Adding a purple corn extract in rats supplemented with chia oil decreases gene expression of SREBP-1c and retains Δ5 and Δ6 hepatic desaturase activity, unmodified the hepatic lipid profile

Sixto Reyna Gallegos[a,b,*], Génesis Torres Arrunátegui[a], Rodrigo Valenzuela[c,d], Miguel Ángel Rincón-Cervera[c], María Elena Villanueva Espinoza[a]

[a] Nutrition Department, Animal Husbandry Faculty, Universidad Nacional Agraria La Molina, Lima, Perú
[b] Carrera de Medicina Veterinaria, Facultad de Ciencias Veterinarias, Universidad Técnica de Manabí, Manabí, Ecuador
[c] Nutrition Department, Faculty of Medicine, Universidad de Chile, Santiago, Chile
[d] Lipid Center, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile

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ABSTRACT

Flavonoids upregulate gene expression of PPAR-α and underregulate the gene expression of SREBP-1c, and their intake increases the plasmatic concentration of n-3 LC-PUFAs. However, the biological mechanisms underlying these effects have not been elucidated. In this work, the effect of oral supplementation of ALA from chia (*Salvia hispanica L.*) seed oil and anthocyanins from a purple corn extract (PCE) on gene expression of SREBP-1c, PPAR-α and Δ5 and Δ6 desaturases (Δ5D and Δ6D), the activity of these enzymes in the liver as well as the hepatic lipid profile were evaluated in thirty-six female Sprague Dawley rats whose diet was supplemented with olive oil (OL), chia oil (CH), olive oil and PCE (OL + PCE) or chia oil and PCE (CH + PCE). Gene expression of PPAR-α was significantly higher when supplemented with CH and CH + PCE, SREBP-1c gene expression was higher when supplemented with chia oil. CH supplementation enhanced Δ5D expression whereas no significant differences between treatments were observed concerning Δ6D gene expression. Activities of both desaturases were increased by including olive oil (OL + PCE and OL), and they were found to be higher in CH + PCE respect to CH for both enzymes. The ALA and n-3 LCPUFAs hepatic content was higher with CH, decreasing the levels of AA and n-6 LCPUFAs. It is concluded that the joint action of flavonoids such as anthocyanins and ALA show an anti-adipogenic effect. Desaturase activity was inhibited by ALA and kept by the anthocyanins from PCE, thus anthocyanins would exert a protective effect on the desaturase activity but they would not affect on its gene expression, however, high doses of ALA increased the production of its metabolites, masking the effect of PCE.

1. Introduction

Linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3) are considered essential because they can not be synthesized by mammals [1]. Although it has been suggested that the beneficial effects of n-3 LCPUFAs are not attributable to the ALA [2,3], several biological functions have been attributed to ALA, such as the activation of the protein kinase activated by AMP (AMPK) [4], the decrease in the gene expression of SREBP-1c [5] and the increase in the PPAR-α, CPT-I [4,6] and acyl-CoA oxidase 1 [6], providing an anti-adipogenic effect [4]. In adition, ALA is the most consumed n-3 PUFA in the Western diet [7] being a precursor of EPA and DHA [6], it is also the main source of n-3 LCPUFAs in the absence of a significant consumption of marine products, [8], even considering that the efficiency of bioconversion process

to EPA and DHA is limited [9]. The characteristics of the Western diet create an imbalance in the intake of ALA versus LA, which is clearly favorable to the second [10,11], which causes that despite the higher affinity of the delta-6-desaturase (Δ6D) for ALA than for LA, arachidonic acid (AA, 20:4 n-6) synthesis predominates, inhibiting the metabolic conversion of ALA to EPA and DHA [12].

Polyphenols including phenolic acids and flavonoids [13] and their consumption have been linked to the prevention of Noncommunicable diseases (NCDs) in humans [13,14] and animals [15]. It has been reported that flavonoids such as anthocyanins activate AMPK in the liver, which increases the gene expression of PPAR-α, acetyl-CoA oxidase (ACO) and carnitine palmitoyl transferase I (CPT-I) and inhibits acetyl-CoA carboxylase (ACC) [16,17], while attenuating the expression of SREBP-1c and its target molecules as the fatty acid synthase, thus

inhibiting lipid synthesis and stimulating lipolysis [18]. The mechanisms proposed for anthocyanins and their effects on lipid metabolism have similarities with those of n-3 PUFAs [16], and interactions between flavonoids and n-3 LCPUFAs have been reported [19]; thus, the combination of these fatty acids and procyanidins determines a synergistic anti-inflammatory effect [20]. However, there is scarce and conflicting information regarding the action of flavonoids on LCPUFAs metabolism and bioconversion of ALA to EPA and DHA [19,21,22] and the biological mechanisms underlying these effects have not been clarified. An increased synthesis of n-3 LCPUFAs from ALA would imply greater activity of the delta 5 (Δ5D) and delta 6 (Δ6D) desaturases [19].

