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Lycopene inhibits proinflammatory cytokine and chemokine expression in adipose tissue☆

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Abstract

Obesity is associated with a low-grade inflammation which is correlated with an increased secretion of pro-inflammatory cytokines and chemokines by adipose tissue, suspected to contribute to the development of insulin resistance. Because lycopene is mostly stored in adipose tissue and possesses anti-inflammatory properties, we hypothesize that lycopene could reduce the production of proinflammatory markers in adipose tissue. In agreement with this hypothesis, we observed a decrease of inflammatory markers such as IL-6, MCP-1 and IL-1β at both the mRNA and protein level when explants of epididymal adipose tissue from mice fed with a high-fat diet were incubated with lycopene *ex vivo*. The same effect was reproduced with explants of adipose tissue preincubated in lycopene and then subjected to TNFα stimulation. The contribution of adipocytes and preadipocytes was evaluated. In both preadipocytes and differentiated 3T3-L1 adipocytes, lycopene preincubation for 24 h decreased the TNFα-mediated induction of IL-6 and MCP-1. Finally, the same results were reproduced with human adipocyte primary cultures. The molecular mechanism was also studied. In transient transfections, a decrease of the luciferase gene reporter under control of NF-κB responsive element was observed for cells incubated in the presence of lycopene and TNFα compared to TNFα alone. The involvement of the NF-κB pathway was confirmed by the modulation of IKKα/β phosphorylation by lycopene.

Altogether, these results showed for the first time a limiting effect of lycopene on adipose tissue proinflammatory cytokine and chemokine production. Such an effect could prevent or limit the prevalence of obesity-associated pathologies, such as insulin resistance.

Keywords: Adipose tissue; Adipocyte; Lycopene; Nutrition; Gene expression; Cytokines; Chemokines; Inflammation

1. Introduction

Adipose tissue is a complex tissue composed by several cell types (adipocytes and the stroma vascular fraction including preadipocytes, macrophages, endothelial cell, *etc.*). Initially, adipose tissue was considered to be an inert tissue that stores triacylglycerols and releases free fatty acids. Now it is recognized as an endocrine organ. Indeed, it notably secretes a large amount of proteins called adipokines (which include cytokines and chemokines), which contribute to the maintenance of whole body homeostasis [1–3].

Obesity is characterized by a chronic low-grade inflammation in adipose tissue that is believed to contribute to the genesis of insulin resistance, which leads to type 2 diabetes, a risk factor for cardiovascular diseases (CVD) [4–6]. This low-grade inflammatory state is notably characterized by the activation of inflammatory pathways (JNK and NF-κB) in adipose tissue [7,8] and increased production of cytokines and chemokines such as interleukin 6 (IL-6), interleukin 1 beta (IL-1 β), tumor necrosis factor alpha (TNFα) and monocyte chemotactic protein 1 (MCP-1), and of acute phase proteins such as C-reactive protein (CRP) [4,5,9–11].

Lycopene is a non-provitamin A carotenoid found in various fruits, such as tomatoes and tomato products, papaya and watermelon [12]. This molecule has been reported to display anti-inflammatory effects in several models of diseases linked to inflammation [13–16]. Its beneficial health effects have been extensively studied, especially in the prevention of prostate cancer [17–20]. Moreover, it has been recently reported that higher lycopene intakes were associated with a lower waist circumference and visceral and subcutaneous fat mass [21], suggesting its impact on adipose tissue metabolism, as reported for β-carotene [22].

Since lycopene has been shown to possess anti-inflammatory properties and because obesity is linked to a low-grade inflammation state in adipose tissue, the aim of the present study was to evaluate the ability of this carotenoid to prevent inflammation in this tissue. The contribution of adipocytes and preadipocytes has been studied. Experiments were performed by using two models of inflammation of mouse adipose tissue: high-fat diet (HFD) and TNFα treatment. We showed that lycopene prevents inflammation *via* a decrease of IL-6,

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MCP-1 and, in some cases, IL-1β at both the mRNA and protein level. Moreover, we demonstrated the involvement of the NF-κB pathway as a key part of the molecular mechanism in this regulation.

