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ORIGINAL PAPER

Avocado Paste Phenolics Mitigate a High-Fat Diet-Induced Plasma HDL Decrease in Male Wistar Rats, by Altering the mRNA Expression of Hepatic SCARB1

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Abstract

Avocado paste (AP) is the main industrial byproduct of its processing, and retains various phenolic compounds (PCs). PCs are known to normalize the plasma lipid profile, but those from avocado byproducts have been minimally studied. We report the normalizing effects of an AP-derived phenolic extract (PE) on the plasma lipid profile of male Wistar rats. A standard (SD) and high-fat diet (HFD) were formulated, and the same diets were supplemented with 1 g/kg of diet of PE (SD + PE and HFD + PE). Rats were fed these diets during an 8-week period. The HFD induced signs of dyslipidemia, but PE treatment countered the decrease in HDL. Relative mRNA expression (real-time PCR) of the hepatic HDL receptor (SCARB1) increased in both groups (SD + PE and HFD + PE), while the LDR receptor (LDLR) increased in SD + PE group. The mRNA expression of apolipoproteins APOA1 and APOB was unaffected. We conclude that PCs from AP can counter a diet-induced decrease in plasma HDL by acting on the mRNA expression of its hepatic receptor.

Keywords *Persea americana* · Byproducts · Apolipoproteins · Dyslipidemia · Liver

Introduction

An organism's plasma lipid profile is multifactorial; diet has a marked influence since certain dietary patterns have been associated with maintaining a healthy profile or inducing anomalies. For example, diets rich in cholesterol and fats and deficient in fiber and micronutrients have been shown to induce multiple dyslipidemias, such as hypercholesterolemia, hypertriglyceridemia, among others [1]. Conversely, diets that include an appropriate amount and type of lipids and are rich in fiber and micronutrients can maintain or restore a healthy lipid profile [2]. Dyslipidemias are intricately associated with diabetes mellitus and can contribute to the development and progression of the disease (as well as some of its comorbidities) if not properly addressed [3]; thus, they remain a challenge for both diabetic patients and the overall population.

The lipid profile is clinically assessed according to the concentration of high- and low-density lipoproteins (HDL and LDL, respectively), and that of the cholesterol and triacylglycerols (TAGs) that they contain. The physiological role of a lipoprotein is dictated by its apolipoprotein content, where apolipoprotein A1 (APOA1) is found as part of HDL, while apolipoprotein B (APOB) is found in LDL and other non-HDL particles [4, 5]. Their presence also makes it possible to regulate the lipoproteins' plasma concentration, since specific hepatic receptors recognize and uptake them as required, most notably, the scavenger receptor class B member 1 (SCARB1) and the LDL receptor (LDLR), which

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Table 1 Detailed composition of experimental diets (g/kg)

Ingredient	SD	HFD	SD + PE	HFD + PE
Corn starch[a]	399	113	399	113
Sucrose	30	30	30	30
Cellulose	60	60	60	60
Casein[b]	257.30	258.30	257.30	258.30
Vegetable oil	23.75	80.45	23.75	80.45
Vegetable shortening	23.75	80.45	23.75	80.45
Vitamin mix[c]	18	18	18	18
Salt mix[d]	60	60	60	60
Choline chloride[e]	2	2	2	2
Water	126.2	297.80	125.2	296.80
Phenolic extract (PE)[f]	–	–	1	1

Detailed composition of the diets is included as Supplementary Material

Q6 *SD* standard diet, *HFD* high-fat diet, *PE* phenolic extract

Bio-Serv # 3200[a], # 1100[b], # F800[c], # 6105[e]. Ethanolic extract extracted as described in the main text[f]

are the main receptors of HDL and LDL, respectively [6, 7]. Thus, an organism's lipid profile is subject to change in response to the effects exerted on the lipoproteins or their receptors by dietary components.

Bioactive compounds in the diet can potentially normalize it, in particular, phenolic compounds (PCs) [8]. They are ubiquitous in fruits and vegetables and have been associated with multiple health benefits [9], while some authors have noticed that their byproducts (such as peel and seeds) are also rich sources [10]. In fact, byproducts can often contain a higher concentration of PCs than the ones found in the edible pulp, but are seldom used as PC sources, since they are commonly discarded into landfills or are otherwise underutilized.

Mexico is the main worldwide producer of avocados (*Persea americana* Mill.), whose lipid-rich composition makes it a source of vegetable oil, yielding avocado paste (AP) as final byproduct. A significant percentage (close to 90%) of PCs can be retained and concentrated in AP, making it a significant source of underutilized PCs [11]. For example, Zuñiga-Martínez et al. [12] report the potential of PCs from AP to inhibit intestinal cholesterol uptake (in silico), while Corella-Salazar et al. [13] show that they can regulate satiety in vivo. We have previously determined the bioactivities of other vegetable byproducts (mango peel) in vivo [14], however, the effects of avocado byproducts on the serum lipid profile remain to be studied.