Approximately 80% of total phenols present in a purple corn extract correspond to anthocyanins, cyanidin 3-glucoside mainly, pelargonidin 3-glucoside and peonidin 3-glucoside [23], while ALA constitutes more than 60% of the fatty acid composition of chia oil [24–26]. Because there is limited information concerning the joint action of anthocyanins and ALA on lipid metabolism, the current study elucidates, at leats parcially, how anthocyanins from a purple corn extract (PCE) affects lipid metabolism at hepatic level when lactating rats are supplemented with chia oil, raising the hypothesis that this combination affects gene expression of SREBP-1c, PPAR-α, Δ5D, Δ6D, the activity of these enzymes, and lipid profile in hepatic tissue, compared to a conventional diet.

2. Materials and methods

2.1. Animals and diets

Thirty-six (36) female Sprague-Dawley rats, weaned, with an average age of 23.31 ± 0.467 days and a liveweight of 46.42 ± 5.88 g, were acquired from the Animal facility biotery of the Laboratory of Food Nutrition Assessment (LENA) of the Animal Husbandry Faculty of the Universidad Nacional Agraria La Molina. The animals received an isocaloric and isoproteic diet (Table 1) in a flour presentation, acquired from the Research and Social Projection in Food Program of the Universidad Nacional Agraria La Molina. The lipid profile of the diets and olive and chia oil supplemented, was estimated using food tables FEDNA 2010 [27] and the information provided by Jimenez et al. [28] it is appreciated in the Table 2. At 28 days of age, nine rats were randomly assigned to each one of the four treatments according to the supplemented oil and the presence of PCE: olive oil (OL), chia oil (CH), olive oil and PCE (OL + PCE) or chia oil and PCE (CH + PCE). Food and water were administrated at discretion

Table 1
Composition and nutritional content of the control diet.
Source: Programme for Food Research and Social Projection. UNALM.

Ingredientes	
Corn	65.72%
Soybean meal	26.29%
Soybean oil	6.32%
Calcium carbonate	1.16%
DL-metionine	0.21%
Vitamins + minerals pre-mix	0.1%
Cl. Coline 60%	0.1%
Sodium chloride	0.1%
Nutritional content	
Dry matter	88.07%
Protein	18.00%
Fiber	2.70%
Fat	8.88%
Metabolizable energy	3.50 Mcal/kg
Total phosphorus	0.33%
Calcium	0.50%
Sodium	0.05%
Linoleic acid	4.71%

Table 2
Lipid profile of the diets and supplementation with oils (g/100 g).

Fatty acids	Treatments			
	OL	CH	OL + PCE	CH + PCE
C14:0	0.00	0.001	0.00	0.01
C16:0	1.73	1.5	1.72	1.48
C16:1, 9	0.03	0.02	0.03	0.02
C18:0	0.61	0.45	0.60	0.45
C18:1, 9	8.76	2.79	8.67	2.77
C18:2 n-6 (AL)	5.32	6.42	5.27	6.36
C18:3 N-3 (ALA)	0.54	4.98	0.53	4.93
Total LCPUFA	0.40	0.4	0.4	0.40

Composition of fatty acids from combination of dietary intake (OL, CH, OL + PCE and CH + PCE) and supplementation with olive or chia oil.

throughout of the experiment, being food consumption and body weight monitored daily and weekly, respectively. The PCE powder with a concentration of anthocyanins of 4.01% (EEC-E163) was acquired from GlobeNatural (anthocyanins MS2 4%, batch number 013070) and supplied in the diet at a dose of 1%, equivalent to 401 mg of anthocyanins per food kg. The anthocyanins composition of purple corn it is appreciated in the Table 3 [29]. The addition of the PCE into the food was performed daily to prevent anthocyanin deterioration [16,30]. The PCE was stored in a dry, cool environment, at temperatures below 30 °C. The extra virgin olive oil was obtained from the local market (Alba del Perú Laboratories, batch 1001153) and chia seeds were provided by Chía Perú, whose taxonomic classification was certified by the herbarium of the National University of San Marcos. The oil, obtained by cold pressing at the Pilot Factory of the Food Industry Faculty of the Universidad Nacional Agraria La Molina, was centrifuged for 15 min at 1000 rpm, sediments were removed and the oil was stored in refrigeration (between 4 °C and 8 °C) and protected from light. Supplementation with chia oil and olive oil was estimated at 20% of the energy supplied daily in the food of the different treatments, crude soybean oil was used in the diet as a source of energy. The corresponding dose of chia oil and olive oil was provided using cannulas from modified hypodermic needles No. 14GX 11/2″ to prevent lacerating the oral cavity of rats. These cannulas were coupled to hypodermic syringes of 1 ml volume. Cannulas cleaning was performed daily, while the syringes were discarded once used. PCE, olive oil and chia oil were supplied throughout the experiment.