2. Materials and methods

2.1. Chemicals

All-*trans* lycopene was kindly provided by Catherine Caris-Veyrat (INRA Avignon). Mouse TNFα and other chemicals, dexamethasone, IBMX and insulin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-phospho-IKKα/β antibody was purchased from Cell Signaling Technology (Ozyme, France).

2.2. Animal experiments

The care and use of mice were in accordance with French guidelines and approved by the local experimental animal ethics committee. Adult male C57BL/6j mice were housed in a temperature-, humidity- and light-controlled room. They were given a standard chow diet and water *ad libitum*. For the HFD, mice (n=6) were fed for 6 weeks with a HFD containing 35% fat, as previously described [23], leading to diet-induced obesity.

2.3 Ex vivo cultures of adipose tissue explants

Adipose tissue explants were recovered from mouse epididymal white adipose tissue, rinsed in saline buffer and placed in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 humidified atmosphere. The medium was changed after 1, 3 and 24 h. Treatment of explants of adipose tissues of mice subjected either to a HFD or to normal chow (control) with lycopene (2 μM; the concentration achieved in human plasma by consumption of tomato sauce [24]) was performed for 24 h. For adipose tissue from mice fed the normal chow, TNFα stimulation (15 ng/ml for 3 h, previously demonstrated to have a maximal effect on proinflammatory cytokine expression; unpublished personal data) was applied after a 24-h lycopene treatment.

2.4. Cell culture

3T3-L1 preadipocytes (ATCC, Manassas, VA, USA) were seeded in 3.5-cm-diameter dishes at a density of 15×10^4 cells/well. Cells were grown in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 humidified atmosphere, as previously reported [25]. To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (Day 0) were stimulated for 48 h with 0.5 mM isobutylmethylxanthine, 0.25 μmol/L dexamethasone and 1 μg/ml insulin in DMEM supplemented with 10% FBS. The cultures were then treated with DMEM supplemented with 10% FBS and 1 μg/ml insulin. To examine the preventive effect of lycopene, 3T3-L1 adipocytes were incubated for 24 h with 2 μM of