The present work reports the plasma lipid-modulating effects of an AP extract rich in PCs in an in vivo model. The effects of AP PCs were studied in parallel in animals that consumed a balanced diet or a high-fat diet (HFD). Our goals were to explore avocado byproducts as sources of bioactive PCs, and determine their potential effects on the serum lipid profile in animals consuming two distinct dietary patterns, a macronutrient-balanced diet and an HFD.

Materials and Methods

Sample Processing

AP used was donated by an avocado processing plant in Jalisco, Mexico, and consisted of a homogeneous mixture of defatted pulp, peel and seed, generated after avocados were cold-pressed. A solid-liquid extraction was performed on freeze-dried AP, using ethanol:water (80:20 v/v) as solvent. AP was homogenized (1:20 w/v) in ethanol:water, and sonicated for 30 min (Bransonic Ultrasonic, Danbury, CT, USA) in cool water (<20 °C). Afterwards, the mixture was centrifuged (9400 × *g* for 15 min, 4 °C), and the PC-rich supernatant was recovered. The solid residue was extracted twice following the aforementioned procedure, but with a 1:10 w/v residue-to-solvent ratio. After liquid extraction, supernatants were filtered and dried with rotary evaporation and freeze-drying to remove the ethanol and water, respectively. This procedure yielded concentrated dry PCs, which were then stored at −20 °C until they were incorporated into the diets.

The PC profile of the AP sample used in the present study was analyzed previously, and contains concentrated ferulic acid, protocatechuic acid, *p*-coumaric acid, quercetin, kaempferol and gallic acid [12].

Animal Experimentation

Male Wistar rats ($n = 24$) were provided by the University of Sonora. They were individually housed in hanging metal cages, under standard conditions with ad libitum access to food and water. The experimental protocol was reviewed and approved by the Bioethics Committee of the Research Centre for Food and Development (CIAD) (CE/014_1/2019), and followed national and international guidelines applicable for animal experimentation. Due to a lack of previous experiments that demonstrate the effects of this byproduct on the lipid profile and lipoproteins, a homogenous sample of young, healthy male rats was considered to avoid possible confounding effects of sex; any effects on female animals cannot be confirmed or denied with the present data.

Four diets were designed and prepared from individual ingredients, which were then used to feed the animals. (1) A control standard diet (SD), (2) a high-fat diet (HFD), (3) the SD supplemented with AP phenolic extract (SD + PE, 1 g extract/kg diet), and (4) an HFD supplemented with AP phenolic extract (HFD + PE, 1 g extract/kg diet). The dose

of PE was chosen according to previous experiments [15, 16], where similar effects were documented in male Wistar rats. Detailed composition of the experimental diets is shown in Table 1. Diets were isocaloric (2.92 kcal/g), and provided 57% of kcal from carbohydrates, 28% protein and 15% from lipids for the SD, and 22% of kcal for carbohydrates, 28% from protein and 50% from lipids for the HFD.

Animals were weighed immediately upon arrival (150–200 g) and were acclimatized for 14 days, during which they were fed with the SD. They were then randomly assigned into 4 weight-matched groups ($n = 6$); each group was fed one of the previously mentioned diets for a total of 8 weeks. After the animals were fasted overnight, a blood sample was collected at the beginning of the experimental period, into EDTA-coated plasma-separating tubes. After the experiment, the animals were again fasted overnight and anesthetized with an intraperitoneal dose of sodium pentobarbital (120 mg/kg body weight). After checking for a total absence of somatosensory reflexes, a blood sample was collected (cardiac puncture), a small sample of the liver was excised (50–100 mg of tissue), and the animals were euthanized. The liver sample was added into a polypropylene tube with 1 ml of TRI reagent (Sigma-Aldrich, St. Louis, MO, USA), and was then stored at −80 °C until later use.

Plasma Lipids

Blood samples collected as described in the "Animal experimentation" section were centrifuged (650 × *g*, 20 °C, 10 min), the plasma was recovered into 1.5 ml polypropylene tubes, and was used to analyze the lipid profile. Total cholesterol, TAGs and HDL were quantified using commercially available kits (1010-430 for total cholesterol, 2100-430 for TAGs and 0590-040 for HDL; Stanbio, Boerne, TX, USA), according to manufacturer's instructions. Colorimetric reactions were read in a microplate reader (FLUOstar Omega, BMG Labtech, Cary, NC, USA). LDL concentration was calculated using Friedewald's equation:

$$\mathrm{LDL} = \text{total cholesterol} - \left(\frac{\text{triacylglycerols}}{5}\right) - \mathrm{HDL}$$

Relative mRNA Expression of Main Hepatic Lipoproteins and Their Receptors

Liver samples stored in TRI reagent were used to quantify the relative mRNA expression of APOA1, APOB, SCARB1 and LDLR, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference gene. Total RNA was extracted and reverse-transcribed according to manufacturer's instructions.