2.2. Reproductive management and eutanasia

When the rats reached the tenth week of age, groups of three rats from the same treatment were mated with males of the same genetic line. Between the fourth and fifth postpartum day the litter size was homogenized to 6 pups. At day 16, nursing mothers were sacrificed by

Table 3
Identity and relative abundance of anthocyanins from a purple corn extract Escribano et al. [29].

Anthocyanins	(%)
Dímers*	1.8
cyanidin-3-glucoside	54.3
pelargonidin-3-glucoside	6.1
peonidin-3-glucoside	14.7
cyanidin-3-(6″-malonylglucoside)	11.6
Pelargonidin-3-(6″-malonylglucoside)	3
peonidin-3-(6″-malonylglucoside)	5.5%
cyanidin-3-(6″-ethylmalonylglucoside	2.6
pelargonidin-3-(6″-ethylmalonylglucoside)	0.2
peonidin-3-(6″-ethylmalonylglucoside)	0.1

*Dimer formed by direct condensation between a flavan-3-ol and cyanidin-3,5 diglucoside.

Table 4
Parameters of growth, liver weight and visceral fat in relation to body weight.

Parameters	OL	CH	OL + PCE	CH + PCE	P value
Initial weight, d 28 (gr)	70.2 ± 6.8 (a)	67.1 ± 6.8 (a)	65.0 ± 6.7 (a)	65.6 ± 7 (a)	0.379
Euthanasia weight (gr)	244.67 ± 20.69 (a)	249.33 ± 21.31 (a)	230.89 ± 22.39 (a)	241.00 ± 13.93 (a)	0.260
Food consumption (gr)/día *	1.26 ± 0.04 (a)	1.26 ± 0.03 (a)	1.27 ± 0.028 (a)	1.25 ± 0.03 (a)	0.815
% liver weight	3.85 ± 0.31 (a)	4.093 ± 0.23 (a)	4.042 ± 0.347 (a)	3.893 ± 0.22 (a)	0.221
% visceral fat	2.98 ± 0,83(a)	2.48 ± 0,55 (a)	2.53 ± 0,87 (a)	2.88 ± 0,46 (a)	0.341

Values represent the mean ± SD of 9 rats per treatment. Different letters indicate significant differences between treatments ($p < 0.05$; one-way ANOVA and Tukey's test)* Logarithmic data transformation.

exsanguination after 12 h of overnight fast and the application of a mixture of xylazine (25 mg/Kg) and ketamine (150 mg/Kg) intraperitoneally applied. Their livers were removed and weighed, a portion of which was placed in previously identified cryogenic vials and frozen in liquid nitrogen until analysis. The research protocol (N° 2014-001) was approved by the Ethics Committee and Animal Welfare of the Faculty of Veterinary Medicine at the National University of San Marcos.

2.3. Genetic expression analysis

Isolation of total RNA and subsequent PPAR-α, SREBP-1c, Δ5D and Δ6D (obtained from the liver of lactating rats) polymerase chain reaction was performed according to Reyes-Farías et al. [31]. The following primers (forward and reverse) were used: for PPAR-α (3′-5′-GGTGCA TTTGGGCGTAACTC) and (3′-5′-CCACAGAGCACCAATCTGTGA); for SREBP-1c: (5′TGGAGCGAGCATTGAACTGT-3′) and (3′-5′-GTGGTAGC CATGCTGGAACT); for Δ5D: (5′-3′CCACTACGCTGGTCAGGATG) and (5′-3′AGCGCCTTATTCTTGGTGGG); and for Δ6D: (5′-3′TTACCAAATG GTCCCAGCGG) and (5′-ATCTGAGAGCTTTTGCCCCG-3′). The relative expression between groups was calculated by the $2^{(-\Delta\Delta Ct)}$ method.

2.4. Enzymatic activity of Δ5D and Δ6D

Liver samples previously frozen in liquid nitrogen (500 mg) were homogenized in a buffer solution pH 7.9 containing 10 mmol/L HEPES, 1 mmol/L EDTA, 0.6% Nonidet P-40, 150 mmol/L NaCl, and protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 1 µg/ml apronitine, 1 µg/ml leupeptin, and 1 mmol/L orthovanadate). The samples were centrifuged, first at 4 °C and 2000 rpm for 30 s, followed by centrifugation of the supernatant at 5000 rpm for 5 min, and finally at 100,000 rpm for 60 min to obtain the necessary extracts to measure the activity of the desaturases. The activity of the Δ6D desaturase was evaluated based on the amount of gamma-linolenic acid (GLA, 18:3 n-6) produced from 18:2 n-6 while Δ5D desaturase activity was based on the amount of 20:3 n-6 converted to 20:4 n-6 according to the methodology described by Valenzuela et al. [32]. These assessments were carried out simultaneously. Results were expressed as nmol/mg of protein/minute.