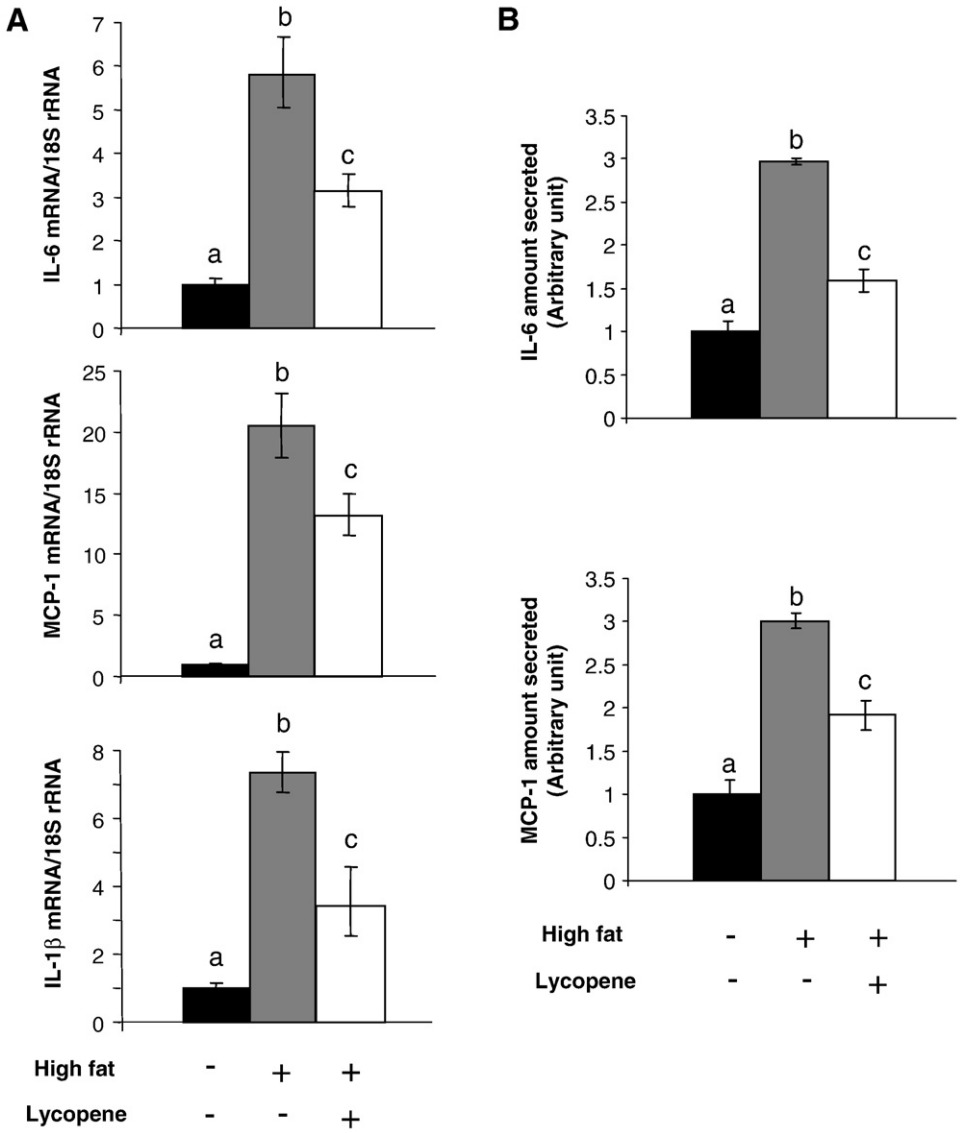

Fig. 1. Lycopene decreases proinflammatory cytokine and chemokine expression of adipose tissue explants from mice subjected to a HFD in *ex vivo* culture. (A) Adipose tissue from mice fed with a HFD for 6 weeks was recovered and incubated *ex vivo* with lycopene (2 μM) for 24 h. RNAs were extracted and reverse transcribed with MMLV. Real-time PCR was performed using specific primers. 18S rRNA was used as the endogenous control. Data were expressed as relative expression ratio. (B) Dosage of IL-6 and MCP-1 excreted in the medium performed using Luminex technology. Mean±S.E.M.; bars not sharing the same letter were significantly different, P<.05.

lycopene dissolved in tetrahydrofurane at 0.01%, as previously reported [26]. Then, adipocytes were incubated with TNFα (15 ng/ml) for 3 h. All treatments were performed on Day 8. The data are the mean of three independent experiments each performed in triplicate.

Human preadipocytes were provided by Promocell (France) and cultured following the company's instructions. Mature adipocytes (Day 14) were incubated with lycopene (2 μM, 24 h) followed by a 3-h incubation with TNFα (15 ng/ml).

2.5. RNA isolation and qPCR

Total cellular RNA was extracted from 3T3-L1 cells and mouse epididymal fat pads using TRIzol reagent according to the manufacturer's instructions. The cDNA was synthesized from 1 μg of total RNA 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, USA) as previously described [27]. For each condition, expression was quantified in duplicate, and 18S mRNA was used as the endogenous control in the comparative cycle threshold (C_T) method [28]. Data were expressed as relative expression ratio.

2.6. Cytokine quantification

Cell culture medium was recovered after the different treatments. The amount of proteins was determined using a Luminex kit (mouse adipocyte multiplex immunoassay, LINCOplex kit, Linco), according to the manufacturer's instructions.

2.7. Transient transfection experiments

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) [29] was transfected in 3T3-L1 cells seeded in 24-well plates at a density of 2.7×10^4 cells/well. The transfection was performed using Lipofectamine 2000 Plus Reagent (Invitrogen). After overnight incubation with transfection mixes, the medium was replaced by DMEM supplemented with 10% FBS and 2 μM lycopene. The treatment was for 24 h and was followed by a 3-h incubation with TNFα. Cells were lysed for luciferase activity using a luciferase assay system (Promega, Madison, WI, USA), which was normalized to β-galactosidase activity as previously described [30]. The transfection experiments were performed in triplicate and repeated at least two times independently.

