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Caffeic acid phenethyl ester reduces the activation of the nuclear factor κB pathway by high-fat diet-induced obesity in mice

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ABSTRACT

Objective. The aim of this study was to investigate the effect of CAPE on the insulin signaling and inflammatory pathway in the liver of mice with high fat diet induced obesity.

Material/Methods. Swiss mice were fed with standard chow or high-fat diet for 12-week. After the eighth week, animals in the HFD group with serum glucose levels higher than 200 mg/dL were divided into two groups, HFD and HFD receiving 30 mg/kg of CAPE for 4 weeks. After 12 weeks, the blood samples could be collected and liver tissue extracted for hormonal and biochemical measurements, and insulin signaling and inflammatory pathway analyzes.

Results. The high-fat diet group exhibited more weight gain, glucose intolerance, and hepatic steatosis compared with standard diet group. The CAPE treatment showed improvement in glucose sensitivity characterized by an area under glucose curve similar to the control group in an oral glucose tolerance test Furthermore, CAPE treatment promoted amelioration in hepatic steatosis compared with the high-fat diet group. The increase in glucose sensitivity was associated with the improvement in insulin-stimulated phosphorylation of the insulin receptor

Abbreviations: CAPE, caffeic acid phenethyl ester; HFD, high-fat diet; AUGC, area under glucose curve; OGTT, Oral glucose tolerance test; IRS, insulin receptor substrate; JNK, c-jun N-terminal kinase; IKKβ, inhibitor of nuclear factor kappa B kinase beta subunit; IκB, inhibitor of nuclear factor kappa B; NFκB, nuclear factor kappa B; TNF-α, tumor necrosis factor-α; FFA, free fatty acids; PKCζ, protein kinase Cζ; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; IKK, inhibitor of NFκB kinase; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; UNICAMP, University of Campinas; AST, plasma aspartate aminotransferase; ALT, alanine aminotransferase; PFA, paraformaldehyde; H&E, hematoxylin and eosin; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; IRS-1, insulin receptor substrate-1; IRS-2, insulin receptor substrate-2; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; anti-pY, anti-phosphotyrosine; IR, insulin receptor; NE-PER, Nuclear and Cytoplasmic Extraction Reagents Kit; SEM, standard error of the mean; ANOVA, one-way analysis of variance.

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substrate-2, followed by an increase in Akt phosphorylation. In addition, it was observed that CAPE reduced the induction of the inflammatory pathway, c-jun-N- terminal kinase, the nuclear factor kappa B, and cyclooxygenase-2 expression, respectively.

Conclusions. Overall, these findings indicate that CAPE exhibited anti-inflammatory activity that partly restores normal metabolism, reduces the molecular changes observed in obesity and insulin resistance, and therefore has a potential as a therapeutic agent in obesity.

1. Introduction

Obesity is characterized by an altered balance between energy intake and expenditure due to eating habits and lifestyle. Therefore, the incidence of obesity in children has been increasing worldwide and closely associated with higher risks of cardiovascular disease, diabetes, insulin resistance, cancer, and other pathophysiological changes in adult life [1,2].

Suffice to say, type 2 diabetes resulting from obesity is currently considered an inflammatory disease, leading to new perspectives for understanding these pathologies [3]. Factors such as tumor necrosis factor-α (TNF-α), free fatty acids (FFA), and oxidative stress inhibit insulin signaling and induce insulin resistance by activating serine/threonine kinases capable of phosphorylating proteins such as the insulin receptor substrate (IRS). Serine phosphorylated IRSs are implicated in the reduction of protein activation in the insulin signaling cascade [4,5]. In addition, c-jun N-terminal kinase (JNK), protein kinase Cζ (PKCζ), inhibitor of nuclear factor kappa B kinase beta subunit (IKKβ), mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR) kinases are not only involved in the mechanism of negative feedback, but also are considered strong candidates for participating in the molecular alterations involved in insulin resistance [6]. Among the serine/threonine kinases, the phosphorylation of IKKβ leads to the activation of nuclear factor kappa B (NFκB), a transcription factor involved in immune/inflammatory responses and a potent inducer of insulin resistance [6,7]. In an experimental model of obesity and insulin resistance, anti-inflammatory agents such as aspirin-like drugs, produced a favorable effect on insulin resistance in high-fat diet (HFD) fed mice expressing a heterozygous knockout of IKK2 (catalytic subunit of the inhibitor of NFκB kinase – IKK complex) [8].

Several synthetic and natural compounds have been shown to inhibit the activation of NFκB and exert anti-inflammatory effect both in vitro and in vivo [9–11]. Caffeic acid phenethyl ester (CAPE), one of the active compounds of propolis, has been proven to inhibit NFκB [12,13]. Despite involving mechanisms which are not entirely clear, research has shown that CAPE plays an important role in anti-inflammatory, anticarcinogenic, and immunomodulating activity in different animal models [14,15]. Some studies have reported that the anti-inflammatory action of CAPE is due to the inhibition of arachidonic acid release in the cell membrane, which suppresses the activity of cyclooxygenase-1 and -2 (COX-1 and COX-2) and inhibits the activation of COX-2 gene expression [13,16].

In a study with cultured cells, CAPE inhibited the induction of NFκB activation by a wide variety of inflammatory agents such as TNF-α, phorbol ester, ceramide, okadaic acid, and hydrogen peroxide. This inhibition occurred by blocking the interaction of NFκB with DNA and not by inhibiting IκBα degradation [10]. In neurons, CAPE presented a pharmacological profile similar to other anti-inflammatory drugs, such as salicylic acid and dexamethasone, and similar to antioxidants such as pyrrolidine dithiocarbamate. Also, it reduced nuclear translocation of NFκB and secretion of TNF-α and nitric oxide production in brain tissue [17].

In this study, we investigated the effect of CAPE on the inflammatory pathway and insulin signaling in the liver of HFD-induced obese mice.

2. Methods

2.1. Experimental protocols

In all the experiments we used 3-week old male Swiss inbred strain mice, originally imported from the Jackson Laboratory (Bar Harbor, ME, USA) and currently bred at the University of Campinas (UNICAMP) Breeding Center (Campinas, SP, Brazil). All experiments were performed in accordance with the guidelines of the Brazilian College for Animal Experimentation and approved by the Ethics Committee of the UNICAMP (protocol n° 888-1).

The animals were maintained on a 12-h artificial light-dark cycle and housed in individual cages. After a 3-day acclimatizing period, the mice were randomly divided into two groups. In each experiment the control group (n = 10) was fed standard rodent chow (Nuvilab CR-1 autoclaved; Nuvital S/A, Colombo, PR, Brazil) ad libitum and the HFD group (n = 20) was fed fat-rich purified diet AIN93 ad libitum for 12 weeks (Table 1).

Blood samples were taken at 9:00 am, after a 4-h fast, on the seventh and on the eighth weeks of HFD administration. After the eighth week, animals in the HFD group presenting with serum glucose levels higher than 200 mg/dL (55% of the

Table 1 – Composition of control and high-fat diets.

Composition	Nuvilab CR-1 (autoclaved)	Hyperlipidemic modified AIN 93 (HFD)
Carbohydrate (%)	56% vegetable starch	20% vegetable starch
Protein (%)	30% vegetable protein	20% casein
Lipid (%)	14% unsaturated oils	60% – 88.64% lard, 11.36% vegetable oils
Caloric content (Kcal/g)	2.9	5.5