2.5. Analysis of hepatic fatty acids

Fatty acids analysis of liver samples were performed by gas-liquid chromatography (GLC). Samples were assessed for lipid extraction according to Bligh and Dyer (Bligh and Dyer [33] and transformed into fatty acid methyl esters (FAME) with methanolic boron trifluoride (12% methanolic solution) according to Firestone [34], and frozen in liquid nitrogen untilanalysis. The GLC of FAME war performed using Hewlett-Packard equipement (model 6890A), with a capillary column (Agilen HP-88, 60 m × 0.25 mm; I.D. 0.25 mm) and flame ionization detector for FAME detection. Hidrogen was the carrier gas. The retention times of FAME were compared to a standard mixture (Nu-Check Prep). C23:0 was used as internal standard. Fatty acids were expressed as mg/100 g liver.

2.6. Statistical analysis

All results are presented as mean ± standard deviation (SD). The normal distribution of the data was analyzed with the Ryan-Joiner test (similar to Shapiro-Wilk) and homogeneity of variance with the Levene test. When it was necessary, we performed a logarithmic transformation prior to the statistical analysis, which consisted in a one-way analysis of variance (ANOVA) to evaluate the effect of treatments on growth parameters and with a 2 × 2 factorial arrangement to determine the effects of the supplementation with anthocyanins, the type of oil and the interaction of both factors on PPAR-α, SREBP-1c, Δ5D and Δ6D gene expression, the activity of the desaturases as well as the liver lipid profile. The level of statistical differences was preset at $p < 0.05$ and the differences among means were estimated using the multiple comparison test of Tukey. The corresponding analysis was performed using the statistical package Minitab (version 16 State College, PA, U.S.A.). The graphs was performed with GraphPad Prism 5 (GraphPad Prism Sofware, Inc. San Diego, CA, USA).

3. Results

3.1. Weight gain and food consumption

The initial weight, age of euthanasia, food intake (g/day), liver weight (g/100 g body weight), and visceral fat (g/100 g body weight) were not significantly different between treatments (Table 4).
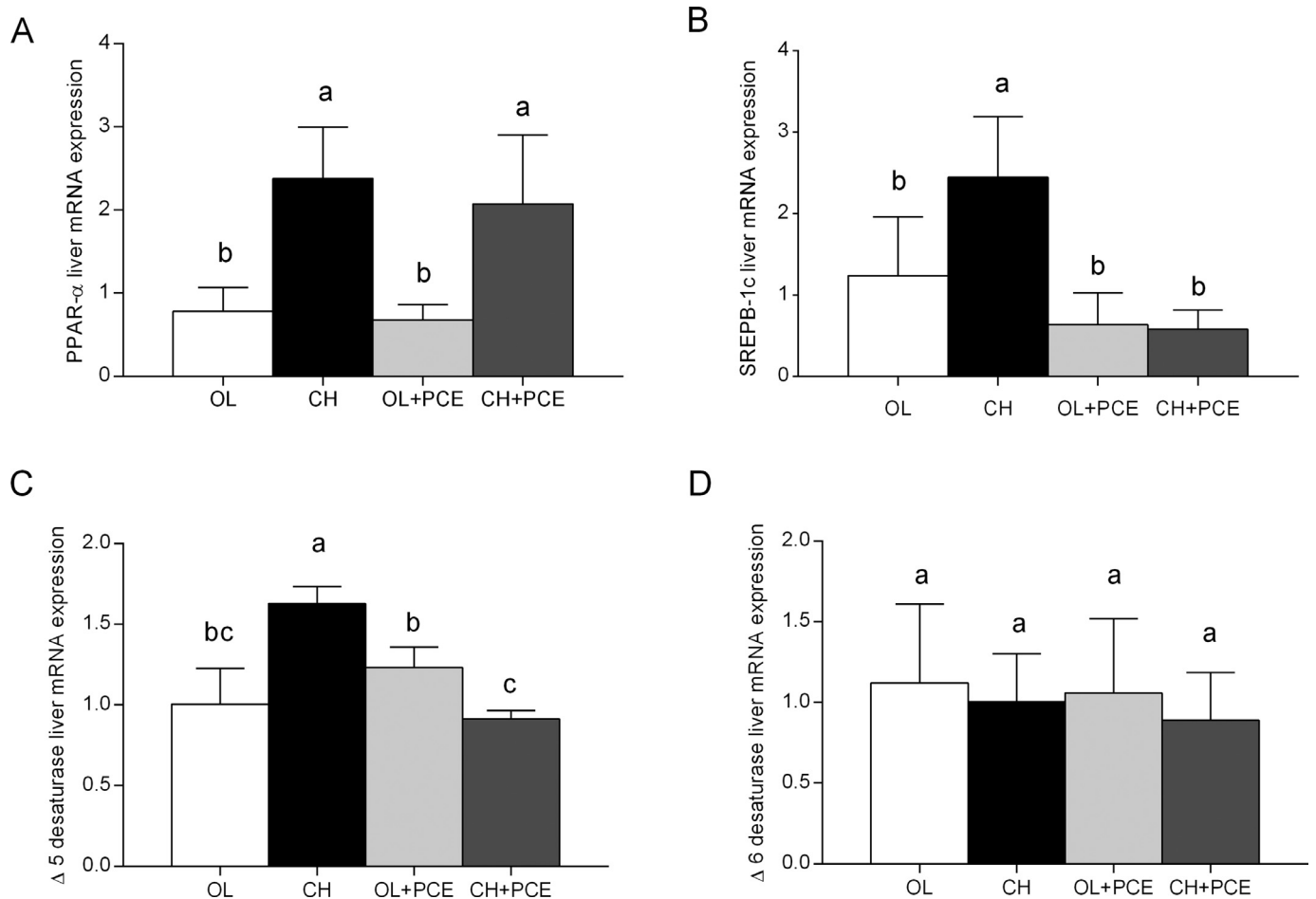
3.2. Genetic expression

