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Research paper

Oleanolic acid improves diet-induced obesity by modulating fat preference and inflammation in mice

Fatima Zohra Djeziri [a], Meriem Belarbi [a], Babar Murtaza [b], Aziz Hichami [b], Chahid Benammar [a], Naim Akhtar Khan [b, *]

[a] Laboratoire des Produits Naturels, Université Abou-Bekr Belkaïd, Tlemcen, 13000, Algeria
[b] Physiologie de la Nutrition & Toxicologie, UMR U1231 INSERM/Université de Bourgogne-Franche Compté (UBFC), Dijon, 21000, France

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ABSTRACT

Obesity, triggered by high-fat diet (HFD), is associated to altered gustatory perception of dietary lipids. Oleanolic acid (OLA), a triterpene, has been reported to exert anti-obesity effects in animal models. Hence, we investigated the role of OLA in the modulation of oro-sensory perception of lipids in control and HFD-induced obese mice. As expected, OLA-treated obese mice exhibited a decrease in body, liver, and visceral adipose tissue weights. OLA treatment improved glucose tolerance, insulin level, plasma lipopolysaccharide (LPS), and hepatic cholesterol and triglyceride concentrations. OLA-treated obese mice exhibited higher fat preference compared to untreated obese mice, probably due to the increase in mRNA encoding CD36, a fat taste receptor, in mouse taste bud cells (mTBC). This phenomenon was associated with fatty-acid induced increases in free intracellular calcium concentrations, $[Ca^{2+}]i$, induced in mTBC from OLA-treated obese mice. OLA also influenced the expression of mRNA encoding pro-inflammatory cytokines (IL-1β and IL-6) and some lipogenic genes (PPARα, SREBP1, FAS, ChREBP, and G6Pase) in liver and adipose tissue. These findings reveal that OLA improves gustatory perception of lipids and exerts protective effects in obesity.

1. Introduction

Obesity has become a worldwide epidemic, characterized by an excessive accumulation of fat in the adipose tissue. According to World Health Organization (WHO) [1], there are more than 1.9 billion of adults who are overweight, and more than 650 million of adults are clinically obese. Obesity is believed to increase the risk for type II diabetes, hypertension, dyslipidemia, liver dysfunction, and various types of cancers [2–4]. It is widely accepted that eating fat-rich food may lead to high calorie intake that might contribute to increased fat accumulation. Indeed, a number of studies have demonstrated a link between increased fat accumulation and chronic inflammation, thus promoting insulin resistance in obese subjects [5,6].

Obesity is also associated with high preference for dietary lipids in rodents and humans [7]. One of the plausible mechanisms implicated in the attraction for dietary lipids is that there might be a 6th taste modality, devoted to the oro-sensory detection of long-chain dietary fatty acids [7]. Indeed, several clinical studies have shown that obese children [8], teenagers [9] and adult participants [10,11] exhibit high detection thresholds for dietary fatty acids. The diet-induced obesity has also been associated with low lingual detection of fatty acids in mice [12]. Hence, low oro-sensory detection of lipids will further contribute to high dietary fat intake in obese subjects.

The Mediterranean diet pattern has been a subject of great interest due to its association with low weight gain [13–15]. This beneficial effect has been attributed to its richness in olive oil [16,17] and phytonutrients such as antioxidants [18–21]. Olive oil, the principal source of fat in the Mediterranean diet, exerts beneficial effects in health and disease as it contains, in high amounts, monounsaturated fatty acids, mainly oleic acid, and bioactive components, including tocopherols, phospholipids, biophenols and

Abbreviations: STD, Standard diet; HFD, High-fat diet; $[Ca^{2+}]i$, free intracellular Ca^{2+} concentrations; OLA, Oleanolic acid; IGTT, Intraperitoneal glucose tolerance test; TL, Total lipid; TG, Triglyceride; LPS, Lipopolysaccharide; FA, Fatty acid; mTBC, mouse taste bud cells; SCD1, Stearoyl-CoA desaturase-1.

* Corresponding author. Physiologie de la Nutrition &Toxicologie, UMR U1231 INSERM/AgroSupDijon/Université de Bourgogne-Franche Compté (UBFC), 6, boulevard Gabriel, Dijon, 21000, France.
E-mail address: Naim.Khan@u-bourgogne.fr (N.A. Khan).

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triterpenic acids [22–24]. The main triterpene of virgin olive oil is oleanolic acid [25]. Pérez-Camino and Cert [26] have reported that oleanolic acid (OLA) is present in the olive fruit at 420 ± 20 mg/kg, while olive oil might contain up to a concentration of 57.2 ± 7.4 mg/kg. OLA has also been reported to be present in edible fruits and vegetables [27]. Raisins have been reported to contain 79.0 mg/100 g of OLA [28]. As far as the biological properties of OLA are concerned, this triterpenoid has been shown to exert antioxidant, antidiabetic and hepatoprotective actions [29–31]. Interestingly, de Melo et al. [32] have demonstrated that OLA exerts anti-obesity effects by improving visceral adiposity and glucose tolerance in obese mice.

Since Mediterranean diet exerts beneficial effects in obesity, and fat intake is altered in this pathology, it was thought worthwhile to undertake the present study to assess the effects of OLA on the modulation of fat preference in diet-induced obesity in the mouse.

