RESEARCH ARTICLE

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The Beneficial Effects of Red Sun-Dried *Capsicum annuum* L. Cv Senise Extract with Antioxidant Properties in Experimental Obesity are Associated with Modulation of the Intestinal Microbiota

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Scope: ***Capsicum annuum*** **L. cv Senise is a sweet pepper containing health promoting compounds that can be modified by ripening and drying. This study focuses on finding the peppers with the best antioxidant properties, which are evaluated on an experimental model of obesity.**
Methods and Results: **Phytochemical profile and antioxidant activity are evaluated on several peppers obtained from the same cultivar at different ripening stages. Red sweet peppers show the highest content in polyphenols, β-carotene, lycopene, and capsinoids, and demonstrate the best antioxidant activity in vitro. Mice fed a high fat diet are orally treated with an extract from these peppers (Capsicum annuum extract [CAE]) (1, 10, and 25 mg/kg/day). It promotes weight loss and improves plasma markers related to glucose and lipid metabolisms. CAE also ameliorates obesity-associated systemic inflammation reducing the expression of pro-inflammatory cytokines in adipose and hepatic tissues and improving the expression of different markers involved in the gut epithelial barrier function. These effects are associated with a modulation of the intestinal microbiome, which appears altered.**
Conclusions: **The extract can be considered a new potential approach for the treatment of obesity, complementary to dietary restrictions.**

1. Introduction

Obesity is considered a major public health problem affecting over a third of the world's population nowadays.[1] It is a metabolic disorder characterized by the accumulation of excessive body fat that increases the risk to develop type 2 diabetes mellitus, non-alcoholic fatty liver diseases, cancer, and cardiovascular diseases.[2,3] The prolonged exposure to excessive stored fat causes remodeling and hypertrophy of adipocytes, impaired secretion of adipokines, and production of pro-inflammatory cytokines. In fact, obesity is considered a chronic low-grade inflammatory condition.[4] This seems to be initially triggered by an altered intestinal barrier function that facilitates bacterial translocation and the onset of systemic endotoxemia, which impairs the metabolic function.[2] In fact, high fat diet (HFD) feeding impairs intestinal integrity and increases endotoxemia due to the

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translocation of bacterial lipopolysaccharides (LPS) from gut microbiota into the blood.[5] Moreover, plasma LPS induces systemic low-grade inflammation and insulin resistance.[6] In this sense, the gut microbiota would play a key role. In fact, it has been extensively described that the gut microbiota participates in the development of obesity, both in animals and humans.[7] Obesity is characterized by a higher abundance of *Firmicutes* and a smaller proportion of *Bacteroidetes*.[7] Besides, the bacterium *Akkermansia muciniphila* is reported to protect against obesity and inflammation in HFD-fed mice by improving intestinal barrier integrity and altering adipose tissue metabolism.[8] However, gut microbiota not only impacts the gut immune function,[9] but also inflammation in peripheral tissues. Studies using germ-free mice colonized by *Escherichia coli* have shown a high macrophage infiltration in adipose tissue and an increased expression of proinflammatory cytokines.[10] Furthermore, mice fed with LPS display enhanced adipose tissue inflammation and reduced insulin sensitivity.[6] These findings reveal that gut microbiota may contribute to metabolic tissue inflammation thereby affecting host metabolism.

Management of obesity entails lifestyle modifications, including severe dietary changes, which are essential for both its prevention and treatment. However, these are usually difficult to maintain, so the use of adjuvant strategies, like drug therapy, helps to obtain long-term weight loss.[11] Although different antiobesity drugs are available, most of them have limited effectiveness and many adverse effects. For instance, sibutramine reduces food intake and increase satiety by inhibiting the neuronal reuptake of the neurotransmitters noradrenaline and serotonin, but it may also causes insomnia, dry mouth, or formation of thrombi; the lipase inhibitor orlistat reduces fat absorption and promotes weight loss, but it may produce bloating, steatorrhoea, faecal incontinence, and even increased risk of colon cancer.[12,13] In consequence, there is a clear demand for the development of new efficient and safe approaches to treat and/or prevent obesity. This could be the case of plant extracts and/or functional foods, which contain different compounds, like polyphenols, able to modulate the inflammatory response and preserve the intestinal barrier function.[14,15] Among the natural products with reported beneficial effects in obese patients, red pepper (*Capsicum annuum* L.) (*Solanaceae* family) should be considered. In fact, it is consumed as food additive and it has been traditionally used for the treatment of cough, toothache, sore throat, parasitic infections, rheumatism, and different gastrointestinal complaints, including dyspepsia, loss of appetite, gastroesophageal reflux disease, and gastric ulcer; and for its wound healing, antiseptic, antioxidant, and immunomodulatory properties.[16] These beneficial effects have been ascribed to the presence of bioactive compounds, especially capsaicinoids, carotenoids, or phenolic derivatives.[17] Moreover, considering different studies, both in experimental animals and in humans, red pepper and capsaicin, its most representative active compound, also have potential therapeutic properties for the management of the metabolic syndrome, including dyslipidemia, hyperglycemia, and obesity.[16] In fact, human studies have revealed that capsaicin decreases food intake and increases energy expenditure, facilitates fat oxidation, reduces appetite, increases satiety, and modulates the expression of some hypothalamic peptides implicated in food intake, thus resulting in a significant weight loss.[18–20] However, the intake of capsaicin, alone or as a component of hot pepper, for long periods of time, results in bad compliance, mainly due to the stomach burning feeling that it may generate.[21] An alternative could be the use of a sweet, non-pungent red pepper, like a cultivar named CH-19, which has shown beneficial effects in reducing energy intake to prevent body weight gain in humans.[22]

The sweet dried pepper *C. annuum* L. cultivar Senise is the most representative product of Basilicata region (Italy), being typical in the culture and gastronomical tradition of the valley between Sinni and Agri rivers. Senise peppers are sold fresh and dried under the sunlight, threaded onto strings called "serte," or as powder. Loizzo et al. (2013)[23] demonstrated that the drying process of *C. annuum* cv Senise influences the phytochemical profile, as well as the in vitro antioxidant and hypoglycemic activities. The aim of this study was to evaluate the variations in the chemical composition and their antioxidant activity of several extracts of *C. annuum* cv Senise obtained at different ripening stages. This served us to select an extract, the one with the best antioxidant properties, to assay its antiobesity activity and its impact on the gut microbiome in HFD-fed mice.

2. Experimental Section

2.1. Chemicals and Reagents

All chemicals were purchased from Sigma-Aldrich Quimica SL (Madrid, Spain), unless otherwise stated.