2.8. Western blot

Whole cell lysates were resuspended in lysis buffer [20 mM Tris, pH 7.4, 150 mM NaCl, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 100 mM NaF, 1% (final) Triton X-100]. Protein extracts (40 μg) were boiled for 5 min in Laemmli buffer and loaded onto a 10% SDS PAGE gel for migration (200 V for 1 h). After blocking with 5% bovine serum albumin (BSA) (w/v) in Tris buffered saline (137 mmol/L NaCl, 20 mmol/L Tris, pH 7.6) plus Tween 20 at 0.05% (v/v) (TBST) solution, the membrane was incubated overnight at 4°C with the primary antibody. Then, protein was transferred onto a polyvinylidene difluoride membrane (100 V for 30 min). The membrane was blocked for 1 h at room temperature in TBST with 5% BSA. The primary antibody was incubated with the membrane in TBST buffer overnight at 4°C. The membrane was washed three times with the TBST solution and then incubated with the secondary antibody. After three washings with the TBST solution, the bound HRP-conjugated antibody was detected by chemiluminescence using Immobilon Western chemiluminescent HRP substrate (Millipore, Billerica, MA, USA). The resulting light was detected on autoradiographic film.

2.9. Statistical analysis

Data are expressed as means±S.E.M. Significant differences between the control and treated group were determined by unpaired Student's *t* test or ANOVA, followed by the Tukey–Kramer *post hoc* test using Statview software (SAS Institute, Cary, NC, USA). $P<.05$ was considered to be statistically significant.

3. Results

3.1. Lycopene decreased proinflammatory cytokine and chemokine expression in ex vivo cultures of adipose tissue explants from HFD-fed mice

To evaluate the effect of lycopene on inflammation in adipose tissue, mice were fed with a HFD for 6 weeks (model of diet-induced obesity) or submitted to a normal chow diet as the control. Epididymal adipose tissue was dissected, and explants were pre-incubated with lycopene (2 μM) for 24 h. The expression of different markers of inflammation such as IL-6, MCP-1 and IL-1β was evaluated by real-time qPCR. As expected, HFD induced a strong increase of IL-6, MCP-1 and IL-1β mRNA levels compared to the chow diet (5.8-, 20- and 7.3-fold, respectively). In agreement with our assumption, preincubation of explants with lycopene decreased the expression of mRNA of the different markers, by −50%, −36% and −50%, for IL-6, MCP-1 and IL-1β, respectively, as compared to the HFD group (Fig. 1A). Concerning the secretion of these adipokines, we observed an increase of IL-6 and MCP-1 proteins in the medium of explants from HFD mice as compared to those fed on the control diet. Surprisingly, IL-1β was not detected in the medium. As reported at the mRNA level, lycopene preincubation decreased the amount of IL-6 and MCP-1 proteins in the medium (by −50% and −35% for IL-6 and MCP-1, respectively) (Fig. 1B). Taken together, these data suggested that lycopene can prevent the transcription and secretion of inflammatory markers in adipose tissue after HFD.

3.2. Lycopene decreased proinflammatory cytokine and chemokine expression in ex vivo cultures of mouse adipose tissue explants incubated with TNFα

TNFα expression is increased in the adipose tissue of obese people and has been demonstrated to be a major mediator of the low-grade