Gene expression of PPAR-α showed significant differences among treatments, being higher in CH and CH + PCE groups and lower in OL + PCE and OL. Differences in gene expression of SREBP-1c were also observed, which were higher in CH respect to the remaining treatments, particularly those including PCE (CH + PCE and OL + PCE). Likewise Δ5D expression was higherafter supplementation with chia oil, however no differences among treatments were observed when Δ6D gene expression was assessed (Fig. 1). Due to mRNA degradation of some samples, different numbers of data for gene expression of PPAR-α, SREBP-1c, Δ5D and Δ6D were observed.

3.3. Enzyme activity

Although the activity of Δ5D and Δ6D was higher with olive oil than with chia oil, no differences were observed between OL + PCE and OL treatments. The decrease in the Δ5D and Δ6D activity was lower when PCE was added, evidencing greater enzymatic activity of Δ5D and Δ6D in CH + PCE than in CH (Fig. 2). This suggests a protective effect of PCE on the activity of these enzymes against high doses of ALA present in chia oil.

3.4. Fatty acid composition of hepatic triacylglycerols

The total content of saturated, monounsaturated and arachidonic fatty acids were higher in rats supplemented with olive oil (OL and

Fig. 1. Hepatic expression mRNA of PPAR-α (A), SREBP-1c (B), Δ5 desaturase (C) and Δ6 desaturase (D) by qPCR from the experimental groups OL, CH, OL + EMM and CH + EMM. Values represent the mean ± SD. (N = 7, 5, 6, and 6 rats/experimental group for PPAR-α gene expression, SREBP-1c, Δ5D and Δ6D). Different letters indicate significant differences between treatments. Treatments identified with the letter a, the gene expression is greater than those represented by the letter b, and these that the treatments noted with the letter c ($p < 0.05$; ANOVA with factorial arrangement 2 × 2 and Tukey's test).

OL + PCE treatments); the hepatic concentration of n-6 LCPUFAs was higher in and OL + PCE treatment compared to treatments supplemented with chia oil. The supplementation with chia oil determined higher concentration of n-3 fatty acids (ALA, EPA and DHA) and total n-3 LCPUFA. Total PUFAs was similar between treatments (Table 5).

4. Discussion

Anthocyanins, chia oil or a combination of both products had no effect on food intake, final weight and liver weight in relation of body weight (g/100 g BW) of the rats, which is consistent with results reported by Vauzour et al. [21] who did not observe differences between treatments for these parameters, after including purified anthocyanins in the diet of male rats, and it is also consistent with data reported by Lefevre et al. [35], by providing a semi purified anthocyanin extract to male mice fed with a diet rich in fat and cholesterol, however PCE significantly decreased the weight of liver tissue. Another study attributed to anthocyanins the decrease in body and liver weight in obese mice fed with high-fat food [36]. Also, it has been reported that the inclusion of chia seeds in the diet reduced abdominal fat in dyslipidemic rats [37] and that the consumption of chia flour significantly (but discretely) decreases the body mass in overweight or obese humans [38]. These data suggest that the intake of anthocyanins and ALA by supplementing with PCE and chia oil exerts an anti-adipogenic effect mainly in overweight or obese individuals.