2. Materials and methods

2.1. Materials

The standard diet was purchased from SAFE (France). The palm oil was obtained from Huilerie Vigean (France). Oleanolic acid, oleic acid, and xanthan gum were procured from Sigma-Aldrich (USA). Glucose was from Merck (France). TRIzol was purchased from Invitrogen (USA). The rat/mouse insulin ELISA kit was obtained from EMD Millipore (USA). The commercial cholesterol and triglyceride (TG) assay kits were from DiaSys Diagnostic Systems (Germany). The High Capacity cDNA Reverse Transcription Kit was bought from Applied Biosystems (Lithuania). Reference standard 68A was from Nu-Chek Prep (USA). All of the solvents and other chemicals were obtained from Sigma (USA).

2.2. Mice and diets

C57BL/6J female mice, aged between 6 and 10 weeks (16–20 g), were obtained from Janvier Labs (France). They were housed individually in a controlled environment with a 12 h light/dark cycle with food available *ad libitum*. The palm oil supplied the main fat component in high-fat diets. The different diets and their fatty acid compositions are indicated in Table 1 and Table 2. The diets were prepared weekly and stored at 4 °C until further use. The study was conducted as per Declaration of Helsinki and European ethical guidelines for the care and use of animals for experimentation. All the experimental protocols were approved by the Regional Ethical Committee of the University of Burgundy (Dijon, France).

2.3. Diet-induced obesity

C57BL/6J female mice were grouped at random (n = 6/group) and fed with either of the following diets: standard diet (STD) or high-fat diet (HFD). Another group of mice, maintained on HFD, was given oleanolic acid in water feeders at 0.005% (w/v) for 16 weeks. This group was termed as HFD + OLA. The OLA stock solution was prepared in 3% (v/v) Tween-80 at 0.05% (w/v). STD and HFD-fed mice received the same vehicle, *i.e.*, Tween-80 (0.3% v/v). OLA or vehicle was changed twice a week. The body weight, food, and water intake were measured, weekly.

After 16 weeks of feeding the diets, animals were fasted overnight and then sacrificed by cervical dislocation, without anesthesia to avoid any further stress. Blood glucose concentration was measured to assess glycaemia. Plasma was immediately stored at −80 °C until analysis. Tongues were rapidly removed and placed in Tyrode solution for papillae isolation. Liver and visceral adipose tissue were removed, weighed (expressed in mg/10 g of body weight), and stored at −80 °C.

Table 1
Composition of the diets.

Content (%)	STD	HFD
Proteins	66.8	40.07
Starch	16.10	14.6
Fats	3.10	35.3
Cholesterol	–	0.03
Cellulose	3.9	2.7
Vitamins	5	3.4
Minerals	5.1	3.9
Energy (Kcal/100 g)	359.5	536.65
Fat energy (% of total energy)	8	60

Standard diet (STD); high-fat diet (HFD).

Table 2
Acid composition of the diets.

Fatty acids (%)	STD	HFD
SFA	18.82	45.85
MUFA	26.38	40.02
PUFA	54.8	14.13

Standard diet (STD); high-fat diet (HFD).
SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids.

2.4. Two-bottle preference test

The preference for lipid solution was performed by using two-bottle preference test as described elsewhere [33]. Before starting the experiment, mice were accustomed to water drinking in two bottles for a week. During the period of overnight (12 h), each mouse was subjected to two-bottle choice between control and experimental solutions. In the experimental solution, 0.2% (w/v) of oleic acid was added. The xanthan gum at 0.3% (w/v) was used as a control solution. The liquid intake was calculated by weighing the bottles before and after the experiment (as g/12 h).

Table 3
Sequences of different primers.

Gene	Primer sequence
β-Actin	Forward: ATGGAGGGGAATACAGCCC
	Reverse: TTCTTTGCAGCTCCTTCGTT
CD36	Forward: GGCCAAGCTATTGCGACATG
	Reverse: CCGAACACAGCGTAGATAGAC
PPARα	Forward: AGAGCCCCATCTGTCCTCTC
	Reverse: ACTGGTAGTCTGCAAAACCAAA
PPARγ	Forward: ATCTTAACTGCCGGATCCAC
	Reverse: AGGCACTTCTGAAACCGACA
SREBP-1	Forward: TCAACAACCAAGACAGTGACTTCCCTGGCC
	Reverse: GTTCTCCTGCTTGAGCTTCTGGTTGCTGTGTG
FAS	Forward: GGCTCTATGGATTACCCAAGC
	Reverse: CCAGTGTTCGTTCCTCGGA
ACC1	Forward: CGGACCTTTGAAGATTTTGTGAGG
	Reverse: GCTTTATTCTGCTGGTGTAACTCTC
ChREBP	Forward: CTGGGGACCTAAACAGGAGC
	Reverse: GAAGCCACCCTATAGCTCCC
G6Pase	Forward: CGACTCGCTATCTCCAAGTGA
	Reverse: GTTGAACCAGTCTCCGACCA
IL-1β	Forward: GGGCCTCAAAGGAAAGAATC
	Reverse: TACCAGTTGGGGAACTCTGC
IL-6	Forward: GACAACCACGGCCTTCCCTA
	Reverse: GCCTCCGACTTGTGAAGTGGT
TNFα	Forward: GGCAGGTCTACTTTGGAGTCATTGC
	Reverse: ACATTCGAGGCTCCAGTGAATTCGG