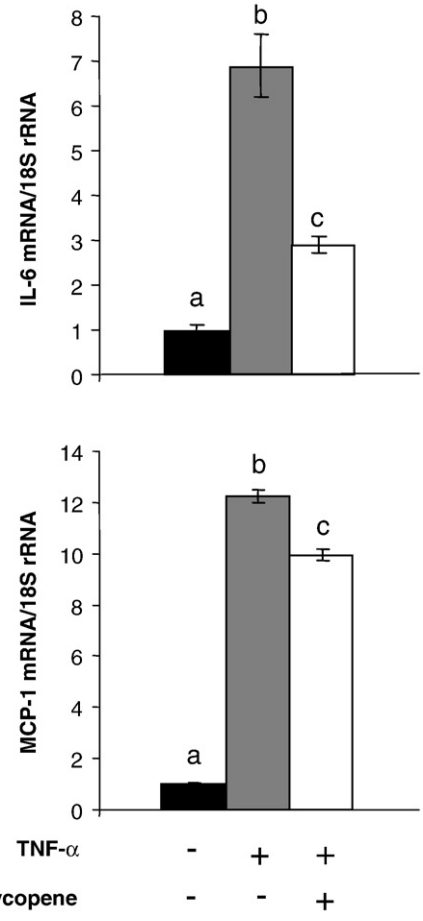

Fig. 2. Lycopene decreases proinflammatory cytokine and chemokine expression induced by TNFα in *ex vivo* culture of adipose tissue. Explants of adipose tissue of C57BL/6j mice fed with a control diet were recovered and cultured under *ex vivo* conditions. After a 24-h preincubation with lycopene (2 μM), adipose tissue explants were incubated with TNFα for 3 h. Then, RNAs were extracted and reverse transcribed with MMLV. Real-time PCR was performed using specific primers. 18S rRNA was used as the endogenous control. Data were expressed as relative expression ratio. Mean±S.E.M.; bars not sharing the same letter were significantly different, $P<.05$.

inflammatory state [4,31]. Thus, we evaluated the preventive effect of lycopene on *ex vivo* cultures of mouse adipose tissue explants subjected to TNFα treatment. For this purpose, explants were preincubated for 24 h with lycopene and subjected to TNFα (15 ng/ml) treatment for 3 h. As expected, TNFα induced an increase of IL-6 (about 7-fold) and MCP-1 (about 12-fold) mRNA levels compared to the control explants (Fig. 2). The 24-h preincubation with 2 μM lycopene significantly reduced the expression of these adipokines (about −58% and −20%, respectively, for IL-6 and MCP-1); no impact on IL-1β expression was observed (data not shown).

3.3. Lycopene decreased TNFa-mediated proinflammatory cytokine and chemokine expression in 3T3-L1 adipocytes and preadipocytes

Because both adipocytes and preadipocytes are known to contribute to the production of proinflammatory cytokines and chemokines [32,33], 3T3-L1 preadipocytes and differentiated 3T3-L1 adipocytes (mature adipocytes) were preincubated with lycopene for 24 h and then incubated with TNFα (15 ng/ml) for 3 h to estimate the involvement of adipocytes and preadipocytes in the preventive effect observed. In preadipocytes, TNFα increased the mRNA expression of IL-6 (about 12-fold) and MCP-1 (about 20-fold). Preincubation with lycopene reduced the expression of these cytokines (about −40% for IL-6 and −30% for MCP-1) (Fig. 3A). The same pattern was seen in mature adipocytes, in which a decrease of these markers was observed in the same range: about a 37% reduction for IL-6 and 43% for MCP-1 mRNA expression (Fig. 3B).

3.4. Lycopene decreased TNFα-mediated proinflammatory cytokine and chemokine expression in human adipocytes

To validate our results in humans, we applied the same protocol of TNFα stimulation in human adipocytes differentiated in culture. We observed an increase of IL-6 (16-fold), MCP-1 (27-fold) and IL-1β (17-fold) mRNA levels upon TNFα treatment. These inductions were strongly reduced by the preincubation of human adipocytes with lycopene (about −30%, −22% and −50% for IL-6, MCP-1 and IL-1β, respectively) (Fig. 4).

3.5. Lycopene reduced the TNFα-mediated activation of the NF-κB pathway

NF-κB is a well-known transcription factor involved in the regulation of proinflammatory cytokines and chemokines [34,35]. Thus, we examined the involvement of the NF-κB signaling pathway in our adipose tissue inflammatory model and the ability of lycopene to modulate it. To this purpose, 3T3-L1 adipocytes were transiently transfected with a reporter plasmid containing the luciferase gene under the control of four NF-κB response elements. As expected, TNFα (15 ng/ml; 3 h) induced an increase of the luciferase gene expression, which was reversed by lycopene preincubation (2 μM; 24 h) (Fig. 5A). To confirm the involvement of the NF-κB pathway, the level of phosphorylation of IKKα/β was measured in 3T3-L1 cells. As expected, the TNFα treatment resulted in an increase of phosphorylated IKKα/β, which was strongly decreased by lycopene preincubation (2 μM; 24 h) (Fig. 5B). Altogether, these data

