Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 125 (2024) 109549

Journal of Nutritional Biochemistry

RESEARCH PAPER

Body weight gain and control: beneficial effect of extra virgin olive oil versus corn oil in an experimental model of mammary cancer

Raquel Moral[a,*], Garyfallia Kapravelou[a], Marta Cubedo[b], Montserrat Solanas[a], Eduard Escrich[a,*]

[a] Department of Cell Biology, Physiology and Immunology, Physiology Unit, Faculty of Medicine, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain
[b] Department of Statistics, Universitat de Barcelona, Barcelona, Spain

Received 31 October 2023; received in revised form 2 December 2023; accepted 13 December 2023

Abstract

Obesity is a known risk factor for breast cancer, the most common malignancy among women worldwide. We have previously described different effects of high-fat diets on mammary experimental carcinogenesis. In this work, we analyzed the animal growth data obtained in six experimental assays, in healthy and carcinogen-induced rats undergoing different dietary interventions. The animals were fed with three experimental diets administered at different periods of development: a control low-fat diet, and two isocaloric high-fat diets (rich in corn oil or in extravirgin olive oil -EVOO-). Weekly weight throughout the development of 818 animals have been compiled and reanalyzed using adjusted mathematical models. Molecular mechanisms have been investigated: ethanolamides in small intestine, neuropeptides controlling satiety in hypothalamus, and proteins controlling lipid metabolism in adipose and mammary tissues. The results indicated that the effect of diets depended on type of lipid, timing of intervention and health status. The high corn oil diet, but not the high EVOO diet, increased body weight and mass, especially if administered from weaning, in healthy animals and in those that received a moderate dose of carcinogen. The potential protective effect of EVOO on weight maintenance may be related to anorexigenic neuropeptides such as oxytocin and lipolysis/deposition balance in adipose tissue (increasing phospho-PKA, HSL, MGL and decreasing FAS). In animals with cancer, body weight gain was related to the severity of the disease. Taken together, our results suggest that EVOO has a beneficial effect on body weight maintenance in both health and cancer.

Keywords: Dietary lipids; Extra virgin olive oil; Body weight; Breast cancer; Molecular mechanisms; Lipolysis.

1. Introduction

The Mediterranean diet was acknowledged as an Intangible Cultural Heritage of Humanity by UNESCO in 2010. This diet includes a variety of food patterns from the Mediterranean region and is characterized, among other factors, by the consumption of olive oil as the principal source of fat [1]. A wealth of evidence supports the health benefits of this diet, associated with a reduced risk of mortality, and incidence of major chronic diseases, such as cardiovascular disease, cancer andobesity [2]. These results are of great importance since the prevalence of chronic diseases is a global public health problem. For example, worldwide obesity prevalence has nearly tripled from 1975 to 2016 and continues to rise [3]. Prospective studies have found an inverse association between adherence to the Mediterranean diet and obesity risk, weight gain, and abdominal obesity [4–6].

Several studies have also associated overweight and obesity with an increased risk of breast cancer [7]. This cancer is also an important public health problem and a leading cause of mortality in women worldwide [8]. Although the risk for breast cancer is multifactorial, there is a substantial contribution of environmental factors such as diet, attracting great interest since they are modifiable risk factors [7,9]. Epidemiological and especially experimental studies have found a link between dietary lipids and this neoplasia. This influence depends, foremost, on the total amount of fat consumed, but also on the specific type of dietary lipid [10]. Diets rich in n-6 polyunsaturated fatty acids (PUFA), as well as diets rich in saturated or trans fatty acids, enhance experimental tumorigenesis,

Abbreviations: AEA, N-arachidonoyl ethanolamide; DMBA, 7,12-dimethylbenz(a)anthracene; EVOO, extravirgin olive oil, Exp, experiment; FA: fatty acids, HCO, high corn oil; HOO, high extravirgin olive oil; LA, linoleic acid; LF, low-fat; MUFA, monounsaturated fatty acids; OA, oleic acid; OEA, oleaoyl ethanolamide; PUFA, polyunsaturated fatty acids.

* Corresponding authors at: Edifici M, Avinguda de Can Domènech, Bellaterra 08193 (Cerdanyola del Vallès), Barcelona, Spain. Tel.: +34 93 5811341.
E-mail addresses: Raquel.Moral@uab.cat (R. Moral), Eduard.Escrich@uab.cat (E. Escrich).

https://doi.org/10.1016/j.jnutbio.2023.109549

whereas n-3 PUFA has shown inhibitory effects, and the effects of monounsaturated fatty acids (MUFA) remains unclear [10]. In humans, different dietary patterns have been associated with cancer risk, and Mediterranean diet has been reported to decrease breast cancer risk and mortality [11,12]. Although it is more difficult to establish associations with dietary components, prospective cohort studies have suggested a protective effect of olive oil consumption in hormone-independent breast tumors [13]. The beneficial effects of healthy dietary patterns on the development of this disease can account, at least in part, by their influence on body composition. Body weight and adiposity have been associated with breast cancer mortality and recurrence [7,14]. This cancer has been linked as well to other parameters related to nutrition and body growth, such as early menarche, which is an established risk factor for this disease [7]. Thus, nutritional exposures in early life that influence growth, sexual maturation, and adiposity may have an impact on later risk for this neoplasia.

Due to the difficulty of obtaining data with controlled variables in humans, animal models are widely used to get an insight into the influence of dietetic factors in health. Using the experimental model of mammary cancer chemically induced with 7,12-dimethylbenz(α)anthracene (DMBA) in the female Sprague-Dawley rat [15], we found a differential effect of diets high in seed oils (rich in n-6 PUFA) and in extra virgin olive oil (EVOO) on clinical behavior and histopathological features of experimental mammary tumors. While a diet rich in n-6 PUFA had a clear stimulating effect, the influence of a diet high in EVOO was compatible with a potential protective effect [10,16–18]. Such differential influence can be aroused by multiple and complex mechanisms, including body growth and adiposity [19,20]. To get an insight into the effects that diets rich in n-6 PUFA or in EVOO can exert on body mass, in this work we analyzed animal growth data obtained in six experimental assays, in healthy and carcinogen-induced rats undergoing different dietary interventions. For this, the weight data throughout the development of 818 animals belonging to those previous experiments have been compiled and re-analyzed, using adjusted mathematical models. Further, molecular mechanisms associated with such effects have also been investigated, including changes in hypothalamic factors controlling satiety and lipid metabolism in adipose and mammary tissue.

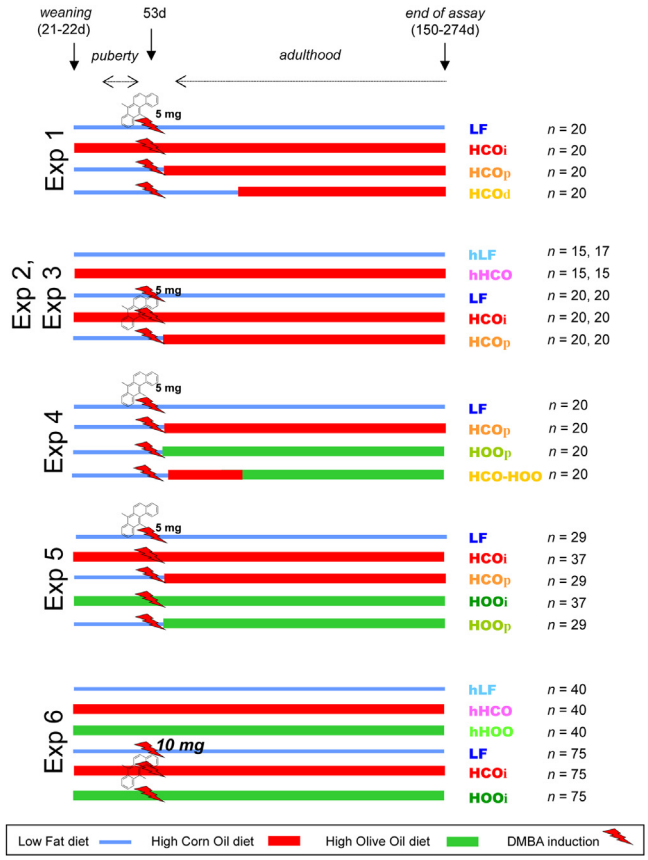

Fig. 1. Experimental designs. Female Sprague-Dawley rats were fed the low-fat control diet (LF), the high-corn oil diet (HCO) or and the high-extra virgin olive oil diet (HOO). Healthy animals (noninduced) were fed the experimental diets from weaning (hLF, hHCO, hHOO groups). Induced animals were fed with the experimental diets at different timings: with the LF diet from weaning (LF group); with the HCO diet from weaning (HCOi), after induction (HCOp) or from mid-adulthood (HCOd); and with the HOO diet form weaning (HOOi), after induction (HOOp), or after a period of intervention with the high corn oil diet (HCO-HOO). Exp: experiment. Exp2 and Exp3 had the same design.

