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RESEARCH ARTICLE

Trans-palmitoleic acid does not modify the inflammasome expression in a rodent model of diet-induced obesity

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Abstract

Background: *Metabolic disorders such as obesity and type 2 diabetes (T2D) coincide with an increased expression of pro-inflammatory factors. The NLRP3 inflammasome is a complex that activates the pro-inflammatory cytokine IL-1β. (NOD-like receptor protein 3). Some nutrients, such as fatty acids, influence inflammatory processes. For example, in clinical studies, higher trans-palmitoyl acid (TP) concentrations coincide with lower adiposity and lower risk of developing T2D. This study aims to evaluate the effect of TP on NLRP3 expression in a rodent model of diet-induced obesity (DIO).* **Methods:** *C57BL/6J mice were fed ad libitum with a control or a high-fat diet (HFD), added with or without TP (3 g/kg diet), for 11 weeks. IL-1β was quantified in serum, and NLRP3-related gene expression was explored in epididymal adipose tissue.* **Results:** *Despite increased weight gain in both high-fat groups, the high-fat TP group gained less weight than the high-fat group. In addition, NLRP3 and caspase-1 expression was higher in the HFD groups, but no differences were observed between the HFD and the HFD TP groups. Serum IL-1β levels were not different among groups.* **Conclusions:** *Diet supplementation with TP prevents weight gain and has a neutral influence over NLRP3 expression and IL-1β concentration in a DIO mice model.*

Keywords: *Trans-palmitoleic acid. Inflammasome. NLRP3. Obesity. Type 2 diabetes.*

El ácido trans-palmitoléico no modifica la expresión del inflamasoma en un modelo murino de obesidad inducida por dieta

Resumen

Introducción: *Las alteraciones metabólicas como la obesidad y diabetes tipo 2 (DT2) coinciden con la expresión aumentada de factores proinflamatorios. Un complejo que induce la activación de la citocina proinflamatoria IL-1β es el inflamasoma NLRP3 (NOD-like receptor protein 3). Algunos nutrimentos, como los ácidos grasos, influencian los procesos inflamatorios. Por ejemplo, en estudios clínicos, mayores concentraciones del ácido trans-palmitoléico (TP) coinciden con una menor adiposidad y un menor riesgo de desarrollar DT2. El objetivo de este estudio fue evaluar el efecto del TP sobre la expresión del inflamasoma NLRP3 en un modelo de obesidad inducida por dieta (OID) en roedores.* **Métodos:** *Se alimentaron ratones C57BL/6J ad libitum con una dieta control o alta en lípidos (AL), adicionada o no con TP (3 g/kg dieta), durante 11 semanas. Se cuantificó la concentración de IL-1β en elsuero de los animales, y en el tejido adiposo epididimal se midió la expresión de los componentes del inflamasoma.* **Resultados:** *A pesar del aumento de peso en ambos grupos de dieta con alto*

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contenido en lípidos, el grupo alto en lípidos TP ganó menos peso que el grupo AL. Por otro lado, la expresión de genes del inflamasoma resultó mayor en los grupos AL, pero no se encontraron diferencias entre los grupos AL y AL TP. Además, no se observaron diferencias en la concentración de IL-1β en suero entre grupos. **Conclusiones:** *La dieta suplementada con TP previno el aumento del peso corporal, pero no modificó la expresión de los componentes del inflamasoma ni la concentración de IL-1β en suero.*

Palabras clave: *Ácido trans-palmitoléico. Inflamasoma. NLRP3. Obesidad. Diabetes tipo 2.*

Introduction

Insulin resistance, which is associated with obesity, is the metabolic abnormality most frequently related to type 2 diabetes (T2D). The underlying mechanisms involved in insulin resistance are decreased insulin receptor expression or its activation, reducing the activation of downstream insulin signaling molecules[1,2] and decreasing glucose uptake in cells such as myocytes and adipocytes[3].