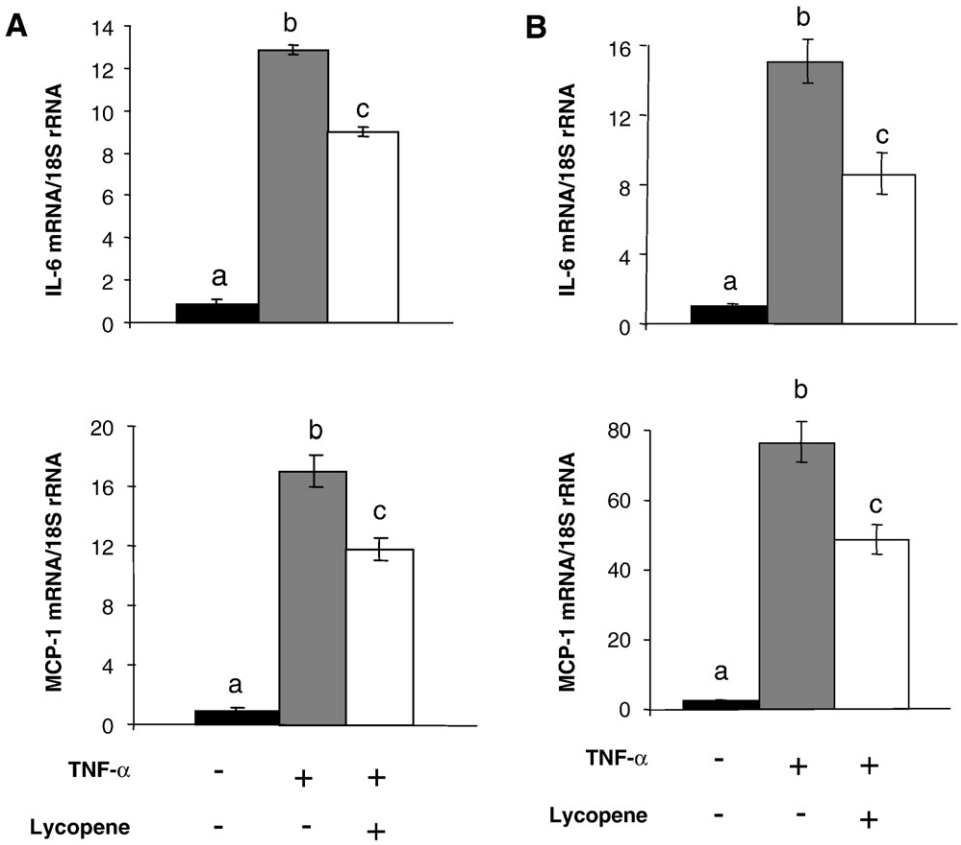

Fig. 3. Mature adipocytes and preadipocytes participate in the anti-inflammatory effect of lycopene. Preadipocytes (A) and mature 3T3-L1 adipocytes (B) were incubated with lycopene (2 μM) for 24 h. A 3-h incubation with TNFα (15 ng/ml) was then performed. RNAs were extracted and reverse transcribed with MMLV. Real-time PCR was performed using specific primers. 18S rRNA was used as the endogenous control. Data were expressed as relative expression ratio. Mean±S.E.M.; bars not sharing the same letter were significantly different, $P<.05$.