2. Material and methods

2.1. Diets

Three semisynthetic diets were designed, a low-fat diet (3.71 kcal/g, with 7.3% calories in the form of fat), and two high-fat diets (4.56 kcal/g, with 39.5% calories in the form of fat). The control low-fat diet contained 3% (weight/weight, -w/w-) of corn oil, the high corn oil diet contained 20% of such oil, and a high olive oil diet contained 3% of corn oil and 17% of extra virgin olive oil. Carbohydrates (dextrose) were 73% calories (67.9% -w/w-) in the low-fat diet, and 40.3% calories (45.9% -w/w-) in both high-fat diets. Protein, in the form of casein, was 18% -w/w- in the low-fat diet (19.4% calories), and 23% -w/w- in both high-fat diet (20.2% calories). All diets had 5% -w/w- cellulose, 5.9% -w/w- salt-mixture and 0.24% -w/w- vitamin mixture. The definition, preparation and suitability of the experimental diets were previously described [21,22,16]. Diets were prepared weekly and stored under nitrogen in the dark at 4ºC (Supplementary Table 1).

2.2. Animals and experimental designs

All animals received humane care under the protocols approved by the Animal and Human Experimentation Ethics Committee at the Universitat Autònoma de Barcelona, following the legislation applicable in this country.

Different independent experimental series were designed in order to study the effects of the diets, administered at different timing, in healthy and DMBA-induced female Sprague-Dawley rats (Charles River Lab.; stain Crl: OFA [SD]; Iffa-Credo -Lyon, France-). Fig. 1 summarizes the design of each experiment (Exp), and Supplementary Fig. 1 compiles and depicts the different experimental groups depending on the type and time of dietary intervention. All animals arrived at 22–23 days of age, were housed 3–4 per cage in a controlled environment maintained 22±2ºC, 50% relative humidity and a 12:12-h light to dark cycle. Carcinogen-induced rats were gavaged with one single dose of 5 mg (experiments 1–5) or 10 mg (experiment -Exp- 6) of dimethylbenz(a)anthracene (DMBA, Sigma-Aldrich) at 53 days of age (Fig. 1). The experimental diets and water were administered ad libitum. Animals were euthanized by decapitation (Exp 1–5) or isoflurane anesthetized and exsanguinated by cardiac puncture (Exp6).

Experiment 1: Animals were randomly distributed into four experimental groups (n=20 each): LF, receiving control diet throughout the experiment; and 3 groups receiving the high corn oil diet, from weaning (initiation group, HCOi), from induction with DMBA (promotion group, HCOp), or from day 157 once the tumors had

appeared (development group, HCOd). All animals were induced with 5 mg of DMBA (Sigma-Aldrich) and they were euthanized at an average age of 214 days [23].

Experiment 2 and 3: Animals were distributed into five experimental groups. Two healthy groups were fed with control (hLF, n=15 -Exp2-, n=17 -Exp3-) or high corn oil diet (hHCO, n=15) from weaning. Three groups were induced with 5 mg of DMBA and fed with control diet from weaning (LF, n=20), or the high corn oil diet from weaning (initiation group, HCOi n=20) or from induction (promotion group, HCOp n=20). Rats were euthanized at an average age of 254 days (Exp2) or 274 days (Exp3).

Experiment 4: Animals were distributed into four groups (n=20 each) and induced with 5 mg of DMBA. All rats received the control diet from weaning to induction. From day 54, they were fed with control (LF), with high corn oil (HCOp), or with high olive oil diet (HOOp) to sacrifice. HCO-HOO group received the high corn oil diet from day 54 to day 80, and the high olive oil diet onwards. Rats were euthanized at an average age of 261 days [16].

Experiment 5: Animals were distributed into five groups: LF (n=37) receiving control diet throughout the experiment; two groups receiving the high corn oil, from weaning (HCOi, n=37) or from induction (HCOp, n=29); and two groups receiving the high olive oil diet, from weaning (HOOi, n=37) or from induction (HOOp, n=29). All animals were induced with 5 mg of DMBA. Animals were euthanized at several ages of development (36, 51, 100 days), and at the end of the experiment (average age 246 days) [24].

Experiment 6: Animals were distributed into six groups. Three healthy groups were fed throughout the study with the control (hLF, n=40), the high corn oil (hHCO, n=40) or the high olive oil (hHOO, n=40) diet. In parallel, three groups of animals induced with 10 mg of DMBA were also fed from weaning with control (LF, n=75), the high corn oil (HCO, n=75) or the high olive oil (HOO, n=75) diet. Animals were euthanized at different ages of development, and at the end of the experiment (average age 150 days).

In all the experiments, 1–2 days before induction, all animals were fed the control diet so that the type of diet did not influence the absorption of the carcinogen. Animal monitoring and body weight measurement was performed at least once a week. The day of the euthanasia the animals were examined, weighted and body length (nose-to-anus) were measured. The phase of the estrous cycle was also determined by vaginal cytology, and animals in the diestrus phase were chosen for euthanasia. Depending on the experiment, after sacrifice different organs (hypothalamus, small intestine, adipose tissue, mammary gland, in addition to the mammary tumors) were collected and flash frozen and stored at -80ºC for the molecular analyses, while blood was centrifuged and the plasma was stored at -20ºC.

2.3. Body weight and mass

Growth studies in each experiment were performed from weekly body weight measurements throughout the assays. On days of euthanasia body mass was determined using the Lee Index [10000 × (g)⅓ / cm] [25,26] and the Body Mass Index (BMI) [g / cm2].

2.4. Ethanolamides quantification by UPLC MS/MS

Samples (proximal and distal small intestine) were prepared by homogenization of 50 mg of tissue in 1 mL cold methanol/acetonitrile (1:1) with 5ng deuterated standards (OEA-d4 and AEA-d8, Cayman) and centrifugation at 10,000 g. Supernatant was separated, speed-vac desiccated and resuspended in 200μL water/methanol (70:30). Sample ethanolamides were purified in the DISCOVERY DSC-18LT columns (Sigma-Aldrich), eluted with methanol and speed-vac dried. Ethanolamides quantification was performed by UPLC MS/MS at the Servei de Metabolòmica de la UAT (Vall d'Hebron). In brief, acetonitrile-resuspended samples were analyzed in duplicates (100μL each) in the Waters Acquity UPLC system coupled with a Xevo TQ-S triple quadrupole mass spectrometer with electrospray ionization interface (Waters) using CORTECS UPLC C18 1.6μm columns (Waters). The mobile phase consisted of 0.1% formic acid milliQ-water (v/v, solvent A) and 0.1 formic acid in acetonitrile (v/v, solvent B) with a flow rate of 0.3 mL min−1. Optimized parameters for the mass spectrometer detector were spray voltage, 3 kV; capillary temperature, 270°C; collision energy, 17 V; argon as the collision gas. For calibration and linearity, six-point concentration standards ranging 0.1–100 ng/mL OEA (Sigma-Aldrich) and AEA (Cayman) were prepared and analyzed in triplicate.

2.5. Analysis of neuropeptides by luminex assay

For the determination of neuropeptides, total protein extracts from hypothalamus were obtained by tissue lysis in 20mM NaHPO4-Na2PO4 pH7.8, 100mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulphate, 0.025% sodium azide, 0.5% Na Deoxycholate, 1 mM phenylmthanesulfonyl fluoride, 5mM NaF, 0.1 mM Na3VO4 and a protease inhibitor cocktail (Sigma-Aldrich), and centrifugation at 14,000g. Peptides (orexin A, substance P, alpha-MSH, oxytocin and neurotensin) were analyzed using the LuminexMAP technology (Milliplex Map Rat/Mouse Neuropeptide Magnetic Bead Panel - Neuroscience Multiplex Assay, Millipore). Protein extracts were precipitated with acetonitrile, dried and reconstituted in buffer assay. Samples were run in duplicated. Peptide quantification was performed by immunoassay on the surface of fluorescent-coated magnetic beads, following manufacturer's instructions. Plate was run on MAGPIX with xPONENT software, and peptide concentration in samples was calculated by analyzing the median fluorescent intensity data using a 5-parameter logistic method.

2.6. Western blot

Total protein extracts were obtained from abdominal adipose tissue (visceral fat, perirenal-retroperitoneal white adipose tissue) abdominal mammary gland, and hypothalamus. Tissues were homogenized in 50mM Tris HCl pH 8.0, 150mM NaCl, 1% Igepal, 0.5% deoxycholic acid, 1% sodium dodecyl sulfate and 10 μl/ml protease inhibitor cocktail (Sigma-Aldrich). Determination of relative protein levels was performed by Western blot analyses. Briefly, samples were subjected to SDS-PAGE on 7.5–12% acrylamide Mini-Protean TGX Stain Free Gels and electrophoretically transferred to nitrocellulose membranes using Trans-Blot Turbo Transfer system (Bio-Rad). The primary antibodies and the dilutions used were: anti-NPY (Cell Signaling, 1:1000), antiphospho PKA (Cell Signaling, 1:1000), anti-ATGL (Thermofisher Scientific, 1:15000), anti-HSL (Thermofisher Scientific, 1:5000), anti-MGL (Abcam, 1:5000). Anti-Cidec (Thermofisher Scientific, 1:1000), anti-Plin (Abcam 1:3000), anti-Fas (Cell Signaling, 1:10000). Membranes were incubated with the secondary peroxidase-conjugated antibodies (Sigma, 1:3000 dilution) and Luminata Forte Western HRP Substrate (EMD Millipore) luminogen. The specific bands were visualized by Chemi-DocTM XRS+ Imaging system using Image Lab Software 5.1 Beta (BioRad Laboratories, Hercules, CA, USA). Densitometric values were first normalized to total protein loaded, and then to an internal control of pooled samples loaded in duplicate in all the blots.