Genetic expression of PPAR-α in the liver was significantly

increased by providing a source of ALA (CH and CH + PCE treatment) (Fig. 1a), probably due to the low content of PUFAs in the olive oil and the high proportion of ALA in chia oil [24]. PUFAs, especially those of the n-3 family, are able to activate PPAR-α [39,40]. Thus, a diet rich in ALA increased gene expression of PPAR-α in goat liver [41], while in epididymal adipose tissue of mices, ALA upregulated PPAR-α via AMPK [4], and in the liver of rats, n-6 PUFAs act as ligands for PPAR-α, increasing its activation [42]. PPAR-α increases β-oxidation through regulation of genes encoding several key enzymes involved in that process [40,43], and decreases the gene expression of ACC and FAS, target genes of SREBP-1c, therefore resulting in inhibition of lipogenesis [43]. PCE intake did not modify gene expression of PPAR-α in the liver. Regarding this issue, Seymour et al. [44] reported that the intake of anthocyanins of blueberry extract significantly increased mRNA levels of PPAR-α in skeletal muscle and abdominal adipose tissue of lean rats, and gene expression of PPAR-α in the abdominal adipose tissue of obese rats. Additionally, cyanidin -one of the most abundant anthocyanins-activates gene expression of PPAR-α in HepG2 liver cells [45]. SREBP-1c gene expression was significantly higher in the CH treatment compared to CH + PCE, whereas no significant differences were found among CH + PCE, OL and OL + PCE. (Fig. 1b), suggesting that ALA increases the expression of PPAR-α and SREBP-1c and that the PCE inhibits gene expression of SREBP-1c. In this regard, it is noted that oils rich in PUFAs decrease "in vivo" the hepatic expression of SREBP-1c [41] by reducing its active nuclear form and the mRNA expression and stability of SREBP-1c [46,47]. In contrast, Harnack et al. [48] observed
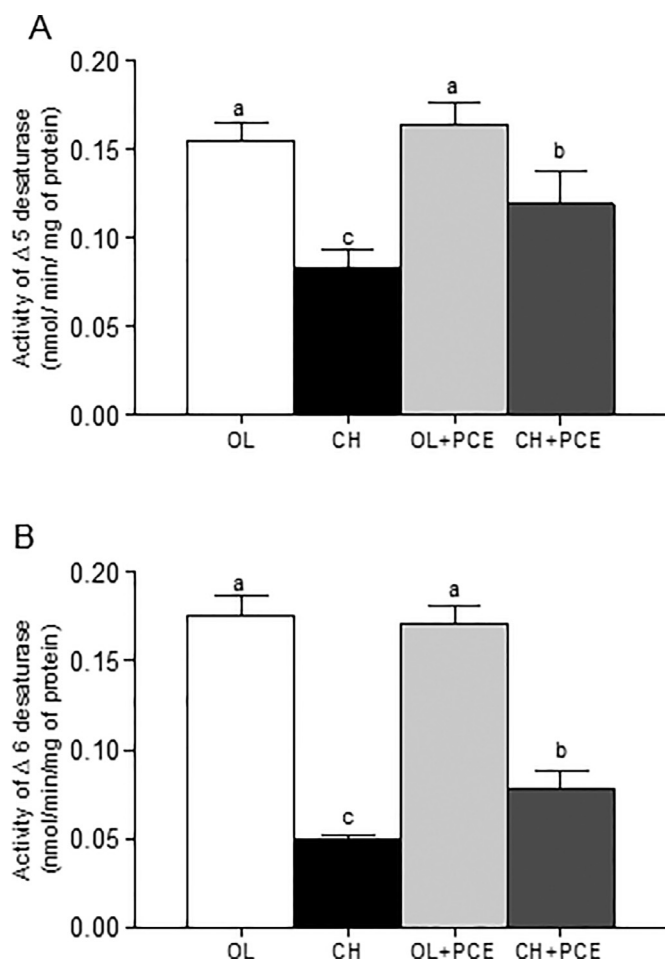
Fig. 2. Hepatic activity of Δ5D (A) and Δ6D (B). Values represent the mean ± SD (n = 6 rats per treatment). Different letters indicate significant differences between treatments. Treatments identified with the letter a, the gene expression is greater than those represented by the letter b, and these that the treatments noted with the letter c ($p < 0.05$; ANOVA with factorial arrangement 2×2 and Tukey's test).

cells of liver tissue [35], considering that the intake of anthocyanins, in a dose-dependent mode, determines the phosphorylation and subsequent activation of AMPK in the liver, increasing the gene expression of PPAR-α [35,16,18] which in turn upregulates target genes for enzymes involved in β-oxidation, such as CPT-I and acyl CoA oxidase (ACOX) [22,16]. In addition, AMPK activation by anthocyanins limits the lipid biosynthesis by inhibiting the expression of ACC and FAS expression indirectly by suppressing SREBP-1c "in vitro" [35,18]. It has also been reported that the activation of PPAR-α inhibits the induction of SREBP-1c [51]. In this experiment we showed that the combined action of ALA and anthocyanins from chia oil and the PCE respectively, determine an anti-adipogenic effect mediated by the gene expression of PPAR-α and SREBP-1c, so that in the CH + PCE treatment, gene expression is increased for PPAR-α and decreased for SREBP-1. It was also observed that the interaction between the oil type and the dose of anthocyanins determines the inhibition of the gene expression of SREBP-1c, particularly in the treatment with chia oil.