2.2. Pepper Fruit Extract

For the analysis, five different ethanol extracts were used, four from the fruits of *C. annuum* cv Senise and one from a commercial fresh red pepper. For the analysis, five different extracts were used. The fruits of *C. annuum* cv Senise were supplied by Azienda La Casa del Lago, Senise, PZ - ITALY in 2016. Fresh peppers were collected according to their ripening stage: Capsicum annuum extract-fresh green peppers (CAE-G), Capsicum annuum extract-fresh intermediate peppers (CAE-I), and Capsicum annuum extract-fresh red peppers (CAE-R); and were analyzed. Capsicum annuum extract-dried-red peppers (CAE-D) were also evaluated since fresh red pepper fruits are commonly consumed dried. A commercial cultivar of fresh red pepper was bought at a market and investigated. More detailed information is available in supplementary materials online.

2.3. Chemical Characterization

2.3.1. Determination of Total Carotenoid Content

β-Carotene and lycopene were determined according to the method described by Nagata and Yamashita (1992)[18] and described in the Supporting material online.

2.3.2. Total Polyphenol Content

The total polyphenol content (TPC) was determined by the Folin–Ciocalteu method,[19] briefly explained in the Supporting material online.

2.3.3. Capsinoid Content by RP-HPLC-DAD

Pepper extracts underwent HPLC-DAD analysis as explained in Supporting material online. Results were expressed as μg of capsiate/capsaicin equivalent per grams of dried extract (μg g^{-1} dry weight [DW]), which were used as standards.

2.4. In Vitro Radical-Scavenging Activity

The radical-scavenging activity of pepper extracts was investigated as described in Supporting material online. Results were expressed as mean ± standard deviation of IC25 or IC50 (mg mL^{-1}), the concentration of extract required to scavenge 25% or 50% of the initial radical.

2.5. Effects of *Capsicum Annuum* Extract on High Fat Diet Fed Mice

The study was carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" as promulgated by the National Institute of Health and the protocols approved by the Ethic Committee of Laboratory Animals of the University of Granada (Spain) (Ref. No. 28/03/2016/030). Male 5-week-old C57BL/6J mice (Janvier, St Berthevin, Cedex, France) were housed in standard conditions (12-h light/dark cycle, temperature 22 ± 1 °C, 55 ± 10% relative humidity) with free access to food and water. The mice were divided into four groups ($n = 10$): control, control-treated with CAE (25 mg kg^{-1}), obese control, and obese-treated with CAE at different doses (1, 10, and 25 mg kg^{-1}). Pepper extract was administered daily by oral gavage for six weeks. Control mice received a standard diet (Global diet 2014; Harlan Laboratories, Barcelona, Spain) whereas obese mice were fed an HFD in which 60% of its caloric content was derived from fat (Purified diet 230 HF; Scientific Animal Food & Engineering, Augy, France). During the experimental period, animal body weight as well as food and water intake were regularly determined.

Briefly, a glucose tolerance test was performed one week before the end of the experiment. Then, blood samples were collected to determine glucose, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and total cholesterol. Liver, adipose, and colonic tissue were extracted after sacrifice to evaluate gene expression by RT-q-PCR (Table S1, Supporting Information). Colonic luminal contents were also collected at endpoint to characterize the microbiota (see supplementary material online for details).

2.6. Statistical Analysis

In vitro experiments were carried out in triplicate and the results were expressed as the mean ± SD. Results of preclinical studies were expressed as the mean ± SEM. Differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA) and post-hoc least significance tests. All statistical analyses were carried out with the GraphPad 5.0 software package (GraphPad Software, Inc., La Jolla, CA, USA), with statistical significance set at $p < 0.05$.

3. Results and Discussion

3.1. Extraction Yield, Carotenoid Content, and Antioxidant Activity

Sweet fresh and dried *C. annuum* cv Senise fruits and a commercial fresh red pepper were extracted by maceration using ethanol as solvent. The extraction yields ranged from 5.13% to 11.70% w/w in CAE-G and CAE-D, respectively. The color of the pepper fruits depend on the presence of carotenoids, which change during ripening. Fresh red peppers showed the highest amount of β-carotene and lycopene, which provides red color (**Table 1**) followed by intermediate and green ripening stages. Red pepper Senise cv contained twice as much of β-carotene than the commercial one. Senise dried peppers had the highest amount of β-carotene (10.13 ± 1.49 mg/100 g) and lycopene (5.31 ± 0.91 mg/100 g) (Table 1). *C. annum* is a source of polyphenols, the widest group of phytochemicals, which are potent antioxidants that collaborate with the endogenous antioxidants, like vitamins and enzymes, in the defense against oxidative stress caused by an excess of reactive oxygen species.[24] In the present study, Senise pepper extracts reported similar total phenolic content ranging from 14.30 ± 0.80 to 20.46 ± 0.80 mg GAE/g of dried extract. The highest value was observed in dried red peppers (Table 1), which disagrees with a previous study that reports higher TPC values in green peppers.[25] In addition, peppers are a source of capsinoids, evaluated for the first time in *C. annuum* cv Senise by HPLC in this study. The capsinoid content was also influenced by ripening (Table 1), and ranged from 5.96 ± 1.07 to 987.72 ± 128.85 μg g^{-1} of DW. The drying process increased capsinoid content, as seen in Table 1, which agrees with previous studies.[26,27] Interestingly, all extracts showed higher capsinoid content than the commercial pepper cultivar at red stage. The differences in the phytochemical profile considering the ripening stage are due to changes in synthesis, transport, and degradation of various metabolites.[28] The phytochemical classes reported in the studied peppers suggest an important radical-scavenging ability. In this study, all extracts showed a dose-dependent scavenging activity against nitric oxide and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals. Senise dried red pepper extract displayed the highest potency as nitric oxide scavenger, with lowest IC25 value of 0.18 ± 0.02 (Table 1); whereas commercial red fresh pepper was the less potent (Table 1). The ABTS assay estimates the antioxidant activity of a given compound by determining its ability to scavenge the cation $ABTS^{\cdot+}$.[29,28] Based on the lowest IC50 values obtained, all Senise extracts showed higher capability in quenching ABTS free radical than the commercial red pepper, although no significant differences were observed among the pepper extracts obtained at different ripening stages (Table 1).

To better analyses the antioxidant capacity of the extracts in vitro, the assays performed were included for the relative antioxidant capacity index.[30] With this method, the total reducing capacity of the samples was considered, so it would reflect the cumulative capacity of both phenolic and non-phenolic compounds.[31] As expected, Senise dried pepper showed the highest index (0.95) and the commercial fresh red pepper the lowest (−1.30) (**Figure 1**). Based on these results, the extract from CAE-D was selected for the in vivo studies

Table 1. β-carotene, lycopene, total polyphenol content, capsaicinoid and capsinoid derivatives, and antioxidant activity in Senise pepper extract versus commercial red pepper. CAE-G: Fresh green pepper; CAE-I: fresh intermediate peppers; CAE-R: fresh red peppers; CAE-D: Dried-red peppers; and CR: commercial cultivar of fresh red pepper.