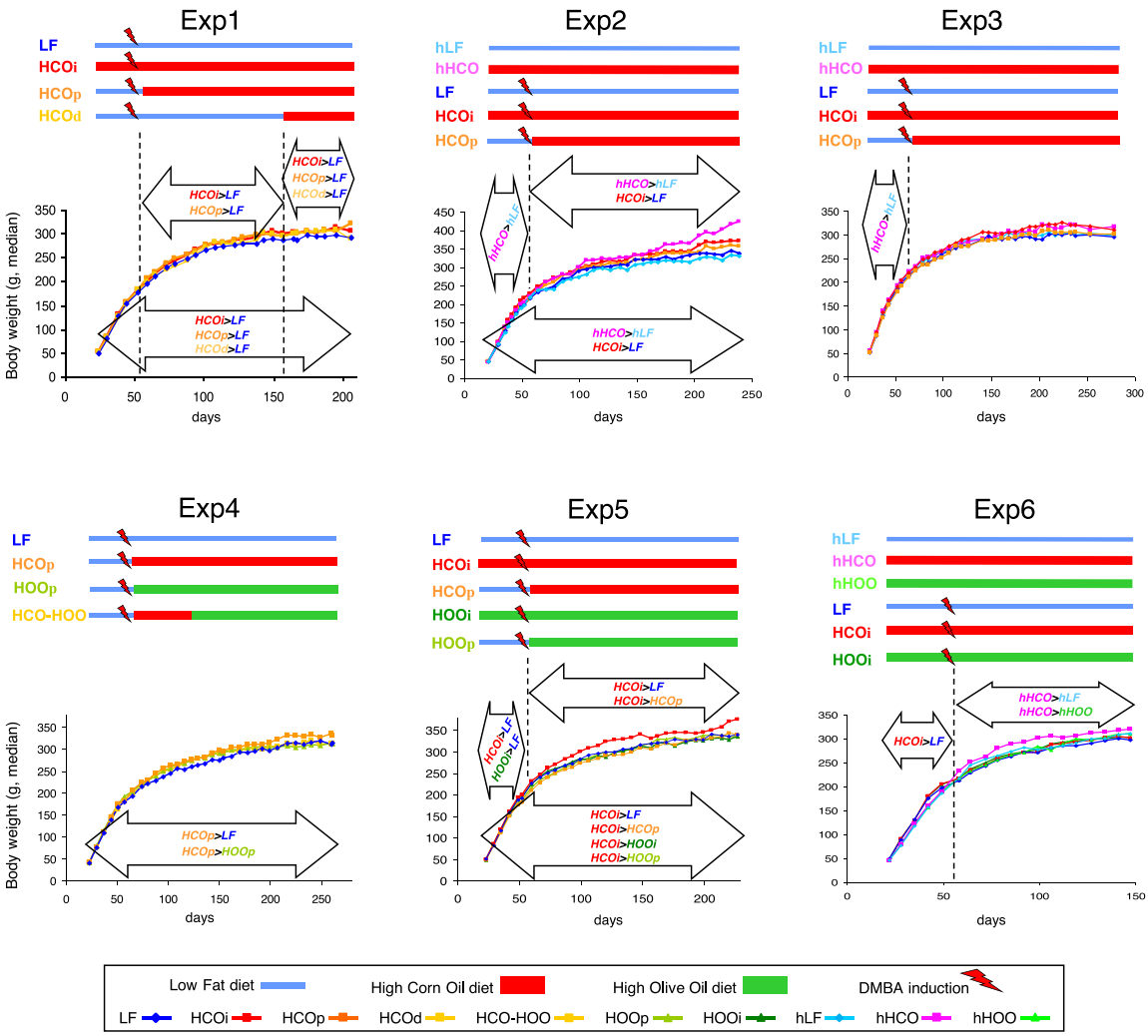

Fig. 2. Body weight over time in each experiment. Values represent medians of the groups. Data was analyzed with Liniar Mixed-effects Model methodology. The arrows cover the time periods in which differences between groups were found. The groups with significant differences are indicated within the arrows. Exp: experiment.

2.7. Statistical analysis

Body weight was analyzed using Linear Mixed-effects Model (LMM) methodology. Data was analyzed in several lifespan periods with different dietary interventions (before induction, after induction, throughout the experiment). LMM considers the fixed effects (common behavior of each group or diet), the random effects (effect of each individual) and that each individual has different weight measurements over time (repeated measurements). For each experiment and period studied, LMM has been used to fit the mathematical model best fitting weight curves values, based on the evolution of the weight of the individuals over time or on a function of time (transformation used in order to normalize the residuals of the model). With this methodology it is possible to incorporate the autocorrelation structure of the residuals, allowing, in a simple way, to correct heteroscedasticity problems of the model by incorporating an adequate variance. Once two or more models have been fitted, to choose the best one we performed the likelihood ratio test to compare nested models two by two. When the models were not nested, the comparison was made using the Akaike's Information Criterion (AIC). After obtaining the best model, we performed analyses to determine if the fixed factor "diet" has a significative effect with an ANOVA test and a Tukey multiple comparisons test. The level of significance considered throughout the study was 0.05 (P-value $<.05$). The statistical analysis has been carried out with the free software R-4.2.1 for Windows and the LME function of the NLME package [27].

All other analyses (body mass, molecular results) were performed with the SPSS software (version 15.0). Data distribution of each variable studied was determined by Kolmogorov-Smirnov test, and the equality of variances among groups was determined by Levene's test. Analysis of parametric quantitative data was performed with ANOVA followed by Tukey's test. Nonparametric quantitative data was analyzed with the Friedman and the Mann-Withney's U test. Qualitative data was analyzed with the Pearson's chi-squared test. Correlations were carried out through Kendall's Tau-b and Spearman's Rho tests. Differences were considered significant when P-value $<.05$.

3. Results

3.1. The effect of high-fat diets on body weight depends of type of fat, developmental period, time of dietary intervention, and health status

Body weight over time of the animals fed the experimental diets at different timing in each experiment is shown in Fig. 2. Moreover, the evolution of each high-fat diet group compared to LF

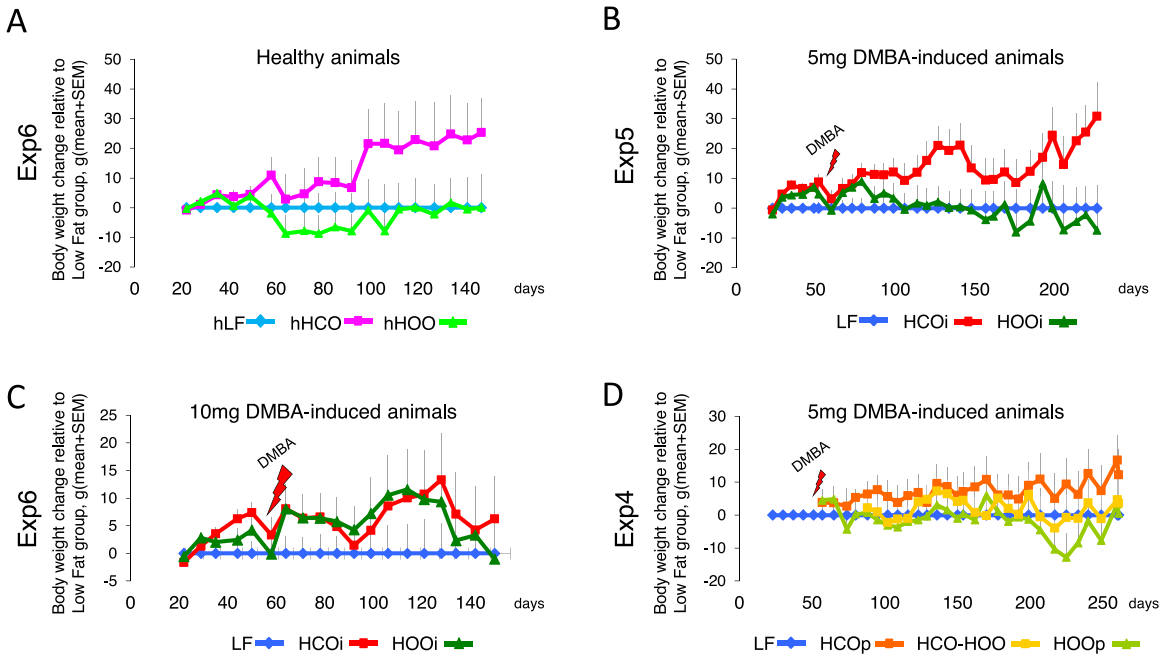

Fig. 3. Body weight change relative to the low-fat group. Body weight change in healthy animals fed the high fat diets throughout the experiment (A). Body weight change in animals induced with 5 mg of carcinogen (B) or 10 mg of carcinogen (C) and fed the high-fat diets throughout the experiment. Body weight change in animals fed the high-fat diets after DMBA-induction (D). Data represent mean+SEM.

control is depicted in Supplementary Fig. 2 (healthy animals) and Supplementary Fig. 3 (carcinogen-induced animals). Analyses were performed comparing groups in different periods (before induction, after induction, and all lifespan, Supplementary Table 1). Results showed differences in body weight depending on the type of diet, time of dietary intervention, and health status. Thus, at prepubertal and pubertal periods (from weaning to induction at 53 days), the HCO diet, compared to the LF diet, increased body weight in most experiments (all assays except Exp1), while the effect of the high EVOO diet was smaller (increased body weight in Exp5 but not in Exp6). When the long-term diet intake was analyzed (from induction onwards or all lifespan), the two high-fat diets showed more clearly a differential effect. In healthy animals, body weights from the hHCO group were higher than those from control hLF (Exp2, Exp6) and hHOO group (Exp6). Similar effects were observed in the animals induced with 5 mg of DMBA, in which dietary intervention with the high corn oil diet from weaning (HCOi group) resulted in increased body weights when compared with low-fat and with high EVOO diets. In some experiments we also observed this effect if the high corn oil diet was administered after induction (HCOp group; Exp1, Exp4). In the animals induced with a higher dose of DMBA (10 mg, Exp6) no effect of the high-fat diets on body weight was observed. On the other hand, the long-term high EVOO diet intake did not increase body weight (Exp4, Exp5, Exp6).