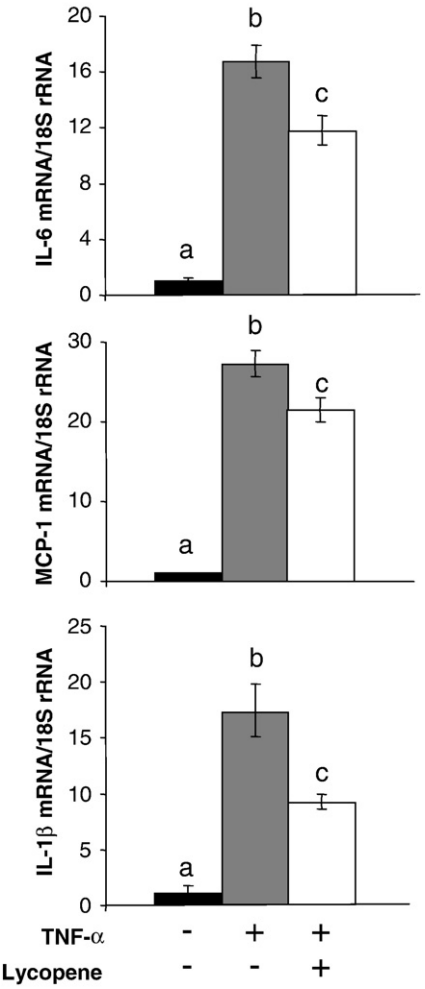

Fig. 4. Lycopene decreases TNFα-mediated proinflammatory marker expression in human adipocytes. Human adipocytes were preincubated with lycopene (2 μM) for 2 h and then incubated with TNFα (15 ng/ml) for 3 h. RNAs were extracted and reverse transcribed with MMLV. Real-time PCR was performed using specific primers. 18S rRNA was used as the endogenous control. Data were expressed as relative expression ratio. Mean±S.E.M.; bars not sharing the same letter were significantly different, $P<.05$.

suggested that lycopene is able to reduce the TNFα-mediated activation of the NF-κB pathway in adipocytes.

4. Discussion

In this study, we showed that lycopene was able to reduce the expression of genes (IL-6, IL-1β and MCP-1) involved in the inflammation of adipose tissue submitted to a stress. Several models (a HFD in mice leading to diet-induced obesity or TNFα treatment in cell cultures and explants) were used to show the ability of lycopene to prevent inflammation. Furthermore, the NF-κB signaling pathway was demonstrated to be modulated by lycopene, providing a mechanistic explanation for the beneficial effect of lycopene on inflammation.

Indeed, we reported for the first time the ability of lycopene to prevent the increase of proinflammatory factors in the adipose tissue of mice in response to a HFD (Fig. 1) or incubated with TNFα (Fig. 2). To this purpose, we developed a methodology based on *ex vivo* culture of explants of adipose tissue. The bioavailability of lycopene is particularly low in mice, compared with humans: the plasma concentration remains critically low (10 nM) after 1 month of supplementation [36]. Thus, with these *ex vivo* cultures, we were able to evaluate the effect of a concentration of lycopene equivalent to that found in human plasma after tomato sauce consumption [24] on proinflammatory cytokine/chemokine expression. It is noteworthy that results obtained on the expression of proinflammatory cytokines such as IL-6, MCP-1 or IL-1β (at mRNA and protein levels) in adipose tissue, in response to HFD or TNFα incubation, are consistent with *in vivo* studies [5,9,11]. In addition, the two *ex vivo* approaches, *i.e.*, HFD and TNFα incubation, revealed a similar range of induction of the different cytokine/chemokine measured. Altogether, these observations contribute to the validation of our *ex vivo* model.

In addition to the global effect of lycopene on adipose tissue inflammatory gene expression, we also showed that both adipocytes and preadipocytes participate in this phenomenon (Fig. 3). These data confirmed that lycopene can modulate the expression of proinflammatory cytokines/chemokines in both cell types in a similar manner to that which may occur in adipose tissue. It is also important to note that these results were also reproduced in human adipocytes (Fig. 4). Moreover, our *in vitro* data argue for a direct role of lycopene in proinflammatory cytokine/chemokine gene regulation, in addition to an indirect effect on the regulation of the expression of these molecules that could occur *ex vivo.*