	β-Carotene [mg/100 g]	Lycopene [mg/100 g]	TPC [mg GAE g^{-1}]	Capsaicinoid derivatives [$\mu g\ g^{-1}$]	Capsinoid derivatives [$\mu g\ g^{-1}$]	ABTS [IC50 mg mL^{-1}]	NO [IC25 mg mL^{-1}]
CAE-G	0.85 ± 0.17^{a}	n.d	20.15 ± 0.73^{a}	n.d.	21.87 ± 1.83^{d}	0.04 ± 0.00^{a}	0.46 ± 0.04^{a}
CAE-I	0.93 ± 0.30^{a}	0.47 ± 0.08^{a}	18.79 ± 0.99^{a}	n.d.	35.99 ± 6.74^{c}	0.05 ± 0.00^{a}	0.44 ± 0.04^{a}
CAE-R	4.29 ± 0.48^{b}	1.74 ± 0.19^{b}	16.46 ± 0.75^{b}	>15	73.59 ± 13.87^{b}	0.05 ± 0.00^{a}	0.62 ± 0.05^{b}
CAE-D	10.13 ± 1.49^{c}	5.31 ± 0.91^{c}	20.46 ± 0.80^{a}	<15	987.72 ± 128.85^{a}	0.05 ± 0.01^{a}	0.18 ± 0.018^{c}
CR	2.41 ± 0.34^{d}	1.04 ± 0.12^{d}	14.30 ± 0.80^{b}	n.d.	5.96 ± 1.07^{e}	0.08 ± 0.01^{b}	0.91 ± 0.08^{d}

Carotenoids are expressed as mg of β-carotene or lycopene per 100 g of fresh weight; TPC: total polyphenol content expressed as milligrams of gallic acid equivalent per grams of extract; Capsaicinoids are expressed as μg of capsaicin equivalent per grams of dried extract; Capsinoids are expressed as μg of capsiate equivalent per grams of dried extract; ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, results are expressed as concentration required for 50% inhibition; NO: nitric oxide assay results are expressed as concentration required for 25% inhibition. Experiments were carried out in triplicate and data were reported as mean ± SD. Values with different letters are significantly different (one-way ANOVA post hoc Tukey's test, $p < 0.05$).

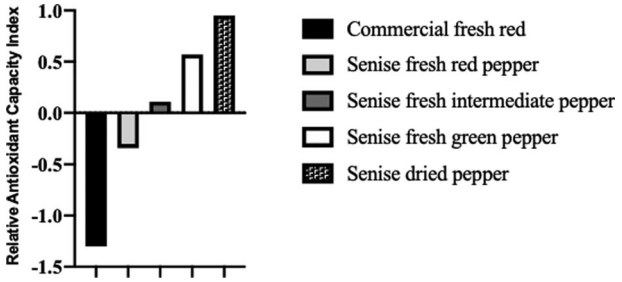

Figure 1. Relative antioxidant capacity index (RACI) values obtained comparing TPC, ABTS, and NO results. TPC: total polyphenolic content; ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; NO: nitric oxide assay.

3.2. Effect of *Capsicum Annuum* Cv Senise Extract on High Fat Diet-Induced Obesity in Mice

3.2.1. Effects of Capsicum Annuum Extract on Body Weight, Glucose Tolerance Test, and Plasma Biochemical Profile

HFD-fed mice showed a higher progressive increase in body weight over the 6 weeks period when compared to standard diet –fed mice. CAE significantly reduced weight in HFD-fed mice, but had no significant effect on mice receiving normal diet (**Figure 2**A). These results support previous studies that proposed antiobesity effects of different extracts of *C. annuum*, both in vitro and in vivo.[32,33] CAE did not produce significant differences in energy intake but reduced energy efficiency in comparison with the corresponding control mice (Figure 2A). These data agree with previous studies that reported beneficial effects of sweet pepper extract CH-19 on body weight in humans, but only in those with positive energy balance.[22]

Moreover, the extract showed a positive impact on glucose homeostasis, as evidenced in the glucose tolerance test. It reduced glucose levels from 15 min onwards to those values in untreated obese mice, which resulted in lower values of the area under the curve (Figure 2B). Accordingly, plasma glucose level was significantly increased in HFD-fed control mice compared to lean mice, but CAE, at 10 and 25 mg kg^{-1}, significantly reduced it. However, no differences were observed in plasma insulin levels among the groups (**Figure 3**B). HOMA-IR index values revealed an improvement in the insulin levels in CAE-treated mice (10 and 25 mg kg^{-1}) (Figure 3A). Previous studies have reported hypoglycemic activities of other *C. annuum* L. cultivars, due to their content in flavonoids, carotenoids, and capsaicinoids, which were ascribed α-glucosidase and α-amylase inhibitory activities.[34,35] Moreover, the capsaicin in chili red pepper extracts has been described to promote other beneficial effects on hyperglycemic conditions, through the activation of transient receptor potential vanilloid subtype 1, which leads to insulin resistance improvement and glucose homeostasis regulation.[36] Of note, it is unlikely that this mechanism could contribute to the improvement of the glucose metabolism in obese mice since the content of capsaicin in the extract is very low (less than 15 ug g^{-1} DW; 130ug g^{-1} fresh weight), at least 30-fold lower than in spicy pepper as reported previously.[37] Determination of capsaicin and dihydrocapsaicin in *Capsicum* fruit samples using high performance liquid chromatography.[37] In consequence, the effect on glucose metabolism may be ascribed to other components, including the carotenoids β-carotene and lycopene, which have been reported to improve insulin resistance in humans[38] as well as in experimental obesity in mice.[39,40] The daily doses of β-carotene and lycopene administered to obese mice were, ≈6 and ≈3 μg/mice, respectively. Although these doses are lower than those used previously by other authors, they could have a synergistic effect that could contribute to the improvement of glucose metabolism.

The macroscopic characterization of the obesity status revealed a significant increase in the amount of adipose tissue in the control HFD-fed mice in comparison with non-obese mice, while CAE (25 mg kg^{-1}) significantly reduced both abdominal and epididymal fat contents (Figure 2C). When considering the plasma cholesterol profile, HFD-fed mice showed higher levels of both LDL-cholesterol and HDL-cholesterol than in lean mice (Figure 3B), with increased LDL/HDL ratio. CAE administration to obese mice reduced this ratio, showing no statistical differences in comparison with non-obese control mice (Figure 3B). The beneficial effects exerted by other *C. annuum* extracts on altered lipid profile have been previously reported in experimental models of obesity in rodents,[16] mostly attributed to the presence of capsaicin in the extracts, able to ameliorate dyslipidemia in obesity