Activation of insulin signaling mediators is reversed by the inflammatory pathway during obesity. Recently, the protein complex NLRP3 inflammasome has been associated with insulin resistance[4]. This complex comprises NLRP (nod-like receptor protein), the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), and procaspase-1. The inflammasome assembly occurs in response to glucose, saturated fatty acids, ceramides, and other compounds. The inflammasome triggers proteolytic cleavage of procaspase-1 into active caspase-1, which converts the cytokine precursors pro-IL (interleukin)-1β into mature and biologically active IL-1β[5,6]. The NLRP3 inflammasome components, the activity of caspase-1, and IL-1β concentrations are increased in visceral adipose tissue of obese rodents and humans[4,7], and these directly correlate to insulin resistance. Moreover, inhibition of the inflammasome has been suggested to improve insulin signaling in adipose tissue, liver, and skeletal muscle and increase insulin secretion in the pancreas[4,8].

Furthermore, fatty acids have been described as NLRP3 modulators[9]. Research has been conducted regarding the influence of saturated, mono-unsaturated, and poly-unsaturated fatty acids (SFA, MUFA, PUFA) over the complex activation. Several studies have shown that while palmitate induces NLRP3, oleate and linoleate oppose palmitate-driven activation of NLRP3[10]. Moreover, dietary fatty acids have been extensively studied not only for their implication in the inflammatory process but for their association with metabolic diseases such as T2D. Such is the case of *trans*-palmitoleic acid (TP), a 16-carbon, *trans*-MUFA (*trans*-C16:1, n-7), considered a biomarker of dairy consumption[11,12]. TP is found in the lipid fraction of whole milk and yogurt and, to a significantly lesser extent, in hydrogenated oils[13].

Clinical studies have demonstrated that increased serum TP is associated with lower risk and incidence of diabetes, lower adiposity, lower fasting insulin, and insulin resistance in individuals of multiethnic origin[11,14]. Animal models, specifically C57BL/6 mice supplemented with TP, confirmed that TP could be incorporated into plasma-free fatty acids, phospholipids, and triglycerides[15]. In addition, TP prevented body weight gain but not insulin resistance in a rodent model of diet-induced obesity (DIO)[16,17]. In endothelial and hepatic cell lines, TP diminished the expression of some pro-inflammatory molecules as cytokines and adhesion molecules[18]. However, pero no modificó, no studies regarding TP supplementation in DIO animal models have been designed to explore *NLRP3* expression. Therefore, the present study aimed to evaluate the effect of TP on *NLRP3* expression and IL-1β serum concentration in a rodent model of DIO.

Methods

Animals

Male C57BL/6J mice (10 weeks old) weighing an average of 23 g were fed experimental diets *ad libitum* and housed individually in a 12h light-dark cycle and controlled environment (Easy Flow, Techniplast, VA, Italy) for 11 weeks. Also, animals had free access to filtered water, high-quality bedding, and nesting environmental enrichment. All animal protocols were approved by the Ethical and Research Committees (HIM 2016/079/SSA1359), Hospital Infantil de México Federico Gómez, and carried out in accordance with the ARRIVE guidelines. Fifteen animals per diet group were randomized to achieve a similar initial body weight average. Four diets were administered: control (control: 15.9 kJ/g, 11% energy from lipids), control plus TP (control TP: 15.9 kJ/g, 11% energy from lipids, TP: 3 g/kg

Table 1. Weekly weight gain and energy intake

Parameter	Control	Control TP	High-fat	High-fat TP	p
Weight gain (g/week)	0.49 ± 0.15[c]	0.54 ± 0.14[c]	1.25 ± 0.23[a]	0.83 ± 0.16[b]	<0.05
Energy intake (kcal/week)	82.6 ± 1.0[a]	84.6 ± 0.79[a]	103.3 ± 2.8[b]	100.6 ± 1.6[b]	<0.0001