Comparison of the two isocaloric high-fat diets also showed a differential effect on body weight evolution, especially in the long-term, in the three experiments including both diets (Exp4, Exp5, Exp6, Fig. 2). Fig. 3 shows the body weight change relative to the low-fat group. In healthy animals, the weight change was clearly higher in the animals fed with the HCO diet, but not in those fed with the isocaloric high EVOO diet (Exp6). A similar profile was obtained in animals induced with 5 mg of DMBA (Exp5), but there were no differences in body weight with a stronger carcinogenic insult (10 mg of DMBA, Exp6). Although less evident, when the diet was administered after induction with 5 mg of DMBA, greater body weight gain was also observed in the animals fed the HCO diet than in those fed the high EVOO diet (Exp4).

The results of body weight and body mass indexes (Lee Index and BMI) at the end of the experiments are shown in Fig. 4. We found increased body mass indexes by effect of the HCO diet when it was administered from weaning, marginally significant in healthy animals from Exp2 (hHCO *vs.* hLF), but more evident in carcinogen-induced animals (Exp1, Exp2, Exp3, Exp5; HCOi *vs.* LF).

3.2. Intestinal levels of ethanolamides

The levels of N-arachidonoyl ethanolamide (AEA) and oleaoyl ethanolamide (OEA), potential mediators of the effects of dietary lipids on body weight control, were determined in the small intestine (proximal and distal thirds) of healthy rats from Exp6.

The diet high in EVOO increased the OEA levels in proximal and distal small intestine, especially compared with the high n-6 PUFA diet (statistically significant at all ages except at 100 days), and also in relation to the control group at 52 days of age (Fig. 5A). Comparison of curves confirmed the different OEA levels by effect of diets in proximal small intestine (lower in hHCO, intermedium in control, higher in hHOO), and in distal small intestine (lower values in hHCO). On the other hand, no differences were found in the AEA levels (Fig. 5B).

3.3. Hypothalamic levels of orexigenic and anorexigenic factors

The levels of the orexigenic factors Orexin A, Susbtance P and NPY (Fig. 6A), and the anorexigenic factors Oxytocin, Neurotensin and alpha-MSH P (Fig. 6B) were determined in the hypothalamus of healthy animals from Exp6. Results showed variable levels depending on dietary intervention and age. Some statistical differences were found in the levels of orexigenic factors, indicating that the high EVOO diet decreased the Substance P by 58 days and NPY by 150 days (Fig. 6A). Regarding the anorexigenic peptides, the

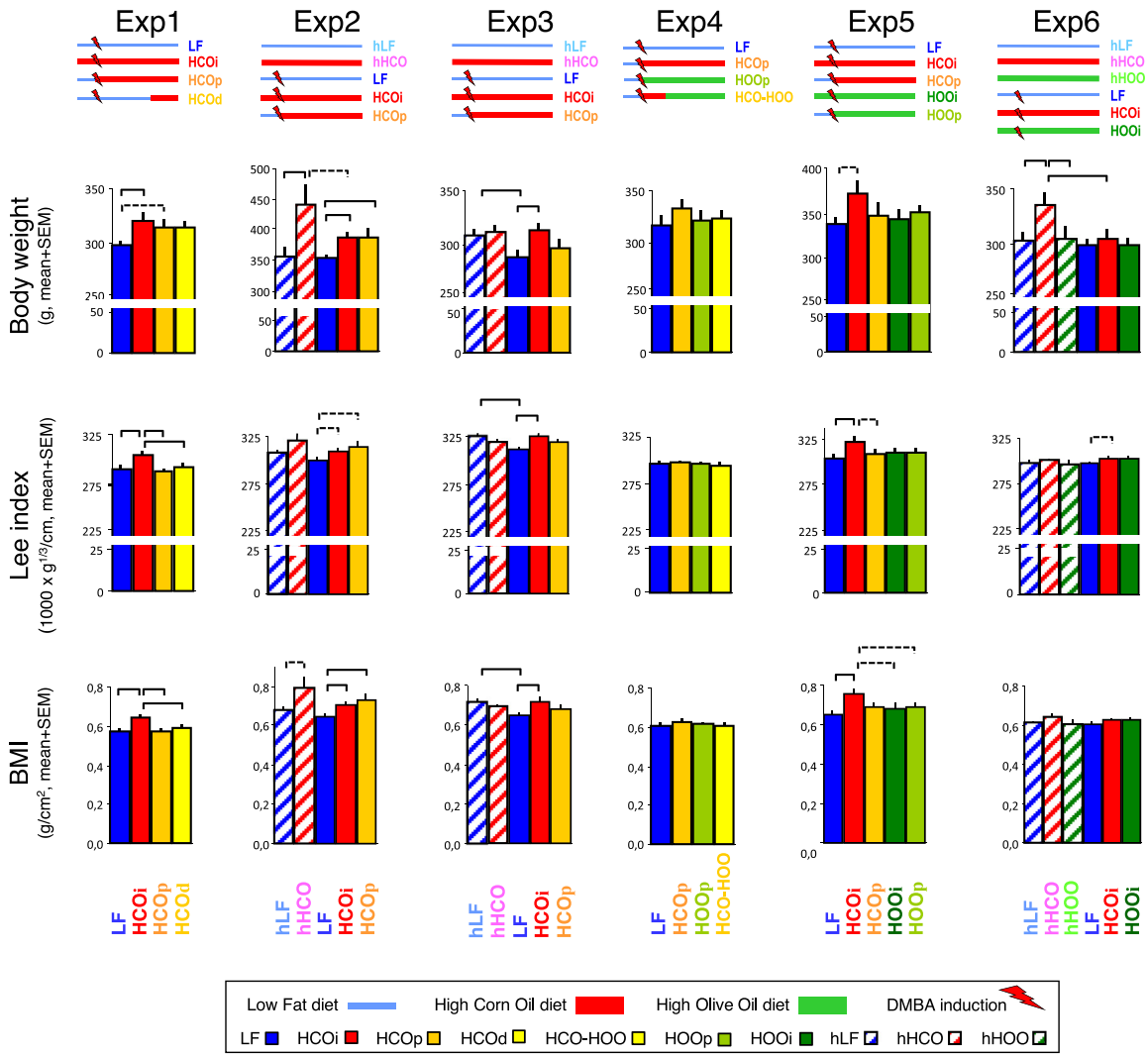

Fig. 4. Body weight and body mass indexes (Lee index and BMI) at the end of the experiments. Mean+SEM, solid lines connecting groups represent statistically significant differences ($P<.05$), dotted lines represent differences close to significance ($P<.1$), Tukey test.

EVOO diet increased Oxytocin by 100 days, while the HCO diet decreased neurotensin by 52 and 58 days of age. The effect of the EVOO diet on alpha-MSH depended on age (Fig. 6B).

3.4. Lipolysis, lipid deposit, and lipogenesis in abdominal adipose and mammary tissues

Levels of key proteins controlling lipolysis (phospo-PKA, ATGL, HSL, and MGL), lipid deposit (Cidec, Plin1, and the ratio pPKA/Plin1) and lipogenesis (FAS) were analyzed in abdominal adipose tissue (Fig. 7) and in mammary glands (Fig. 8) from Exp6.

In adipose tissue of healthy animals, the high EVOO diet increased most of the lipolysis proteins (marginally for pPKA, significantly for HSL and MGL). In induced animals, the EVOO diet also increased the levels of MGL. Moreover, the comparison of healthy and induced animals showed an effect of the cancer disease increasing the levels of these lipolysis proteins (significantly for pPKA in control group, for ATGL and HSL in HCOi group, and for MGL in HOOi group) (Fig. 7A).

The levels of deposit proteins in adipose tissue (Fig. 7B) did not change by the effect of diets in healthy animals, while Cidec, and Plin1 were increased in the induced HOOi group. Cidec was also increased by the effect of the disease in the EVOO group (HOOi vs hHOO), and Plin1 was increased in both induced groups fed high-fat diet (HCOi *vs.* hHCO; HOOi *vs.* hHOO).

Finally, the lipogenesis main protein (FAS, Fig. 7C) was decreased by the effect of the EVOO diet in healthy animals. Moreover, cancer disease upregulated FAS in the animals fed the control diet. Thus, the FAS levels were increased in LF group in comparison to the noninduced control group, and also in comparison to the induced high-fat diet groups.

Fig. 8A depicts the results of lipolysis proteins in mammary gland. No effects of diets were found in healthy animals. In induced animals, the high EVOO diet increased the levels of MGL. Comparison of healthy and induced groups showed an effect of the disease decreasing in general the levels of these enzymes, except for ATGL. In relation to the deposit proteins (Fig. 8B), no differences were observed by the effect of diet in the healthy groups, and we found an increase in Cidec by the EVOO diet in induced animals. On the other hand, comparison of healthy and induced groups indicated a decrease in Cidec (HCOi) and Plin1 (all groups), while an increase in the ratio pPKA/Plin1 (all groups) by effect of the disease. Regarding the lipogenesis (Fig. 8C), FAS was decreased by the EVOO diet in healthy animals, and by both high-fat diets in induced animals. FAS was also decreased in HCOi by effect of the disease.