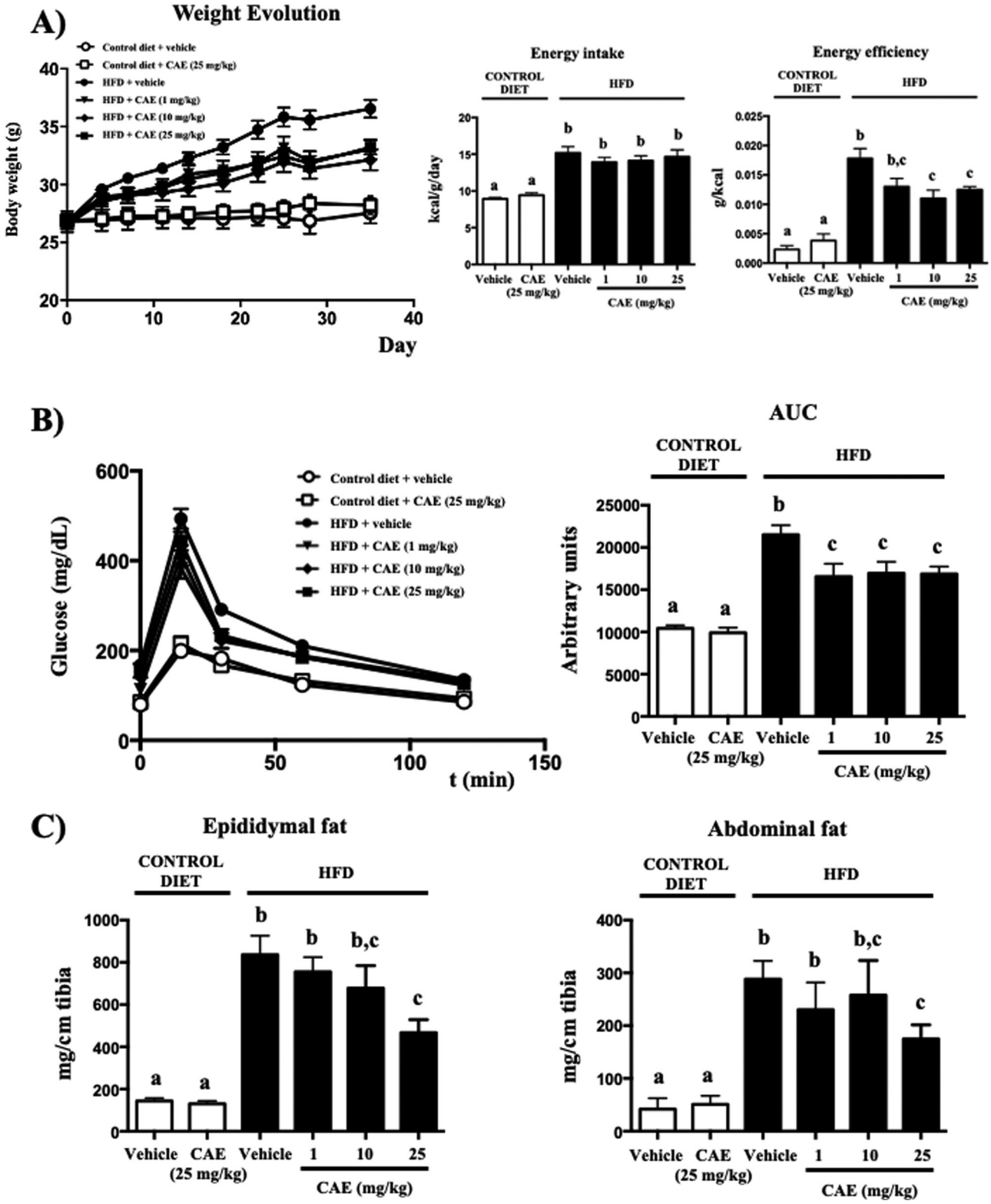

Figure 2. Effects of pepper extract (CAE) (1, 10, and 25 mg kg^{-1}) administration on A) morphological changes (body weight evolution, energy intake, and energy efficiency), B) glucose tolerance test and area under the curve (AUC), and C) abdominal and adipose tissue mass normalized with tibia length. Data are expressed as means ± SEM ($n = 10$). Groups with different letter statistically differ ($p < 0.05$).