Experimental diets were administered *ad libitum* for 11 weeks, and weight and food intake were monitored weekly. Weight gain and energy intake are presented as mean ± standard error of mean (SEM). Unlike superscript letters within a row designate statistical difference; one way-Anova, a>b>c, n = 15-18.
TP: *Trans*-palmitoleic acid.

of diet), high-fat diet (high fat: 19.7 kJ/g, 44% energy from lipids), or a high-fat diet plus TP (high fat TP: high fat: 19.7 kJ/g, 44% energy from lipids, TP: 3 g/kg of diet). Body weight and food intake were monitored weekly. By week 11, animals were euthanized by cervical dislocation. Immediately, serum and epididymal adipose tissue were extracted, frozen in liquid nitrogen, and stored at -80 °C.

Real-time quantitative polymerase chain reaction

According to the manufacturer's instructions, total RNA was extracted from epididymal adipose tissue with the RNeasy mini kit (Qiagen, Hilden, Germany). The quality and integrity of RNA were confirmed by the ratio of absorbance at 260/280 nm and by inspection of the 28S and 18S rRNA bands in agarose gels. RNA was quantified and stored at -80°C. cDNA was generated by reverse transcriptase reactions using Script cDNA Synthesis Kit (Jena Bioscience, Jena, Germany) reagents. The PCR primers were obtained from Applied Biosystems (Foster City, CA) as follows: *NLRP3*, Mm00840904_m1; *Caspase-1(CASP1)*, Mm00438023_m1; *IL-1β*, Mm00434228_m1. Real-time quantitative PCR analysis was performed in 96-well plates using UNG-Master Mix in 10 ul reaction mixtures with a Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA) as described by ABI Prism User Bulletin #2, with the following settings: 50°C for 2 min, 95°C for 10 min, 50 cycles of 95°C for 15 s, and 60°C for 1 min. Samples were performed in triplicate. Results were normalized to the housekeeping gene *RPL32,* Mm02528467_g1, and analyzed with Agilent Aria software version 1.7 by using the relative quantification method (ΔΔCT).

ELISA

The serum concentration of IL-1β was determined with an ELISA (enzyme-linked immunosorbent assay) test kit from Thermo Scientific (EM2IL-1B, Rockford, IL, USA) following the manufacturer's protocols.

Statistical analysis

In order to estimate the sample size, we used our previous values of weight gain in our model, and with an effect size of 0.73, an α error of 0.05, and a statistical power of 0.95. The G*Power software calculated a total sample size of 60 for the four experimental groups. The results are presented as means ± SEM. One or two-way ANOVA, followed by Tukey's significant difference test, were used for analysis (GraphPad Prism, version 7.0). Differences were deemed significant at $p < 0.05$.

Results

Trans-palmitoleic acid supplementation prevented increased weight gain induced by a high-fat diet

As reported before by our group, this model has shown that TP added to the diet prevented further weight gain[19] (Table 1), although weight gain was increased in the high-fat groups in comparison with the control groups. Also, as previously published, total energy intake increased in animals with high-fat diets, and no difference in energy intake was noted after TP consumption.

Trans-palmitoleic acid had neutral effects on inflammasome NLRP3-related gene expression in adipose tissue and circulating IL-1β

Recentlly, we reported that high-fat diet promotes glucose intolerance and insulin resistance[17]. Because insulin resistance in obesity is associated with inflammation and NLRP3 activation, we investigated the