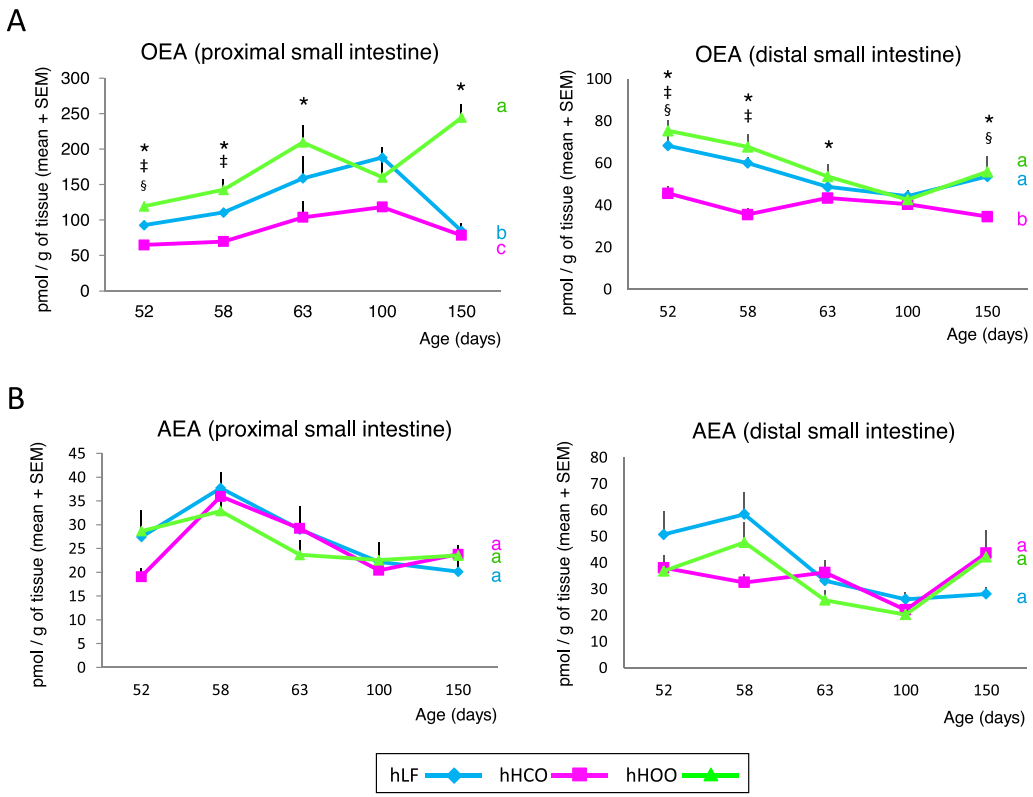

Fig. 5. Ethanothanolamides in proximal and distal small intestine at different ages. A: Levels of oleaoyl ethanolamide (OEA). B: Levels of N-arachidonoyl ethanolamide (AEA). Mean+SEM, *: $P<.05$ hHOO *vs.* hHCO; ‡: $P<.05$ hHOO *vs.* LF; §: $P<.05$ LF vs hHCO, Tukey test ($n=5$ / group / age, Exp6). Curves with different letters indicate significantly different evolution.

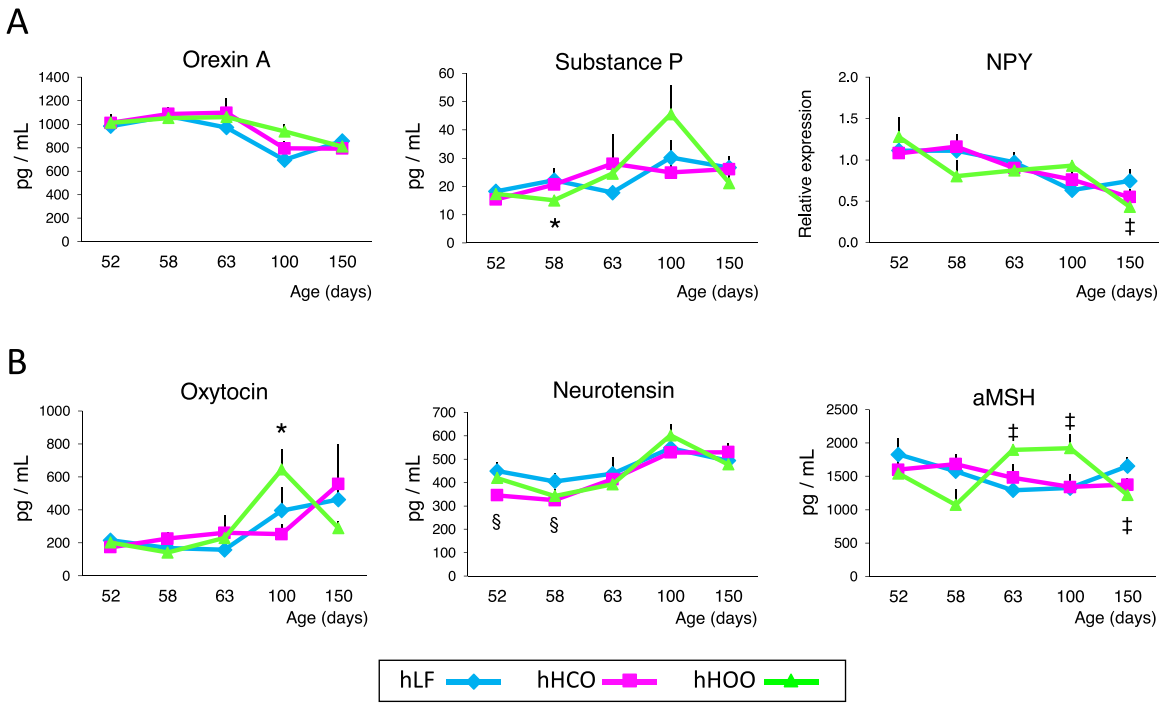

Fig. 6. Neuropeptides in hipothalamus. A: Levels of orexigenic factors determined by luminex (orexin A, substance P -pg/mL-) or Western blot (NPY -relative levels-). B: Levels of anorexigenic factors determined by Luminex (pg/mL). Mean+SEM, *: $P<.05$ hHOO vs. hHCO; ‡: $P<.05$ hHOO vs LF; §: $P<.05$ LF *vs.* hHCO, Tukey test ($n=5$ / group / age, Exp6).

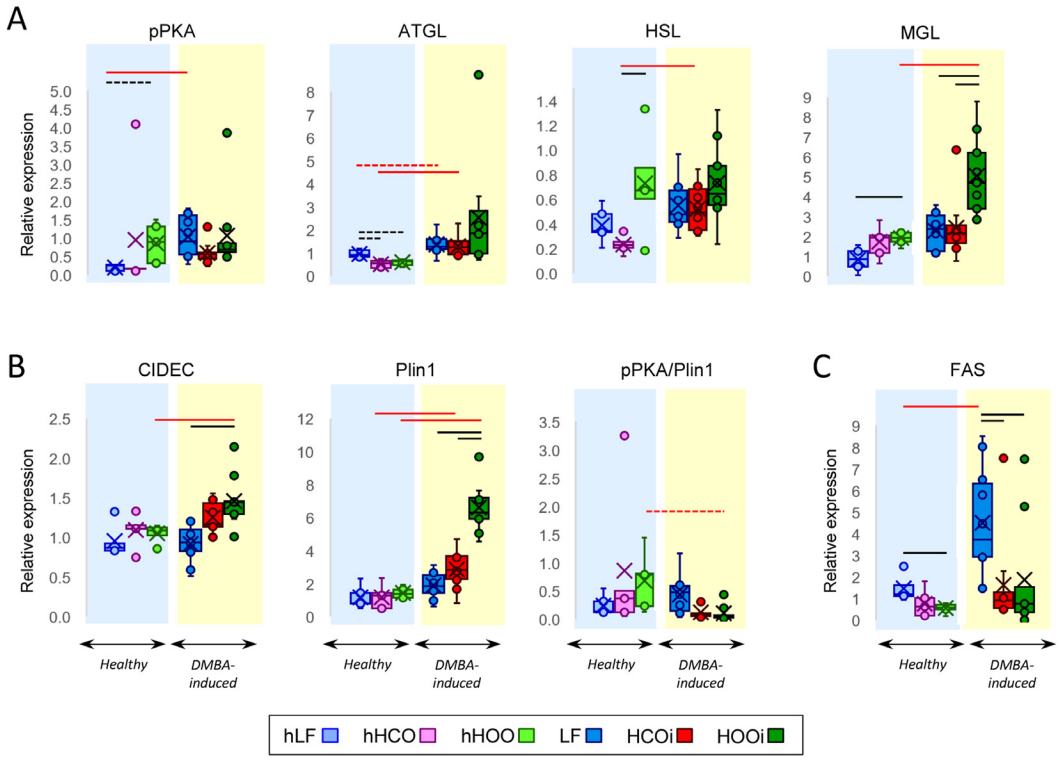

Fig. 7. Lipolysis, lipid deposit, and lipogenesis in abdominal adipose tissue. A: Relative levels of the lipolysis proteins phospho-PKA, ATGL, HSL, and MGL. B: Lipid deposit proteins (Cidec, Plin1) and ratio phospho-PKA/Plin1. C: Relative levels of the lipogenesis protein Fas. Boxplots (median, box 25 and 75 percentile, whisker 10 and 90 percentile); cross (mean). Blue background frame the healthy groups, yellow background frame the DMBA-induced groups. Solid black and red lines connecting groups indicate statistically significant differences ($P<.05$), dashed lines indicate differences close to significance ($P<.1$), Tukey test ($n=5$ / group / age, Exp6). Black lines indicate comparisons between diet groups, red lines indicate comparisons between healthy and DMBA-induced groups.

