

[18] Gregor, M. F., Hotamisligil, G. S., Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.* 2011, *29*, 415–445.

[19] Hotamisligil, G. S., Inflammation and metabolic disorders. *Nature.* 2006, *444*, 860–867.

[20] Bourlier, V., Bouloumie, A., Role of macrophage tissue infiltration in obesity and insulin resistance. *Diabetes Metab.* 2009, *35*, 251–260.

[21] Olefsky, J. M., Glass, C. K., Macrophages, inflammation, and insulin resistance. *Annu. Rev. Physiol.* 2010, *72*, 219–246.

[22] Suganami, T., Nishida, J., Ogawa, Y., A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. *Arterioscler. Thromb. Vasc. Biol.* 2005, *25*, 2062–2068.

[23] Landrier, J. F., Gouranton, E., El Yazidi, C., Malezet, C., et al., Adiponectin expression is induced by vitamin E via a peroxisome proliferator-activated receptor gamma-dependent mechanism. *Endocrinology*. 2009, *150*, 5318–5325.

[24] Landrier, J. F., Gouranton, E., Reboul, E., et al., Vitamin E decreases endogenous cholesterol synthesis and apo-AI-mediated cholesterol secretion in Caco-2 cells. *J. Nutr. Biochem.* 2010, *21*, 1207–1213.

[25] Landrier, J. F., Malezet-Desmoulins, C., Reboul, E., Lorec, M. A., et al., Comparison of different vehicles to study the effect of tocopherols on gene expression in intestinal cells. *Free Radic. Res.* 2008, *42*, 523–530.

[26] Romier, B., Van De Walle, J., During, A., Larondelle, Y., et al., Modulation of signalling nuclear factor-kappaB activation pathway by polyphenols in human intestinal Caco-2 cells. *Br. J. Nutr.* 2008, *100*, 542–551.

[27] Hassan, M., El Yazidi, C., Landrier, J. F., Lairon, D., et al., Phloretin enhances adipocyte differentiation and adiponectin expression in 3T3-L1 cells. *Biochem. Biophys. Res. Commun.* 2007, *361*, 208–213.

[28] Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., et al., Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007, *56*, 1761–1772.

[29] Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., et al., Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008, *57*, 1470–1481.

[30] Allen, C. M., Schwartz, S. J., Craft, N. E., Giovannucci, E. L., et al., Changes in plasma and oral mucosal lycopene isomer concentrations in healthy adults consuming standard servings of processed tomato products. *Nutr. Cancer* 2003, *47*, 48–56.

[31] Fain, J. N., Madan, A. K., Hiler, M. L., Cheema, P., et al., Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 2004, *145*, 2273–2282.

[32] Rotter, V., Nagaev, I., Smith, U., Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J. Biol. Chem.* 2003, *278*, 45777–45784.

[33] Jager, J., Gremeaux, T., Cormont, M., Le Marchand-Brustel, Y., et al., Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* 2007, *148*, 241–251.

[34] Ishizuka, K., Usui, I., Kanatani, Y., Bukhari, A., et al., Chronic tumor necrosis factor-alpha treatment causes insulin resistance via insulin receptor substrate-1 serine phosphorylation and suppressor of cytokine signaling-3 induction in 3T3-L1 adipocytes. *Endocrinology* 2007, *148*, 2994–3003.

[35] Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., et al., Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 2003, *112*, 1796–1808.

[36] Xu, H., Barnes, G. T., Yang, Q., Tan, G., et al., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* 2003, *112*, 1821–1830.

[37] Rafi, M. M., Yadav, P. N., Reyes, M., Lycopene inhibits LPS-induced proinflammatory mediator inducible nitric oxide synthase in mouse macrophage cells. *J. Food. Sci.* 2007, *72*, S069–S074.

[38] Lara-Castro, C., Fu, Y., Chung, B. H., Garvey, W. T., Adiponectin and the metabolic syndrome: mechanisms mediating risk for metabolic and cardiovascular disease. *Curr. Opin. Lipidol.* 2007, *18*, 263–270.

[39] Filippin-Monteiro, F. B., de Oliveira, E. M., Sandri, S., Knebel, F. H., et al., Serum amyloid A is a growth factor for 3T3-L1 adipocytes, inhibits differentiation and promotes insulin resistance. *Int. J. Obes. (Lond).* 2011. doi:10.1038/ijo.2011.193. [Epub ahead of print]

[40] Keophiphath, M., Rouault, C., Divoux, A., Clement, K., et al., CCL5 promotes macrophage recruitment and survival in human adipose tissue. *Arterioscler. Thromb. Vasc. Biol.* 2010, *30*, 39–45.

[41] Yamashita, A., Soga, Y., Iwamoto, Y., Asano, T., et al., DNA microarray analyses of genes expressed differentially in 3T3-L1 adipocytes co-cultured with murine macrophage cell line RAW 264.7 in the presence of the toll-like receptor 4 ligand bacterial endotoxin. *Int. J. Obes.* 2008, *32*, 1725–1729.

[42] McCall, K. D., Holliday, D., Dickerson, E., Wallace, B., et al., Phenylmethimazole blocks palmitate-mediated induction of inflammatory cytokine pathways in 3T3L1 adipocytes and RAW 264.7 macrophages. *J. Endocrinol.* 2010, *207*, 343–353.

[43] Maffei, M., Funicello, M., Vottari, T., Gamucci, O., et al., The obesity and inflammatory marker haptoglobin attracts monocytes via interaction with chemokine (C-C motif) receptor 2 (CCR2). *BMC Biol.* 2009, *7*, 87.

[44] Maquoi, E., Munaut, C., Colige, A., Collen, D., et al., Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. *Diabetes* 2002, *51*, 1093–1101.

[45] O'Hara A, Lim, F. L., Mazzatti, D. J., Trayhurn, P., Microarray analysis identifies matrix metalloproteinases (MMPs) as key genes whose expression is up-regulated in human adipocytes by macrophage-conditioned medium. *Pflugers Arch.* 2009, *458*, 1103–1114.