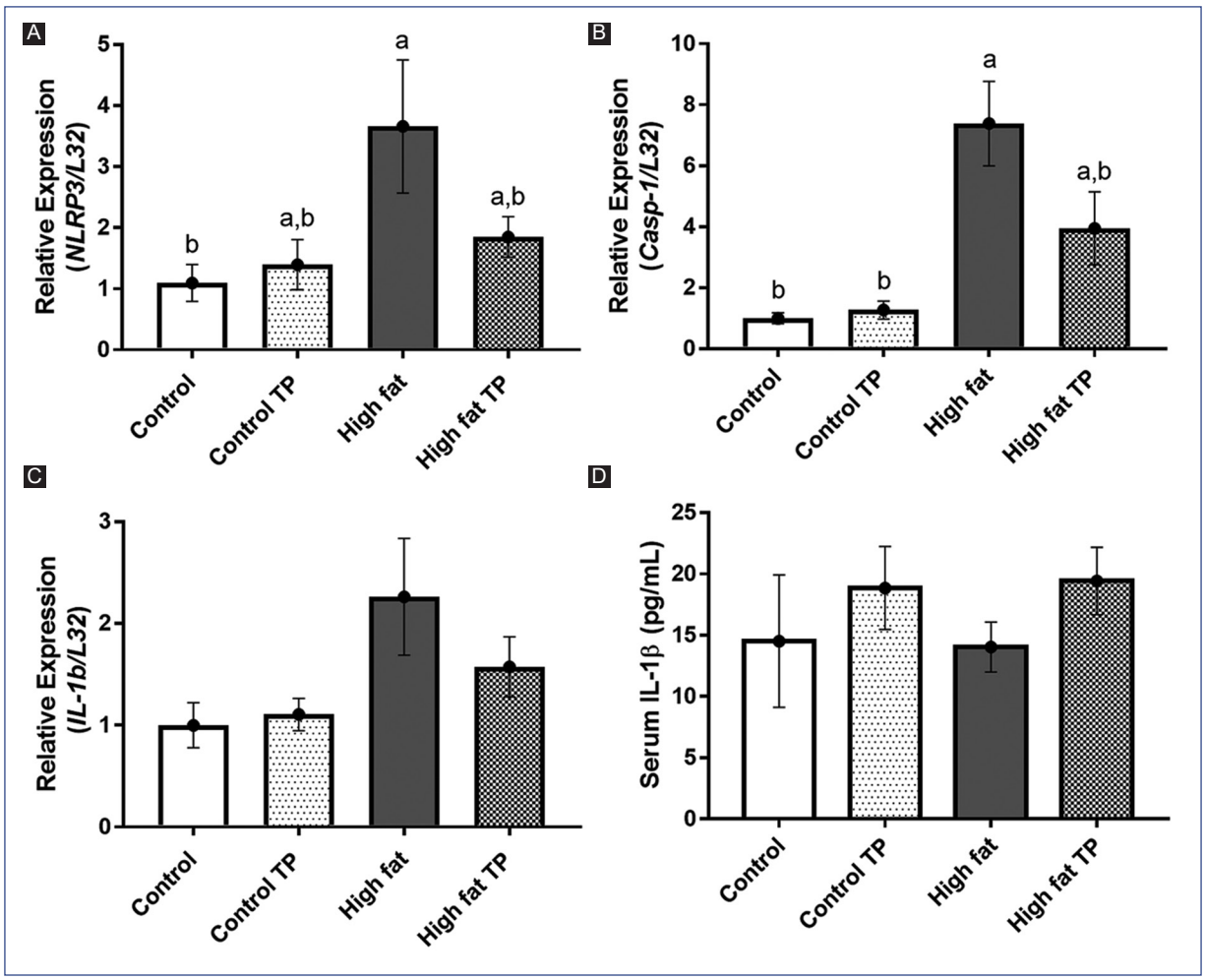

Figure 1. Effect of TP consumption on the NLRP3 inflammasome complex expression in adipose tissue of mice with control and high-fat diet. **A:** inflammasome NLRP3 and **B:** caspase-1 increased in the high-fat group, with no statistical difference after TP administration (a > b, $p < 0.05$ for NLRP3. a > b, $p < 0.005$ for Casp-1). **C:** IL -1β showed a trend to increased expression by high-fat consumption. **D:** serum IL-1β was not significantly different among groups. Data was analyzed by one-way ANOVA and Tukey's *post-hoc* test. a, b indicate significant difference, n = 8 - 15 mice per group. IL: interleukin; NLRP3: NOD-like receptor protein 3; TP: *trans*-palmitoleic acid.