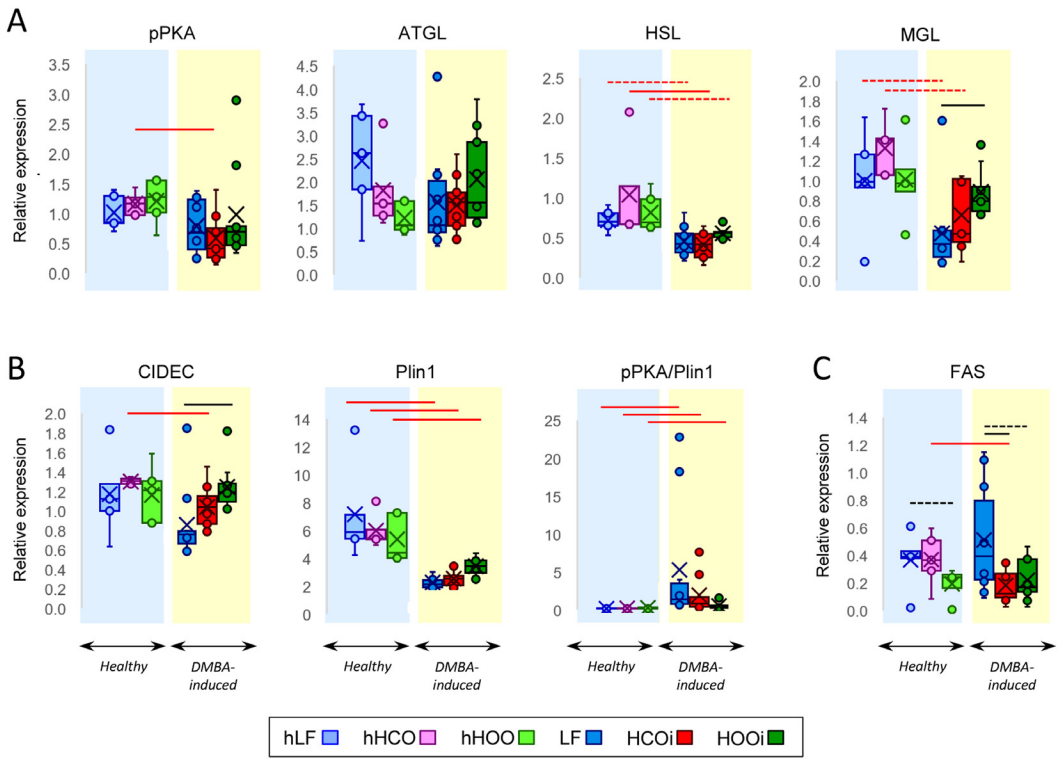

Fig. 8. Lipolysis, lipid deposit and lipogenesis in abdominal mammary gland. A: Relative levels of the lipolysis proteins phospho-PKA, ATGL, HSL, and MGL. B: Lipid deposit proteins (Cidec, Plin1) and ratio phospho-PKA/Plin1. C: Relative levels of the lipogenesis protein Fas. Boxplots (median, box 25 and 75 percentile, whisker 10 and 90 percentile); cross (mean). Blue background frame the healthy groups, yellow background frame the DMBA-induced groups. Solid black and red lines connecting groups indicate statistically significant differences ($P<.05$), dashed lines indicate differences close to significance ($P<.1$), Tukey test ($n=5$ / group / age, Exp6). Black lines indicate comparisons between diet groups, red lines indicate comparisons between healthy and DMBA-induced groups.

4. Discussion

In this work we have demonstrated, both in healthy and tumor-bearing animals, a different effect of two isocaloric high fat diets on body weight, with a diet high in corn oil (rich in n-6 PUFA) increasing body weight and mass, while a diet high in EVOO (rich in MUFA and minor bioactive compounds) had no effect. These diets were not designed to cause obesity, but to demonstrate the specific effects of each lipid, not calories, in promoting experimental carcinogenesis [21,22]. The percentage of oil used in these isocaloric high-fat diets was 20% (w/w, which corresponds to 39.5% calories from fat), a percentage similar to human Western dietary patterns [28], while diet induced-obesity animal models usually provide 45–60% of calories in the form of fat [29].

The effects of the diets have been tested at different periods of development, highlighting their influence after both short and chronic dietary intervention. The HCO diet administered from weaning significantly increased body weight and mass in healthy animals and in those induced with low doses (5 mg) of DMBA. The different effect of both high-fat diets was more evident after chronic administration, but it was also observed after short-term administration. Thus, body weight evolution from weaning to induction, which corresponds to the puberty development, was clearly increased in the animals fed with the HCO diet, while a slighter effect was observed by the high EVOO diet. The stronger effect of the HCO diet at early ages of development, though may be quantitatively small, is of great relevance since it is associated to an advance in the puberty onset, as we previously observed [19]. Obesity and early pubertal maturation are known risk factors of human breast cancer [7,30], and our results suggested that this influence on body weight and maturation are mechanisms underlying the stronger stimulatory effect of the high corn oil diet, in comparison to the high EVOO diet, in mammary carcinogenesis [24,31,32]. Obesity has been linked with chronic systemic low-grade inflammation, driving immune dysfunctions and increased proinflammatory cytokines such as IL-1 [30]. In this sense, although the animals were not obese, we have previously reported that the HCO diet increased leptin and IL1 plasma levels, while the high EVOO diet increased infiltration of cytotoxic T lymphocytes in tumors [20,24,31]. Other authors have also reported that olive oil has a beneficial effect in maintaining body weight in models of obesity in rodents [33–35], but there is little evidence of such effects on tumor-bearing animals.

The effect of the high corn oil diet on body weight also depended on the health status. It is well known that several human and experimental cancers elicit a systemic state of the disease with loss of adipose tissue and skeletal muscle, inducing inflammation and energy imbalance that cause the body to mobilize local and systemic deposits to maintain tumor growth [36,37]. Concordant with this, in animals induced with higher doses of carcinogen (Exp6), in which we observed a more aggressive manifestation of the disease (especially in the group fed the HCO diet), no differences in body weight were found [38]. On the other hand, with the low dose of carcinogen, the long-term effect of the HCO diet was mainly observed if dietary intervention started at early ages (HCOi groups), but some experiments also indicated an effect if the administration started after induction (HCOp groups). Interestingly, in Exp5, in which the HCOp showed no differences in body weight with the control group, HCOp tumors displayed the most aggressive degree of malignancy [39].

Several mechanisms could be at the basis of the different effects of high-fat diets on body weight, such as control of food intake and fat deposition. Oleoyl ethanolamide (OEA) has been suggested as a mediator of the beneficial influence of olive oil on body composition [40]. OEA and other ethanolamides such as arachidonoyl ethanalamide (AEA) are bioactive molecules derived from fatty acid precursors. OEA is formed from oleic acid and has been proposed as a gut-derived satiety factor involved in lipid oxidation and lipolysis through activation of PPAR-alpha [40]. AEA is derived from the n-6 PUFA arachidonic acid. Here we have observed that the diet high in EVOO (rich in oleic acid) increased the levels of OEA in small intestine, while no differences where observed in AEA levels. In hamsters, diets rich in olive oil increased OEA and decreased food intake [41]. Thus, we next investigated hypothalamic factors controlling food intake, including orexigenic (orexin A, substance P, NPY) and anorexigenic (oxytocin, neurotensin, alpha-MSH) factors [42] in healthy animals at several ages. Heterogenous levels of these neuropeptides were found, with few differences that were in line with a decrease in the orexigenic peptides by effect of the high EVOO diet (substance P, NPY) and a decrease in neurotensin (anorexigenic) by effect of the HCO diet. The high EVOO diet also increased the anorexigenic factor oxytocin by 100 days, concordant with previous results [20]. In the literature few studies have addressed the effects of this oils in the hypothalamic control of food intake. In this sense, in mice, a diet rich in olive oil increased the hypothalamic expression of POMC and CART, neuropeptides controlling satiety and energy expenditure [43].