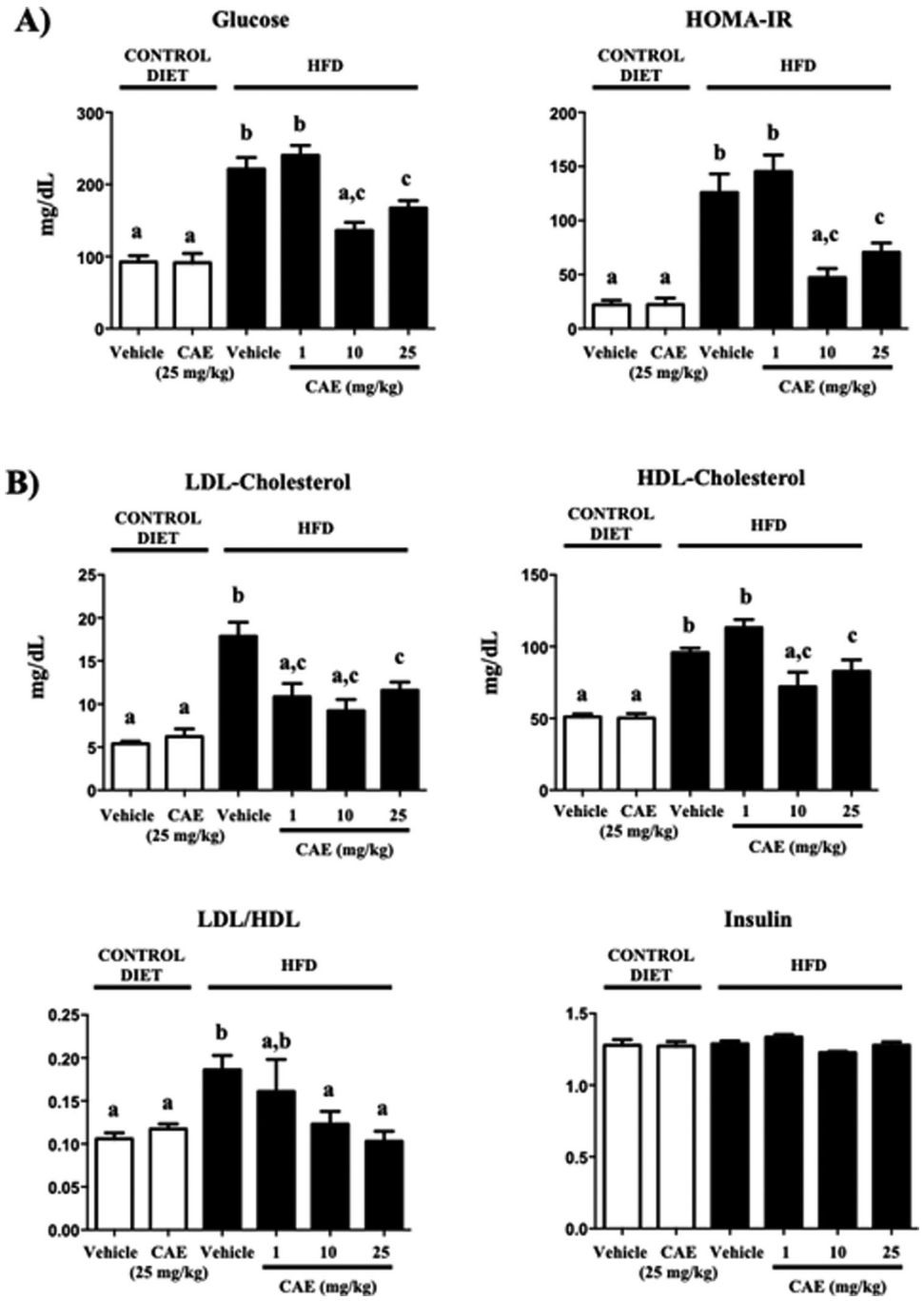

Figure 3. Effects of pepper extract (CAE) (1–25 mg kg^{-1}) on A) plasma glucose concentration and HOMA-IR index, B) LDL- and HDL-cholesterol, LDL/HDL cholesterol plasma ratio in control and HFD-fed mice, and insulin levels. Values are expressed as mean ± SEM ($n = 10$). Groups with different letters statistically differ ($p < 0.05$).

through different mechanisms.[41] However, and as commented before, the low levels of capsaicin in this CAE suggest that other components may be responsible for the improvement of the altered lipid profile in obese mice. In fact, different studies have reported the important role of carotenoids in adipose tissue biology and obesity. Particularly, β-carotene supplementation has been reported to reduce adiposity in mice by inhibiting the expression of peroxisome proliferator-activated receptor γ expression, which participates in adipocyte differentiation and lipogenesis in mature adipocytes.[40] Similarly, lycopene administration to HFD-fed mice significantly reduced adiposity index and improved glucose and lipid homeostasis.[42]

It is well known that the excess of fat in the body is stored in the adipose tissue, thus promoting its remodeling, leading to hypertrophy and dysfunction of the adipocytes, and the subsequent release of pro-inflammatory mediators, including chemokines,

cytokines, and an altered secretion of adipokines, as well as a production of reactive oxygen metabolites.[43] Interestingly, CAE could reduce the damage produced by the reactive oxygen metabolites, due to its scavenging and antioxidant properties described above. In addition, the metabolic alterations observed in obesity are associated with low-grade chronic inflammation in liver and adipose tissue, which is closely related to insulin resistance in obese patients.[44] The results of the present study confirmed these observations, since the expression of the pro-inflammatory cytokines TNFα, IL-1B, and IL-6, as well as the chemokine MCP-1, were significantly increased in liver and/or fat from obese mice in comparison with non-obese control group (**Figure 4**), similarly to that previously reported in humans.[45,46] Moreover, the extract significantly reduced the expression of these markers, ameliorating the inflammatory status in HFD-fed mice (Figure 4). TNFα is produced by several cell types, including the adipocytes and macrophages, and can activate MAPK and NF-κB signaling pathways, thus promoting the production of other pro-inflammatory cytokines, like IL-1β and IL-6.[47] MCP-1 is secreted by adipocytes and has been proposed to play a key role in the initiation of adipose tissue inflammation in obesity, since it stimulates the recruitment of macrophages into the adipose tissue, which are an important cell source of pro-inflammatory cytokines.[48] These cytokines participate in obesity-related systemic insulin resistance by negatively interfering with the cell signaling associated to the insulin receptor in different target tissues, through the inhibition of the insulin-dependent tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate-1.[49,50] This probably alters insulin receptor functionality, reducing the expression and activation of glucose transporters, like GLUT4, thus impairing glucose uptake and resulting in sustained hyperglycemia.[51] In fact, although obese mice show normal levels of insulin, they displayed reduced expression of GLUT-4 in both adipose and liver tissues, thus confirming the situation of insulin resistance (Figures 4). Of note, the administration of CAE to obese mice, especially at the highest dose assayed (25 mg kg^{-1}), significantly increased the expression of GLUT-4 in both tissues (Figure 4), which could account for an increased glucose uptake and the amelioration of the hyperglycemia that occurs in obesity. The presence of β-carotene and lycopene in CAE could contribute to the beneficial effects exerted by CAE on the systemic inflammatory response in obese mice. In fact, these compounds have been reported to display anti-inflammatory properties by reducing the production of pro-inflammatory cytokines, including TNFα, IL-1β, and IL-6, in inflammatory-associated conditions, like obesity or LPS-induced septic shock.[42,52]

As commented above, obesity is characterized by an altered production of adipokines,[43] which was also observed in the present study (**Figure 5**). Thus, *Leptin* expression was significantly increased in adipose tissue from untreated obese mice in comparison with those mice fed the standard diet, which was associated with a reduced expression of its corresponding receptor (Leptin-r), as a manifestation of leptin signaling impairment in obesity. Mostly, human obesity is associated with leptin resistance and the compensatory hyperleptinemia.[53] Similarly, the expression of *Adiponectin* was significantly reduced in HFD-fed mice both in adipose and liver tissues. CAE administration improved the expression of both adipokines in both tissues, as well as of Leptin-r in adipose tissue, especially when the highest dose of CAE was considered (Figure 5). Adiponectin and leptin exert opposite immunomodulatory actions. Adiponectin shows anti-inflammatory and insulin-sensitizing properties, inhibits the phagocytic activity and the production of TNFα in macrophages. Leptin, on the other hand, promotes the production of pro-inflammatory cytokines by T cells, macrophages, and other immune cells.[54] In consequence, CAE may ameliorate the inflammatory status and insulin resistance that characterizes obesity. In fact, carotenoids have been reported to modulate the expression of adipokines in adipocytes in vitro.[55]

As commented above, obesity is considered a subclinical systemic inflammatory condition associated with increased LPS plasma levels and an endotoxemic status.[56] Different studies in obese humans and in experimental obesity in mice have correlated an increased LPS plasma levels with an altered TLR4 signaling, including increased *Tlr-4* gene expression, which can stimulate the production and release of pro-inflammatory cytokines.[57] The present study confirms these observations since the liver expression of *Tlr-4* in untreated HFD-fed mice was significantly higher than in control diet groups (Figure 4). CAE treatment (25 mg kg^{-1} dose), significantly reduced *Tlr-4* expression (Figure 4), thus ameliorating the inflammation-associated endotoxemia process in obese mice. Previously, it has been proposed a link between increased LPS plasma levels in obesity and enhanced intestinal permeability.[58] In fact, this study confirms the existence of an altered epithelial barrier function associated with obesity, since the expression of different colonic markers involved in the epithelial integrity was significantly reduced in HFD-fed mice when compared with non-obese mice (**Figure 6**). They include the mucins Muc-2 and Muc-3, as well as the peptide Tff-3, which is expressed by goblet cells and synergizes with mucins in enhancing the protective barrier properties of the mucus layer.[59] Additionally, ZO-1 is a linker protein in tight junctions in association with the transmembrane protein occludin, with a prominent role in facilitating and maintaining the epithelial integrity.[60] The administration of CAE to HFD-fed mice ameliorated the expression of all these proteins (Figure 6), which could be associated with an improvement in the epithelial barrier function and prevention of the altered permeability reported in obesity, thus contributing to the beneficial effects observed with the extract. Different studies have reported the ability of carotenoids to preserve the intestinal epithelial integrity through an enhancement of the expression of tight junction proteins.[61] In consequence, the presence of either β-carotene and/or lycopene in CAE would restore the impaired permeability that occurs in obesity.