Δ5D gene expression was significantly higher in CH than in CH + PCE. OL + PCE and OL did not differ significantly from each other (Fig. 1c). Increased Δ5D gene expression attributed to ALA would be due to the action on PPAR-α and SREBP-1c (Fig. 1a and b) which in turn induce Δ5D [52]. High levels of ALA oxidation would stimulate the expression of PPAR-α. Furthermore, the action of ALA on MAP kinase increases the expression of SREBP-1c, which would result in a larger expression of Δ5D. In this regard, it was noted that exposure of liver cells to LA/ALA in a 0:1 ratio defines a higher gene expression of PPAR-α, SREBP-1c and Δ5D than ratios of 1:0, 1:1 and 4:1 [48]. Moreover, the PCE would have and anti-adipogenic effect attributable to anthocyanins [29], thus the inhibitory action of PCE on gene expression of SREBP-1c (Fig. 1b) probably inhibits Δ5D, target enzyme of SREBP-1c [53]. Δ6D gene expression was not altered as a result of the action of ALA or PCE (Fig. 1d). In this regard it has been reported that exposure of HepG2 cells at a dose of 100 μM ALA in absence of LA does not alter the levels of Δ6D gene transcription [48] and that larger ALA doses do not alter gene expression of desaturases [53]. Likewise, anthocyanins do not modify Δ6D gene expression; similar results were reported by Graf et al. [21] when the expression of desaturases enzymes in liver of male rats who received anthocyanins in their diet was assessed.

The highest activity of Δ5D and Δ6D desaturases (Fig. 2a and b) was observed in the treatments supplemented with olive oil (OL and OL + PCE), in which the addition of the PCE to the diet did not significantly change the enzymatic activity. When chia oil was included (CH and CH + PCE), a lower Δ5D and Δ6D activity was registered, but this was significantly higher in CH + PCE, suggesting that ALA supplementation reduces the activity of the desaturases, while on the other hand adding PCE inhibits such reduction. Therefore, anthocyanins would exert a protective action on the activity of these enzymes against

that ALA increased gene expression of SREBP-1c "in vitro" probably because ALA increases the gene expression of MAP kinases and these in turn phosphorylate SREBP-1c, stimulating its transcriptional activity [49]. SREBP-1 activates a cascade of enzymatic reactions required for fatty acid endogenous synthesis [45,50], in this regard it has been reported that the anthocyanins attenuate SREBP-1c expression in HepG2

Table 5
Hepatic fatty acid composition (mg/100 g) of rats supplemented with OL, CH, OL + PCE and CH + PCE.

Most relevant fatty acid	OL	CH	OL + PCE	CH + PCE	Oils	PCE	Interaction
C16:0	638.9 ± 147.0 (ab)	600.7 ± 120.3 (b)	840.0 ± 178.5 (a)	620.0 ± 102.0 (b)	0.0381	0.0726	0.1339
C18:0	613.2 ± 112.1 (a)	585.4 ± 107.0 (a)	683.1 ± 89.0 (a)	570.5 ± 54.0 (a)	0.0906	0.4978	0.2972
Total SFA	1283.2 ± 238.3 (b)	1216.4 ± 205.9 (b)	1566.4 ± 271.2 (a)	1217.0 ± 146.0 (b)	0.0353	0.1419	0.1422
C18:1, 9	602.1 ± 183.3 (b)	466.2 ± 172.0 (b)	972.6 ± 302.2 (a)	466.7 ± 114.4 (b)	0.0011	0.0397	0.0402
Total MUFA	673.2 ± 206.3 (ab)	519.0 ± 192.5 (b)	1036.0 ± 314.0 (a)	527.2 ± 128.2 (b)	0.0016	0.0545	0.0651
C18:2, n-6 (AL)	594.0 ± 179.1 (a)	692.3 ± 250.9 (a)	904.0 ± 259.2 (a)	670.0 115.6 (a)	0.4587	0.1259	0.0729
C18:3, n-3 (ALA)	10.4 ± 4.2 (b)	66.7 ± 44.7 (a)	14.9 ± 7.8 (b)	75.4 ± 14.6 (a)	< 0.0001	0.5330	0.8237
C 20: 4, n-6 (AA)	687.2 ± 150.1 (a)	418.4 ± 90.2 (b)	789.3 ± 101.5 (a)	435.1 ± 73.0 (b)	< 0.0001	0.1968	0.3452
C 20: 5, n-3 (EPA)	9.7 ± 2.5 (b)	90.3 ± 38.0 (a)	10.5 ± 1.3 (b)	115.0 ± 26.1 (a)	< 0.0001	0.2212	0.2494
C 22: 6, n-3 (DHA)	127.8 ± 29.3 (b)	239.9 ± 41.2 (a)	123.0 ± 17.0 (b)	238.0 ± 55.2 (a)	< 0.0001	0.8265	0.9164
Total n-6 PUFA	1345.5 ± 327.1 (ab)	1191.7 ± 320.2 (b)	1830.0 ± 374.4 (a)	1180.0 ± 202.0 (b)	0.0070	0.0924	0.0775
Total n-3 PUFA	181.5 ± 31.4 (b)	503.7 ± 120.9 (a)	198.3 ± 27.9 (b)	524.0 ± 95.0 (a)	< 0.0001	0.6172	0.9301
Total PUFA	1520.5 ± 351.3 (a)	1695.3 ± 438.8 (a)	2028.0 ± 402.4 (a)	1703.1 ± 292.6 (a)	0.6459	0.1261	0.1372

Values represent the mean ± SD of 6 rats for treatment. Different letters indicate significant differences between treatments ($p < 0.05$; two-way ANOVA and Tukey's test). Oils, significant influence of oil source in the treatment; PCE significant influence of purple corn; Oils × PCE interacción.