Individual reactions contained 50 ng of total cDNA (previously diluted in nuclease-free water), and were amplified using Taqman reagents for all genes of interest (GenBank; Taqman assay; fluorophore): APOA1 (NM_012738.1; Rn00562483_g1; FAM), APOB (NM_019287.2; Rn01499054_m1; FAM), SCARB1 (NM_031541.1; Rn00580588_m1; FAM), LDLR (NM_175762.2; Rn00598442_m1; FAM), and GAPDH (NM_017008.4, Rn99999916_s1, VIC), all from Applied Biosystems (Waltham, MA, USA).

Three technical replicate reactions were performed in duplex (genes of interest were FAM-labeled, reference gene was VIC-labeled) in a StepOne real-time PCR thermal cycler (Applied Biosystems), and used to quantify the relative mRNA expression, according to the $2^{-\Delta\Delta Ct}$ methodology.

Statistical Analyses

Three technical replicates of the experiments were performed to measure plasma lipids (total cholesterol, TAGs, HDL, and LDL) and gene expression (APOA1, APOB, SCARB1, and LDLR) on each of the six animals of all groups, for a total of 18 data points per group. Normality of the data was determined according to the Anderson-Darling test, and data was then analyzed using a one-way ANOVA with Fisher's test. Analyses were performed in the statistical software Minitab 19 (State College, PA, USA). Results are expressed as mean ± SEM.

Results

Lipid Profile

Figure 1 shows the concentration of plasma lipids at the beginning and end of the experimental period, where the animals had similar initial values for all variables measured. Most changes were significant when comparing the initial values to those recorded at the end of the experimental period. No significant differences in TAGs were found when comparing the untreated and treated groups (SD with SD + PE or HFD with HFD + PE). Regarding cholesterol and LDL, they increased significantly in groups fed the HFD, independently of the presence or absence of PE. HDL decreased in animals fed the HFD, however, it is noteworthy that PE was able to mitigate this change, suggesting a significant effect on this variable.

mRNA Expression

Figure 2 shows the relative mRNA expression of the main lipoproteins present in HDL (APOA1) and LDL (APOB),