3.3. Impact of *Capsicum Annuum* Cv Senise Extract on Gut Microbiota

Many studies have proposed that phenolic extracts can exert prebiotic effects, which help to preserve the configuration of the gut microbiota by promoting beneficial bacteria growth and controlling potential pathogenic bacteria,[62] contributing to the maintenance of host health. Besides, it is widely described that gut microbiota may contribute to the metabolic imbalance and, in fact, many human metabolic conditions, including obesity and

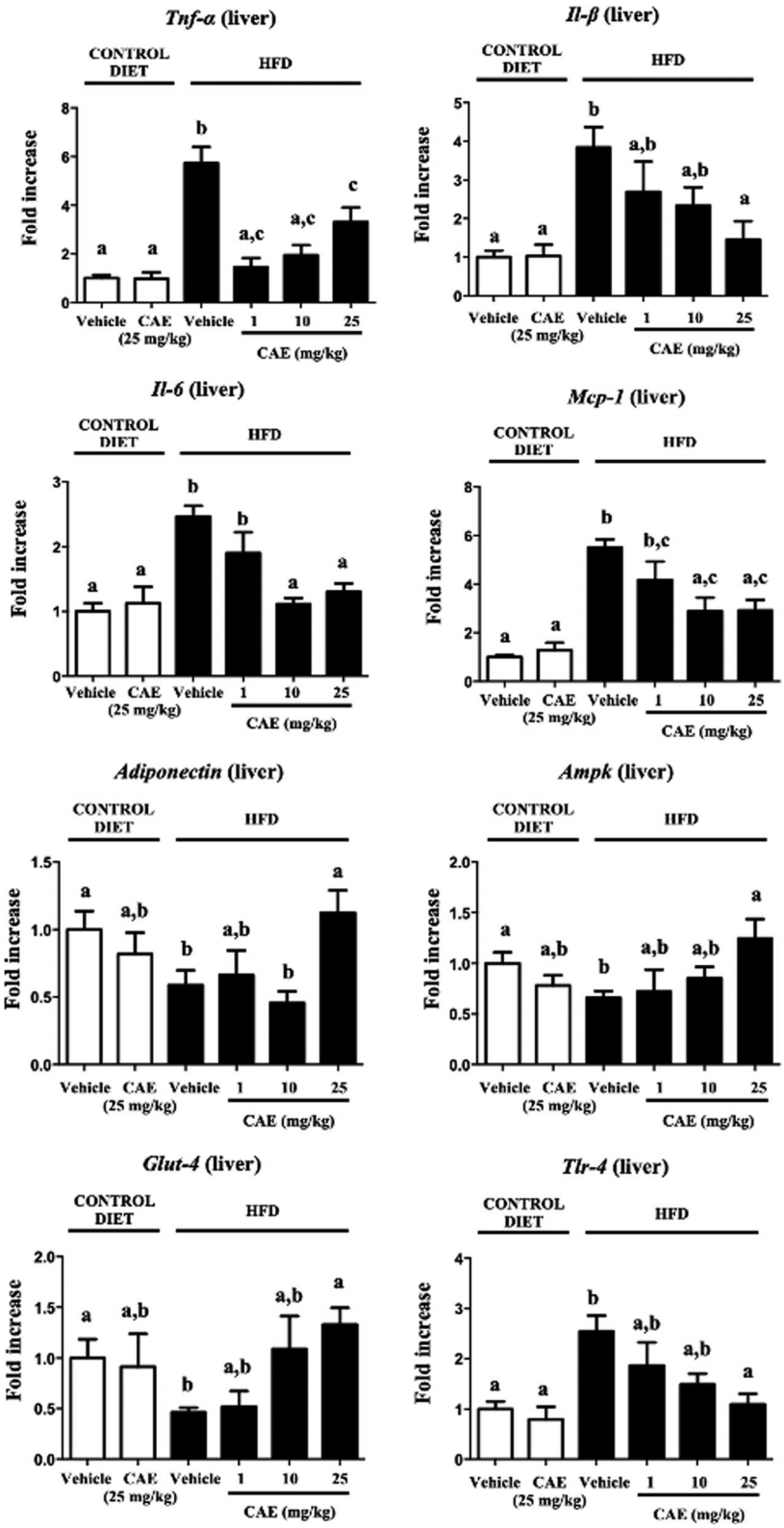

Figure 4. Effect of pepper extract on the gene expression of interleukin (IL)-6, tumor necrosis factor (TNF)-α, Glucose transporter type 4 (GLUT-4), adiponectin, monophosphate activated protein kinase (AMPK) analyzed by real-time qPCR and normalized with the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in liver of control and HFD-mice. Data are expressed as mean ± SEM ($n = 8$–10/group). The groups with different letters are significantly different (one-way ANOVA post hoc Tukey's test, $p < 0.05$).