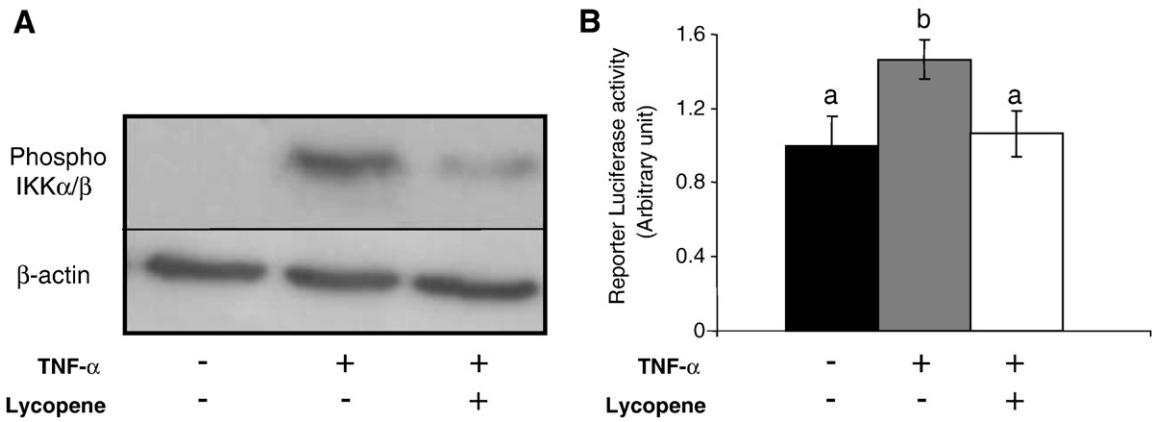

Fig. 5. Lycopene modulates the TNFα-mediated activation of the NF-κB pathway. (A) After a 24-h preincubation with lycopene, cells were treated with TNFα (15 ng/ml) for 5 min. The phosphorylation level of IKKα/β was revealed by Western blot. (B) 3T3-L1 adipocytes were transiently transfected with a reporter plasmid containing the luciferase gene under the control of four NF-kB response elements. Cells were incubated for 24 h with lycopene (2 μM) or not, before incubation with TNFα (15 ng/ml) for 3 h. Dosages of β-galactosidase and luciferase were performed as described in Materials and Methods. Mean±S.E.M.; bars not sharing the same letter were significantly different, $P<.05$.

Interestingly, a similar effect of lycopene in macrophages has recently been reported [16]. Indeed, lycopene suppressed LPS-induced NO and IL-6 production in RAW264.7 cells. These results agree with a previous study that reported an association between lycopene and the decrease of macrophage activation induced by gliadin and interferon γ [37]. Taken together, these data support the anti-inflammatory effect of lycopene in the major cellular subtypes, *i.e.*, adipocytes, preadipocytes and macrophages, which are involved in the production of proinflammatory cytokines/chemokines by adipose tissue.

From a molecular point of view, we reported that an inhibition of the TNFα-mediated activation of the NF-κB signaling pathway was involved in the anti-inflammatory effect of lycopene (Fig. 5). It is noteworthy that other phytochemicals such as procyanidins, anthocyanins, resveratrol or curcumin have been reported to display similar anti-inflammatory properties in various models including macrophages, monocytes or adipocytes, *via* a modulation of the NF-κB pathway [38–41]. The results reported here were also suggested by Feng et al. [16] in macrophages, as well as by several other studies [13,15,19,42,43]. Thus, the blockage of NF-κB activation by lycopene appears not to be tissue specific or cell type specific and could represent a major way by which lycopene controls gene expression in various cell types.

From a physiological point of view, such a regulation could be of particular interest in the field of obesity-related pathologies. Indeed, the proinflammatory cytokines and chemokines shown to be down-regulated by lycopene are deeply involved in the genesis of obesity-associated pathologies, such as insulin resistance and type II diabetes. IL-6 and IL-1β are major proinflammatory cytokines [44–46], whereas MCP-1 induces macrophage infiltration, which largely participates in the low-grade inflammation of adipose tissue [47–49]. Several mechanistic studies demonstrated that these molecules participate in the development of insulin resistance [6,31,46,50,51]. Thus, the decrease in the production of these proinflammatory cytokines/chemokines could have a major impact on the prevalence of obesity-associated pathologies and, consequently, on CVD because these metabolic pathologies are well-known risk factors of CVD. In agreement with this assumption, the EURAMIC study [52] showed that men with the highest concentrations of lycopene in adipose tissue had a 48% reduction in the risk of developing a cardiovascular disease compared with men with the lowest lycopene concentrations. Because lycopene is one of the major carotenoids in human adipose tissue [53] and because of its well-known antioxidant properties [12], the protective effect of lycopene on CVD was found to be associated with its antioxidant capacity. However, we have demonstrated here the strong ability of lycopene to prevent inflammation in adipose tissue at a physiological concentration, and this result may be considered a new mechanistic explanation for the association between lycopene and the low prevalence of CVD.

To conclude, our results demonstrate the ability of lycopene to decrease proinflammatory cytokine and chemokine expression in various adipose tissues and adipocyte models. These results could be of particular relevance in explaining, at least in part, some of the health effects of carotenoids, especially in the context of obesity-related pathologies.

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