ALA. The higher activity of Δ5D and Δ6D in OL and OL + PCE treatments probably reflects lack of inhibition by PUFAs, whose content is low in olive oil, while the inclusion of chia oil, rich in ALA, inhibits the activity of those enzymes, thus showing a negative regulation of n-3 PUFAs on the activity of Δ6D [54]. In this regard, it was noted that an excess of ALA as substrate probably saturates and inhibits the synthetic pathway of n-3 LCPUFAs [55], because Δ6D affinity is higher for ALA than for one of its downstream metabolite: 24:5 n-3 [56]. Additionally the rate of mitochondrial and peroxisomal β-oxidation of ALA is higher than that of other fatty acids [5], this would cause an increase in the availability of acetyl CoA for the Krebs cycle, and therefore, the rate of oxidative phosphorylation, increasing the production of reactive species (ROS) and damage of the cell membrane and organelles, causing peroxidation [57]. Lipid peroxidation and oxidative stress are related to a decrease in the enzymatic activity of Δ5D y Δ6D and depletion of LCPUFAs [58]; anthocyanins inhibit oxidative stress and lipid peroxidation [59].

Thus, a larger ALA intake decreases the levels of EPA [56] and DHA [13]. In this regard, the addition of ALA would favor the biosynthesis of LCPUFAs only when it is incorporated to the diet at levels below 3% of dietary energy, while in higher doses it would inhibit the synthesis of DHA [60]. However, supplementation with chia oil at the dosis used in the present research permitted appreciate how anthocyanins affect the metabolism of LCPUFA. The reduccion of inhibition of Δ5D and Δ6D activity to supplement with PCE, would confirm the influence of anthocyanins on lipid metabolism, specifically on the enzymatic activity of the desaturases and the biosynthesis of LC-PUFAs as previously proposed in studies in rodents [19] and humans [61]. In the present experiment, supplementation with CH increased the levels of ALA, EPA and DHA in liver of rats, while decreased the concentration of AA and total of n-6 PUFA relative to rats supplement with oliva oil and PCE. In this regard, it has been reported that the inclusion of chia oil in the diet of rats increases the serum concentration of ALA and DHA and decreases that of LA and AA [62]. Additionally, supplementation with ALA-rich oils has been reported to increase ALA and n-3 LCPUFAs, and the simultaneous reduction of n-6 LCPUFAs in rat liver, despite the inhibition of the enzymatic activity of desaturases [63]. On the other hand, PCE does not modify the hepatic lipid profile of the rats. In this regards, Vauzour et al. [22] reported that the supply of pure anthocyanins to male rats for 8 weeks did not modify the genic expression of Δ6D and the serum and hepatic content of EPA and DHA. In contrast, Toufeksian et al. [19], reported that purple corn anthocyanins increased plasma levels of n-3 LCPUFA of male rats, in the same way, rye polyphenols increase levels of n-3 LCPUFA in the plasma and liver of male rats [64]. It is likely that on the basis of its antioxidant activity, PCE has an underlying effect on the lipid profile of the liver, by decreasing the inhibition of the desaturase enzymes, probably caused by peroxidation of ALA, due to the dose in which it was supplied. Thus, a high dose of ALA would have a dual effect, inhibiting the activity of the desaturases and at the same time, as substrate of these enzymes for the syntheses of EPA and DHA, masking the protective effect of PCE on the enzymatic activity of the desaturases.

In summary, these results indicate that the combination of a purple corn extract (PCE) containing anthocyanins and chia oil with a high ALA content causes an anti-adipogenic effect, being PCE the responsible of decreasing gene expression of SREBP-1c in the liver, while ALA would increase the expression of PPAR-α. Apparently, the modification of gene expression of PPAR-α and SREBP-1c altered Δ5D gene expression in the liver. On the one hand, it was shown that high doses of ALA reduce the activity of desaturases, and PCE preserves the activity of such enzymes, indicating that increased level of LCPUFA reported in previous works may be due to an increase or protection of Δ5D and Δ6D activity, but not because of a higher gene expression of these enzymes. However, the delivery of high doses of ALA may mask the effect of anthocyanins on the synthesis of LCPUFAs, as an increased activity of desaturases was observed in rats supplemented with olive oil compared to those fed with chia oil. The mechanisms by which PCE preserves desaturase activity against ALA should be further elucidated.

Conflicts of interest

The authors declare no conflict of interest.

Aknowledgment

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