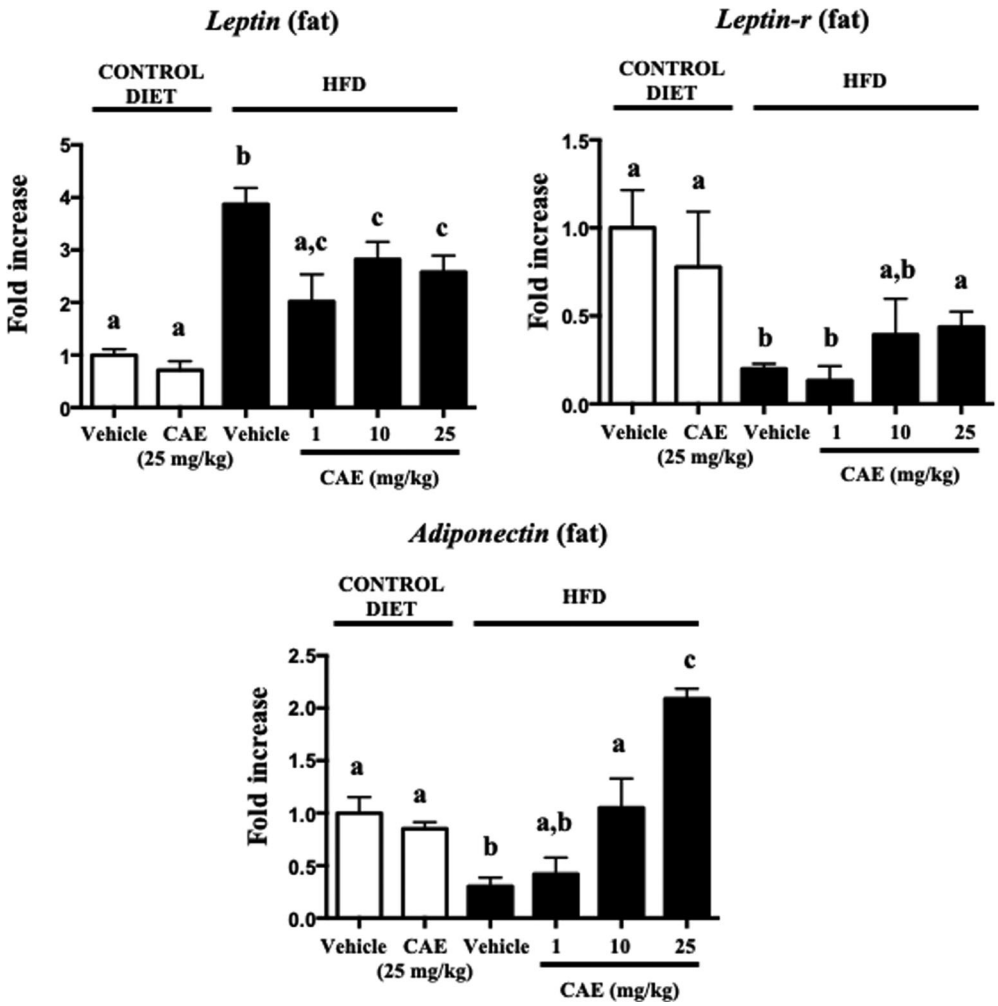

Figure 5. Effect of pepper extract on the gene expression of *Leptin*, *Leptin R*, and *Adiponectin* analyzed by real-time qPCR and normalized with the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in adipose tissue of control and HFD-mice. Data are expressed as mean ± SEM (n = 8–10/group). The groups with different letters are significantly different (one-way ANOVA post hoc Tukey's test, $p < 0.05$).

diabetes, and experimental models, have been linked to an imbalanced gut microbiota, known as dysbiosis.[63] Our results corroborate these observations. The principal coordinate analysis (PCoA) plot, a representative marker of β-diversity, showed that HFD had an impact on the gut microbiota composition as well as CAE treatment (25 mg kg^{-1}) (**Figure 7**A). Moreover, as shown in Figure 7A the PCoA disclosed a higher association between the two high doses of CAE and the lean mice than with HFD-fed mice.

The structural changes of gut microbiota were also evidence when we compared the relative abundance of the phyla. Although the most dominant phyla in all groups were *Bacteroidetes* and *Firmicutes*, the relative abundance was significantly altered by HFD (Figure 7B). CAE treatment (10 mg kg^{-1}) reversed this situation (Figure 7B). An imbalanced ratio of *Firmicutes* and *Bacteroidetes* (F/B ratio) has been associated with risk factors of obesity in both animals and humans[64–66] and is considered as an important parameter to define the health status.[67] Therefore, this ratio reflects gut dysbiosis, and as expected, the F/B ratio was increased in the HFD group compared with the control diet fed group (Figure 7C). Remarkably, CAE significantly reduced the F/B ratio in HFD-fed animals at 1 and 10 mg kg^{-1} doses (Figure 7C). It has been proposed that *Firmicutes* are repressed by polyphenols and their metabolites, thus favoring *Bacteroidetes* in the gut.[68] In the present study, the decrease in the F/B ratio was observed after CAE treatment, which shows a modulatory activity of CAE on the composition of gut microbiota and suggests a positive influence on diet induced gut dysbiosis. Moreover, the degree of operational taxonomic units (OTUs) shared between individual mice and the six groups was summarized in the Venn diagram (Figure 7D). The six groups shared 447 OTUs in their colonic contents. The number of unique OTUs was the highest in the lean mice, which proposes that there was a distinct difference in the composition of the gut microbiome between the non-obese mice and the other five groups. There were 29, 40, and 49 unique OTUs in the HFD group and CAE treatment groups, respectively, indicating that CAE supplementation was able to reshape the composition of the gut microbiota of the obese mice.