On the other hand, we analyzed the effects of these diets on the levels of key proteins in lipid mobilization, deposition, and synthesis processes in visceral abdominal adipose tissue and mammary gland. Complete hydrolysis of triacylglycerides to fatty acids requires the consecutive action of three enzymes: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL). Acute lipolytic response is controlled by PKA. In relation to fat deposition, Perilipin 1 (Plin1) restricts access of lipases to lipid droplets, while CIDEC (or fat-specific protein 27, FSP27), enhances triacylglyceride deposition and lipid droplet size. Finally, fatty acid synthase (FAS) catalyzes the de novo synthesis of fatty acids [44,45]. Our results in abdominal adipose tissue and in mammary tissue suggest different scenarios due to the effect of the diets in health, the effect of the cancer disease, and the effect of dietary lipids in tumor-bearing animals. The results in adipose tissue of healthy groups suggested greater lipolysis due to the influence of the EVOO diet (higher levels of pPKA, HSL, and MGL), while no effect on deposition markers (CIDEC, Plin1). The EVOO diet also decreased the lipogenic enzyme FAS. On the other hand, when comparing the healthy and carcinogen-induced control groups, cancer disease enhanced both lipolysis (increased ATGL) and lipogenesis (clear increase of FAS). This effect could be related to the supply of fatty acids to the tumor, which could send signals to adipose tissue to release fatty acids [36]. The effect of high-fat diets in carcinogen-induced animals did not show clear trends, since we observed upregulation of both lipolysis (MGL) and fat deposition (Plin1 and CICEC) proteins by the high EVOO diet.

In mammary gland, the study of these proteins showed heterogeneous results. This gland is a complex tissue, formed mainly by adipose tissue, but with many other cell types such as epithelial (ductal and alveolar) and myoepithelial cells. The greatest differences found have been due to cancer disease, that decreased the expression of most of the proteins studied. In this sense, Plin1 was down-regulated in all induced groups, which is in agreement with published results in humans [46]. Moreover, the higher ratio activated PKA (pPKA) / Plin1 suggested an increased lipid mobilization in all groups, concordant with a generalized upregulation of lipolysis induced by the disease [36].

Taken together, our results suggest that EVOO has a beneficial effect on body weight maintenance in both health and cancer. In healthy animals, and despite being administered in high quantities, this oil could have effects on satiety control (*e.g.*, more anorexigenic hypothalamic neuropeptides such as Oxytocin), and

on lipolysis/deposition balance facilitating the former (increased pPKA, HSL, MGL, decreased FAS) in abdominal adipose tissue. In animals with cancer, body weight would be influenced by the severity of the disease. High-fat diets could decrease the need to mobilize depots by providing dietary lipids. The beneficial effect of olive oil on body weight can be due to both its high oleic acid content and its minor bioactive compounds. EVOO minor compounds such as polyphenols has shown to induce reduced body weight gain in several experimental models [47–49], and in vitro elicit activation of the AMPK-ATGL-HSL pathway and suppression of FAS [50–52]. In agreement with this, our results showed a clear effect of the EVOO-rich diet in the downregulation of FAS in healthy and tumor bearing animals, both in adipose and mammary tissue.

Finally, the present study has several strengths, mainly related to the large number of data used (from six experiments involving a total of 818 animals) and the long term of dietary intervention (up to 8 months). Furthermore, there is very little data in the literature on the effect of these diets in general, and the olive oil diet in particular, on a disease such as breast cancer, tightly linked with body mass and obesity. However, these results have several limitations. This cohort is a compilation of independent cohorts with their own hypothesis, although all experiments shared a main variable (the experimental diets) and outcome (body weight). Furthermore, between different experiments there may be some variations in significances due to methodological issues, specific composition of the oils, or even the genetic drift of the strain. The assays were designed with special care to avoid stress, so the animals have not been housed individually and individual consumption is not known, nor have fasting samples been obtained. Moreover, the number of samples for molecular studies is small. Even so, the data set demonstrates a different effect of the two isocaloric diets and suggests different pathways and mechanisms of action of a potential protective effect of EVOO against obesity. Thus, the results obtained provide evidence for a qualitative effect linking dietary lipids and body weight beyond the amount of fat consumed. Moreover, dietary factors are important at all ages, but there are critical periods of development that may be relevant for future health risks. In this regard, our results also show a short-term effect of diets, especially at early ages, with a plausible impact on growth and pubertal development, and a long-term effect, increasing the risk of chronic diseases. Although caution should be exercised when extrapolating from experimental data, our investigations support that EVOO, in the context of a healthy diet from early ages and with a moderate intake, is a healthy choice in relation to obesity and breast cancer risk.

Declaration of competing interests

No conflicts of interest.

CRediT authorship contribution statement

Raquel Moral: Conceptualization, Supervision, Formal analysis, Writing – original draft, Writing – review & editing. **Garyfallia Kapravelou:** Investigation, Formal analysis, Writing – review & editing. **Marta Cubedo:** Formal analysis, Writing – review & editing. **Montserrat Solanas:** Formal analysis, Writing – review & editing. **Eduard Escrich:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Funding

This work was supported by grants from “Plan Nacional de I+D+I” (AGL2006-07691; AGL2011-24778); “Fundación Patrimonio Comunal Olivarero (FPCO)” (FPCO2008-165.396; FPCO2013-CF611.084); “Agencia para el Aceite de Oliva del Ministerio de Agricultura, Alimentación y Medio Ambiente” (AAO2008-165.471); “Organización Interprofesional del Aceite de Oliva Español (OIAOE)” (OIP2009-CD165.646), “Departaments de Salut i d’Agricultura, Alimentació i Acció Rural de la Generalitat de Catalunya” (GC2010-165.000), FPCO and OIAOE (FPCO-OIP2016-CF614.087).

Acknowledgments

The sponsors had no role in the study design, data collection, and analysis and interpretation of the results. The authors want to thank all the investigators and staff involved in animal care and research.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2023.109549.

References

[1] Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr 2011;14:2274–84. doi:10.1017/S1368980011002515.
[2] Dominguez LJ, Di Bella G, Veronese N, Barbagallo M. Impact of mediterranean diet on chronic non-communicable diseases and longevity. Nutrients 2021;13:2028. doi:10.3390/nu13062028.
[3] Koliaki C, Dalamaga M, Liatis S. Update on the obesity epidemic: is the sharp rise of the evil empire truly levelling off? Curr Obes Rep 2023;42:600–8. doi:10.1007/s13679-023-00527-y.
[4] Romaguera D, Norat T, Vergnaud A-C, Mouw T, May AM, Agudo A, et al. Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. Am J Clin Nutr 2010;92:912–21. doi:10.3945/ajcn.2010.29482.
[5] Agnoli C, Sieri S, Ricceri F, Giraudo MT, Masala G, Assedi M, et al. Adherence to a Mediterranean diet and long-term changes in weight and waist circumference in the EPIC-Italy cohort. Nutr Diabetes 2018;8:22. doi:10.1038/s41387-018-0023-3.
[6] López-Gil JF, García-Hermoso A, Sotos-Prieto M, Cavero-Redondo I, Martínez-Vizcaíno V, Kales SN. Mediterranean diet-based interventions to improve anthropometric and obesity indicators in children and adolescents: a systematic review with meta-analysis of randomized controlled trials. Adv Nutr 2023;14:858–69. doi:10.1016/j.advnut.2023.04.011.
[7] World Cancer Research Fund/American Institute for Cancer Research. Diet nutrition, physical activity and cancer: a global perspectiveContinuous Update Project Expert Report 2018. Washington DC: World Cancer Research Fund International; 2018.
[8] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17–48. doi:10.3322/caac.21763.
[9] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49. doi:10.3322/caac.21660.
[10] Escrich E, Solanas M, Moral R, Escrich R. Modulatory effects and molecular mechanisms of olive oil and other dietary lipids in breast cancer. Curr Pharm Des 2011;17:813–30. doi:10.2174/138161211795428902.
[11] van den Brandt PA, Schulpen M. Mediterranean diet adherence and risk of postmenopausal breast cancer: results of a cohort study and meta-analysis. Int J Cancer 2017;140:2220–31. doi:10.1002/ijc.30654.
[12] Schwingshackl L, Schwedhelm C, Galbete C, Hoffmann G. Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis. Nutrients 2017;9:1063. doi:10.3390/nu9101063.
[13] Buckland G, Travier N, Agudo A, Fonseca-Nunes A, Navarro C, Lagiou P, et al. Olive oil intake and breast cancer risk in the Mediterranean countries of the European Prospective Investigation into Cancer and Nutrition study. Int J Cancer 2012;131:2465–9. doi:10.1002/ijc.27516.
[14] Pang Y, Wei Y, Kartsonaki C. Associations of adiposity and weight change with recurrence and survival in breast cancer patients: a systematic review and meta-analysis. Breast Cancer 2022;29:575–88. doi:10.1007/s12282-022-01355-z.
[15] Escrich E. Mammary cancer model induced by dimethylbenz(a)anthracene: a good experimental tool for the study of tumour markers. Int J Biol Markers 1987;2:109–19.
[16] Solanas M, Hurtado A, Costa I, Moral R, Menéndez JA, Colomer R, et al. Effects of a high olive oil diet on the clinical behavior and histopathological features of rat DMBA-induced mammary tumors compared with a high corn oil diet. Int J Oncol 2002;21:745–53.