expression of *NLRP3, caspase-1,* and *IL-1β*. Results showed that *NLRP3*, *caspase-1* and *IL-1β* expression increased ($p = 0.0510$) during obesity (Figures 1A-1C, comparing control and high-fat groups), consistent with other studies in obese mice, which indicate that high-fat increases *NLRP3* expression in adipose tissue[20]. No differences were observed in the control TP group; however, in the high-fat diet, a trend towards a decrease in *NLRP3* expression and its target molecules caspase-1 or IL-1β was observed with TP, although there was no significant difference (Figures 1A-1C). Also, we quantified serum IL-1β and found no differences between groups. Although IL-1β gene expression was increased in the high-fat group, IL-1β protein levels, as the effector cytokine, were not changed in serum after high-fat diet. Also, TP did not affect circulating IL-1β after consuming control or high-fat diets (Figure 1D).

Thus, the NLRP3 inflammasome was found to be overexpressed in our DIO model, but TP was not able to modify this expression in adipose tissue. In circulation, IL-1β was not different between groups.

Discussion

Obesity and its metabolic consequences, such as T2D, have become a worldwide health problem. Dietary strategies to avoid adiposity and metabolic abnormalities include modifications in carbohydrate and lipid

type, and content intake[21]. For instance, the effects of diverse fatty acids on metabolism have been extensively studied. Typically, the consumption of *trans*-fatty acids has been associated with cardiovascular risk, but epidemiological data has shown neutral effects of TP on dyslipidemia and cardiovascular disease[22], stroke[14], and blood pressure[11].

Regarding insulin resistance and T2D, clinical studies have found a 42% less risk of T2D associated with higher levels of serum TP[23]. As these chronic abnormalities are linked to inflammation, we aimed to discover if the consumption of TP decreased inflammasome *NLRP3* expression in a rodent model of DIO.

First, obesity was generated by administering 45% of energy as fat in the high-fat groups, and evaluated by weight gain (Table 1). Increased weight gain and adiposity in our model concurred with glucose intolerance and insulin resistance after performing glucose and insulin tolerance tests[16,17]. Subsequently, we explored the gene expression of the inflammasome components *NLRP3, caspase-1* and *IL-1β. NLRP3,* and *caspase-1* were overexpressed in adipose tissue of mice fed high-fat diet, consistent with other studies. In fact, ablation of *NLRP3* in mice prevents obesity and activation of inflammasome in adipose tissue and liver while enhancing insulin signaling[8]. In the present study, no statistically significant differences were observed despite the decreasing trend in the expression of inflammasome-related genes observed in the high-fat TP group. *NLRP3* has not been investigated after oral TP supplementation; however, organelle stress and inflammation in macrophages were prevented by the TP isoform, *cis*-palmitoleate, in a mice study[24]. Other fatty acids have been related to NLRP3, generally demonstrating that MUFAs and PUFAs prevent NLRP3 activity[25], but no studies have suggested an association between trans-MUFAs or other ruminant fatty acids and NLRP3 activation.

Finally, since the activation of NLRP3 induces IL-1β production, we measured serum IL-β but found no differences between groups. Indeed, we expected increased cytokine levels in the high-fat group. However, some authors have proposed that IL-1β may be a paracrine regulator that acts locally and never reaches the blood in mild inflammatory conditions such as obesity[26]. In mice, high-fat diet induced an increased concentration of IL-1β in portal blood but not in the systemic circulation, supporting its potential role as an endocrine mediator in adipose-liver crosstalk[27]. Thus, it is plausible that IL-1β acts locally in this model. Future investigations should be conducted to clarify the effect of TP over alternative inflammation-related proteins in circulation.

In conclusion, we showed that supplementation with TP prevents weight gain and does not modify *NLRP3*-related genes expression and IL-1β concentration in a DIO mice model.

Ethical disclosures

Protection of human and animal subjects. The authors state that the procedures followed were conformed to the ethical standards of the committee on responsible animal experimentation.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on animal experimentation.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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