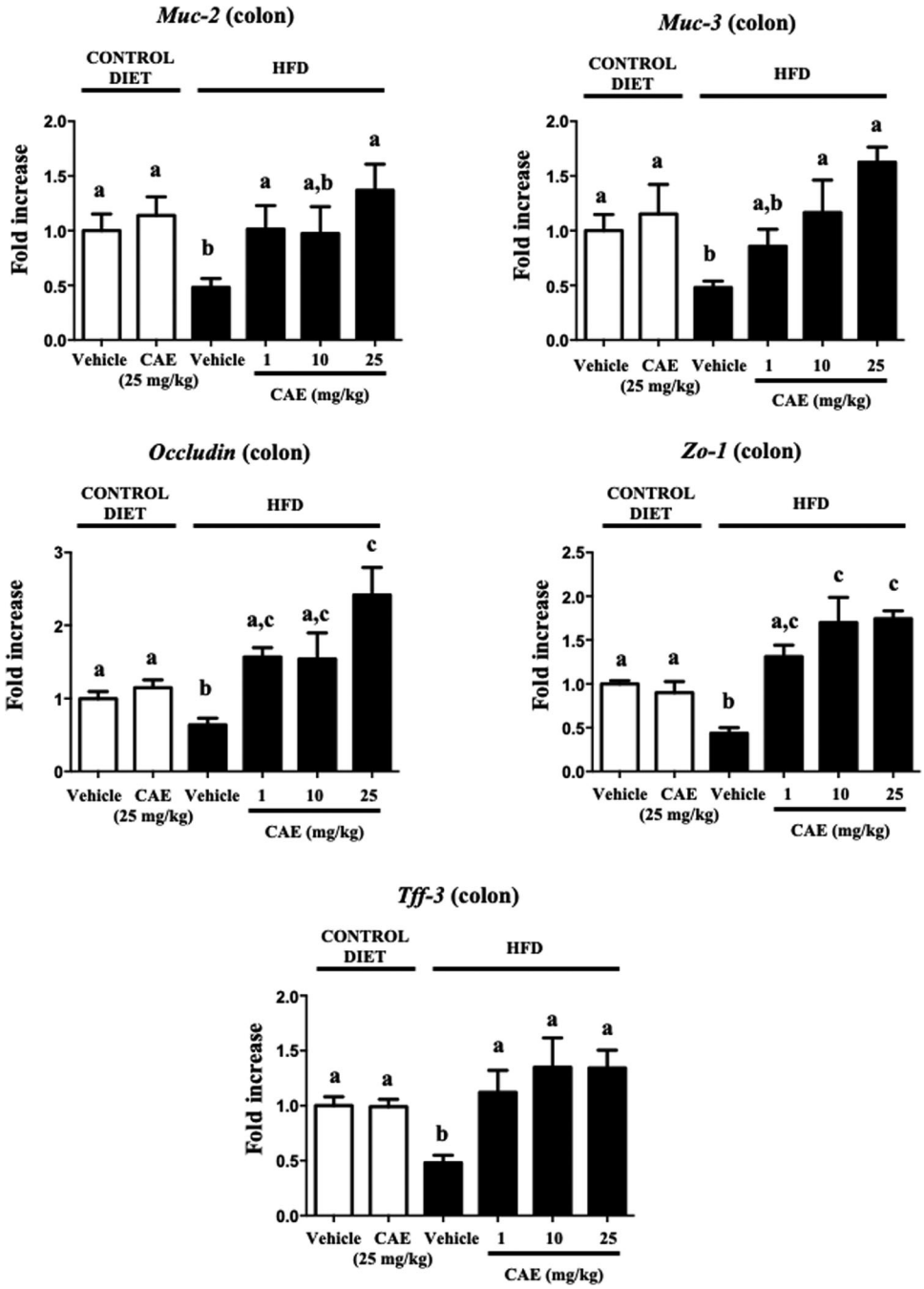

Figure 6. Impact of pepper extract on the gene expression of *Muc-2*, *Muc-3*, *Occludin*, *Zo-1*, *and Tff-3* analyzed by real-time qPCR and normalized with the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in colonic tissue of control and HFD-mice. Data are expressed as mean ± SEM (n = 8–10/group). The groups with different letters are significantly different (one-way ANOVA post hoc Tukey's test, $p < 0.05$).

4. Concluding Remarks

Ripening stage and drying process influenced positively the phytochemical profile and the biological activity of *C. annuum* cv Senise. The dried sweet red pepper commonly used in Basilicata region showed the highest amount of carotenoids, polyphenols, and capsinoids and the best radical-scavenging activity. Besides, the extract was able to ameliorate HFD-induced obesity in mice, improving glucose tolerance and reducing the inflammatory status, maybe due to its antioxidant properties and its ability to modulate gut microbiota composition. Thus, these results suggest a new potential approach in the treatment of obesity, complementary to dietary restrictions, based on an anti-inflammatory effect and the modulation of the microbiome.

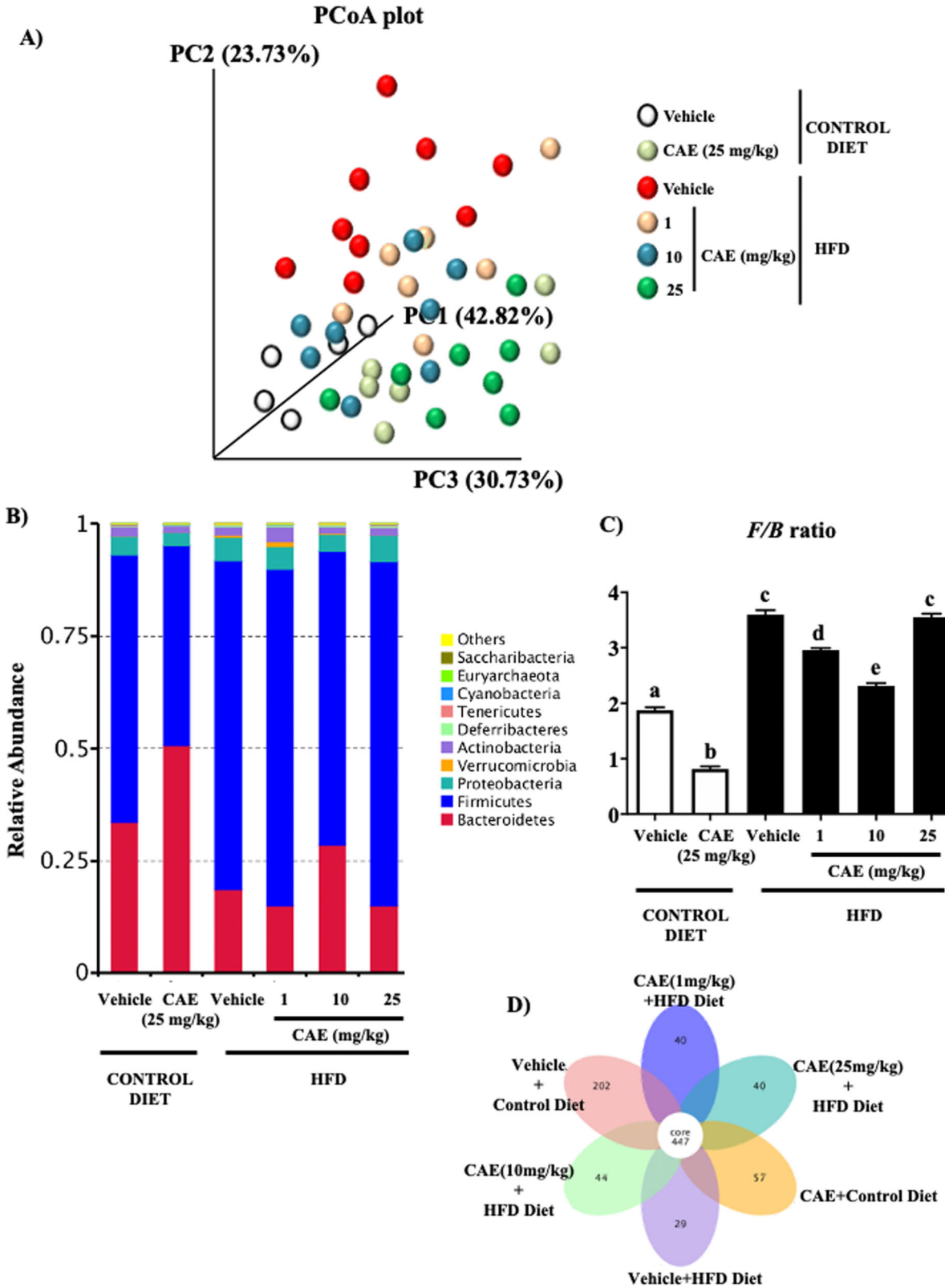

Figure 7. Comparison of faecal microbiota composition between control and high fat diet(HFD)-fed mice: A) Principal component analysis plot based on Bray–Curtis distances, calculated on the metagenomic table of faecal samples of the different groups; B) Relative abundance of the phyla in the different groups; C) The *Firmicutes*/*Bacteroidetes* ratio (*F*/*B* ratio) was calculated as a biomarker of gut dysbiosis, and D) Venn diagram of the OTUs from each group. Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Groups with different letters statistically differ ($p < 0.05$).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

dried red sweet peppers, hypoglycemic effect, hypolipidemic effect, inflammation, microbiomes, obesity

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