[17] Costa I, Moral R, Solanas M, Escrich E. High-fat corn oil diet promotes the development of high histologic grade rat DMBA-induced mammary adenocarcinomas, while high olive oil diet does not. Breast Cancer Res Treat 2004;86:225–35. doi:10.1023/B:BREA.0000036896.75548.0c.
[18] Moral R, Escrich E. Influence of olive oil and its components on breast cancer: molecular mechanisms. Molecules 2022;27:477. doi:10.3390/molecules27020477.
[19] Moral R, Escrich R, Solanas M, Vela E, Costa I, Ruiz de Villa MC, et al. Diets high in corn oil or extra-virgin olive oil provided from weaning advance sexual maturation and differentially modify susceptibility to mammary carcinogenesis in female rats. Nutr Cancer 2011;63:410–20. doi:10.1080/01635581.2011.535956.
[20] Sospedra I, Moral R, Escrich R, Solanas M, Vela E, Escrich E. Effect of high fat diets on body mass, oleylethanolamide plasma levels and oxytocin expression in growing rats. J Food Sci 2015;80:H1425–31. doi:10.1111/1750-3841.12890.
[21] Escrich E, Solanas M, Segura R. Experimental diets for the study of lipid influence on the induced mammary carcinoma in rats: I–diet definition. In Vivo 1994;8:1099–105.
[22] Escrich E, Solanas M, Ruiz De Villa MC, Ribalta T, Muntane J, Segura R. Experimental diets for the study of lipid influence on the induced mammary carcinoma in rats: II–suitability of the diets. In Vivo 1994;8:1107–11.
[23] Escrich E, Solanas M, Soler M, Ruiz de Villa MC, Sanchez JA, Segura R. Dietary polyunsaturated n-6 lipids effects on the growth and fatty acid composition of rat mammary tumors. J Nutr Biochem 2001;12:536–49.
[24] Moral R, Escrich R, Solanas M, Vela E, Ruiz de Villa MC, Escrich E. Diets high in corn oil or extra-virgin olive oil differentially modify the gene expression profile of the mammary gland and influence experimental breast cancer susceptibility. Eur J Nutr 2016;55:1397–409. doi:10.1007/s00394-015-0958-2.
[25] Bernardis LL. Prediction of carcass fat, water and lean body mass from Lee's "nutritive ratio" in rats with hypothalamic obesity. Experientia 1970;26:789–90. doi:10.1007/BF02232553.
[26] Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Nogueira CR, Novelli ELB, et al. Misclassification probability as obese or lean in hypercaloric and normocaloric diet. Biol Res 2008;41:253–9. doi:10.4067/S0716-97602008000300002.
[27] Pinheiro J, Bates DR Core Team. R package version 2022;3:1–158. https://svn.r-project.org/R-packages/trunk/nlme/.
[28] Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, et al. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 2005;81:341–54. doi:10.1093/ajcn.81.2.341.
[29] de Moura e Dias M, dos Reis SA, da Conceição LL, Sediyama CMN de O, Pereira SS, de Oliveira LL, et al. Diet-induced obesity in animal models: points to consider and influence on metabolic markers. Diabetol Metab Syndr 2021;13:32. doi:10.1186/s13098-021-00647-2.
[30] Devericks EN, Carson MS, McCullough LE, Coleman MF, Hursting SD. The obesity-breast cancer link: a multidisciplinary perspective. Cancer Metastasis Rev 2022;41:607–25. doi:10.1007/s10555-022-10043-5.
[31] Escrich R, Costa I, Moreno M, Cubedo M, Vela E, Escrich E, et al. A high-corn-oil diet strongly stimulates mammary carcinogenesis, while a high-extra-virgin-olive-oil diet has a weak effect, through changes in metabolism, immune system function and proliferation/apoptosis pathways. J Nutr Biochem 2019;64:218–27. doi:10.1016/j.jnutbio.2018.11.001.
[32] Solanas M, Grau L, Moral R, Vela E, Escrich R, Escrich E. Dietary olive oil and corn oil differentially affect experimental breast cancer through distinct modulation of the p21Ras signaling and the proliferation-apoptosis balance. Carcinogenesis 2010;31:871–9. doi:10.1093/carcin/bgp243.
[33] Oi-Kano Y, Kawada T, Watanabe T, Koyama F, Watanabe K, Senbongi R, et al. Extra virgin olive oil increases uncoupling protein 1 content in brown adipose tissue and enhances noradrenaline and adrenaline secretions in rats. J Nutr Biochem 2007;18:685–92. doi:10.1016/j.jnutbio.2006.11.009.
[34] Domínguez-Vías G, Segarra AB, Ramírez-Sánchez M, Prieto I. The role of high fat diets and liver peptidase activity in the development of obesity and insulin resistance in Wistar rats. Nutrients 2020;12:636. doi:10.3390/nu12030636.
[35] Álvarez-Amor L, Sierra AL, Cárdenas A, López-Bermudo L, López-Beas J, Andújar E, et al. Extra virgin olive oil improved body weight and insulin sensitivity in high fat diet-induced obese LDLr−/−.Leiden mice without attenuation of steatohepatitis. Sci Rep 2021;11:8250. doi:10.1038/s41598-021-87761-3.
[36] Rybinska I, Agresti R, Trapani A, Tagliabue E, Triulzi T. Adipocytes in breast cancer, the thick and the thin. Cells 2020;9:560. doi:10.3390/cells9030560.
[37] Feng J, Zheng T, Hou Z, Lv C, Xue A, Han T, et al. Luteolin, an aryl hydrocarbon receptor ligand, suppresses tumor metastasis in vitro and in vivo. Oncol Rep 2020;44:2231–40. doi:10.3892/or.2020.7781.
[38] Manzanares MÁ, de Miguel C, Ruiz de Villa MC, Santella RM, Escrich E, Solanas M. Dietary lipids differentially modulate the initiation of experimental breast carcinogenesis through their influence on hepatic xenobiotic metabolism and DNA damage in the mammary gland. J Nutr Biochem 2017;43:68–77. doi:10.1016/j.jnutbio.2017.01.016.
[39] Garcia-Guasch M, Medrano M, Costa I, Vela E, Grau M, Escrich E, et al. Extra-virgin olive oil and its minor compounds influence apoptosis in experimental mammary tumors and human breast cancer cell lines. Cancers (Basel) 2022;14:905. doi:10.3390/cancers14040905.
[40] Bowen KJ, Kris-Etherton PM, Shearer GC, West SG, Reddivari L, Jones PJH. Oleic acid-derived oleoylethanolamide: a nutritional science perspective. Prog Lipid Res 2017;67:1–15. doi:10.1016/j.plipres.2017.04.001.
[41] Sihag J, Jones PJH. Dietary fatty acid composition impacts plasma fatty acid ethanolamide levels and body composition in golden Syrian hamsters. Food Funct 2018;9:3351–62. doi:10.1039/c8fo00621k.
[42] Vardanyan GS, Harutyunyan HS, Aghajanov MI, Vardanyan RS. Neurochemical regulators of food behavior for pharmacological treatment of obesity: current status and future prospects. Future Med Chem 2020;12:1865–84. doi:10.4155/fmc-2019-0361.
[43] Cintra DE, Ropelle ER, Moraes JC, Pauli JR, Morari J, de Souza CT, et al. Unsaturated fatty acids revert diet-induced hypothalamic inflammation in obesity. PLoS One 2012;7:e30571. doi:10.1371/journal.pone.0030571.
[44] Wang C-W. Lipid droplets, lipophagy, and beyond. Biochim Biophys Acta 2016;1861:793–805. doi:10.1016/j.bbalip.2015.12.010.
[45] Grahn THM, Kaur R, Yin J, Schweiger M, Sharma VM, Lee M-J, et al. Fat-specific protein 27 (FSP27) interacts with adipose triglyceride lipase (ATGL) to regulate lipolysis and insulin sensitivity in human adipocytes. J Biol Chem 2014;289:12029–39. doi:10.1074/jbc.M113.539890.
[46] Fletcher SJ, Sacca PA, Pistone-Creydt M, Coló FA, Serra MF, Santino FE, et al. Human breast adipose tissue: characterization of factors that change during tumor progression in human breast cancer. J Exp Clin Cancer Res 2017;36:26. doi:10.1186/s13046-017-0494-4.
[47] Claro-Cala CM, Quintela JC, Pérez-Montero M, Miñano J, Alvarez de Sotomayor M, Herrera MD, et al. Pomace olive oil concentrated in triterpenic acids restores vascular function, glucose tolerance and obesity progression in mice. Nutrients 2020;12:323. doi:10.3390/nu12020323.
[48] Li X, Wei T, Li J, Yuan Y, Wu M, Chen F, et al. Tyrosol ameliorates the symptoms of obesity, promotes adipose thermogenesis, and modulates the composition of gut microbiota in HFD fed mice. Mol Nutr Food Res 2022;66:2101015. doi:10.1002/mnfr.202101015.
[49] Lombardo GE, Lepore SM, Morittu VM, Arcidiacono B, Colica C, Procopio A, et al. Effects of oleacein on high-fat diet-dependent steatosis, weight gain, and insulin resistance in mice. Front Endocrinol (Lausanne) 2018;9:116. doi:10.3389/fendo.2018.00116.
[50] Pacifici F, Farias CLA, Rea S, Capuani B, Feraco A, Coppola A, et al. Tyrosol may prevent obesity by inhibiting adipogenesis in 3T3-L1 preadipocytes. Oxid Med Cell Longev 2020;2020:1–12. doi:10.1155/2020/4794780.
[51] Lepore Maggisano, Bulotta Mignogna, Arcidiacono Procopio, et al. Oleacein prevents high fat diet-induced adiposity and ameliorates some biochemical parameters of insulin sensitivity in mice. Nutrients 2019;11:1829. doi:10.3390/nu11081829.
[52] Menendez JA, Vazquez-Martin A, Oliveras-Ferraros C, Garcia-Villalba R, Carrasco-Pancorbo A, Fernandez-Gutierrez A, et al. Analyzing effects of extra-virgin olive polyphenols on breast cancer-associated fatty acid synthase protein expression using reverse-phase protein microarrays. Int J Mol Med 2008;22:433–9. doi:10.3892/ijmm_00000039.