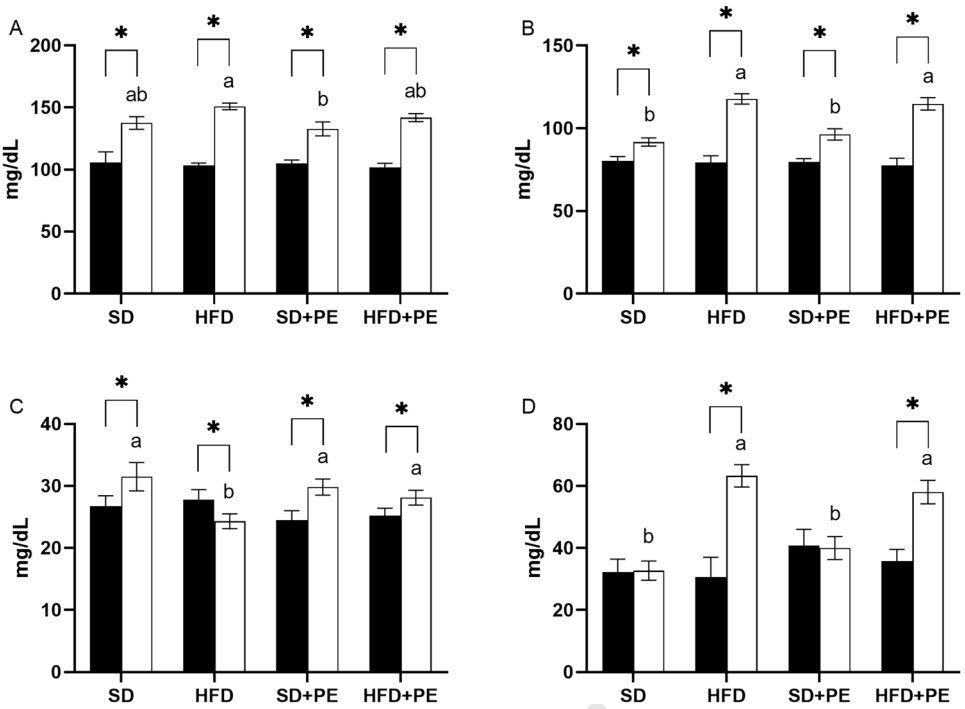

Fig. 1 Plasma concentration of triacylglycerols (TAGs) (**A**), total cholesterol (**B**), HDL (**C**) and LDL (**D**), of male Wistar rats that consumed a standard diet (SD), high-fat diet (HFD) or these supplemented with an avocado paste (AP) phenolic extract (PE). Mean ± SEM ($n = 3$). An asterisk indicates significant differences ($p < 0.05$) at the beginning (black bars) and end (white bars) of the experimental period for the same group. Lowercase letters indicate significant differences ($p < 0.05$) between groups at the end of the experimental period

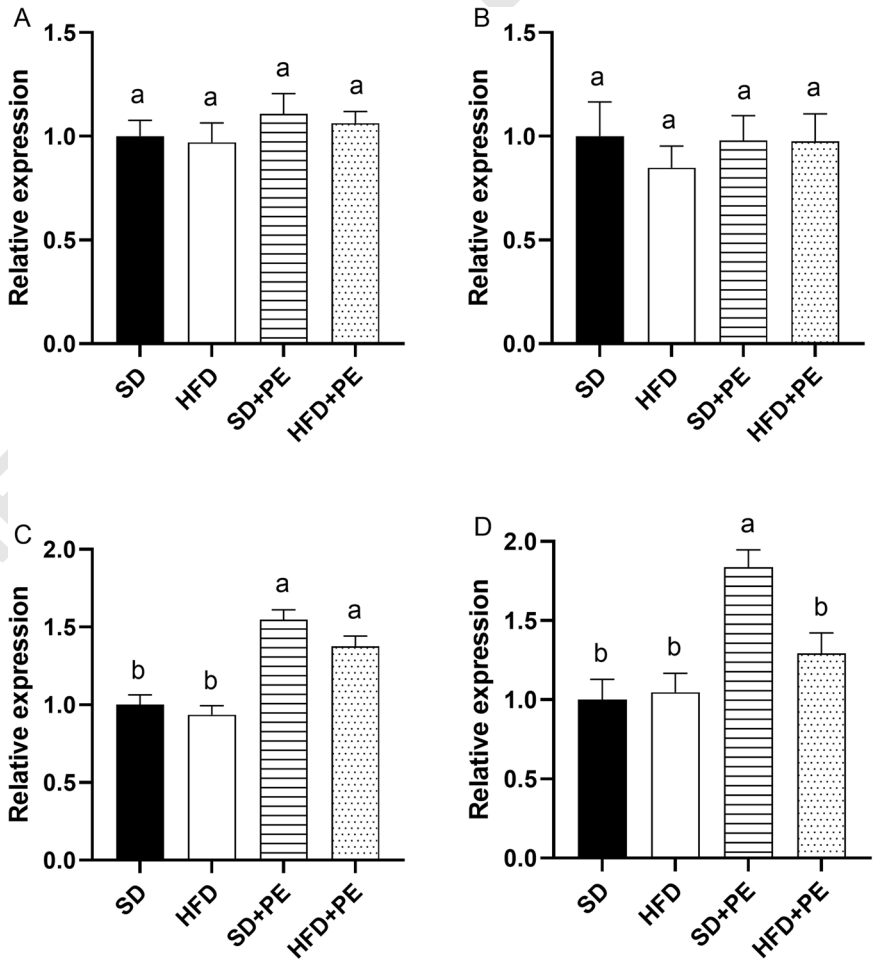

Fig. 2 Relative mRNA expression (using GAPDH as reference gene) of APOA1 (**A**), APOB (**B**), SCARB1 (**C**) and LDLR (**D**), of male Wistar rats that consumed a standard diet (SD), high-fat diet (HFD) or these supplemented with an avocado paste phenolic extract (PE). Mean ± SEM ($n = 3$). Lowercase letters indicate significant differences ($p < 0.05$)

and their main hepatic receptors of (SCARB1 and LDLR). No statistically significant ($p > 0.05$) changes were found on the relative expression of APOA1 and APOB. Relative expression of SCARB1 was similar in the untreated groups (SD and HFD), while a significant increase was found in treated groups (SD + PE and HFD + PE). Relative expression of the LDLR was increased in the SD + PE group only, with all others having statistically similar values.

Discussion

Lipid Profile

Regarding the effects of the HFD, similar findings were reported in a murine model, where HFDs were able to negatively alter the lipid profile; these changes are indicative of the negative effects of consuming such a diet, which acts by promoting dyslipidemia due to enhanced lipid accumulation and hindered reverse cholesterol transport [16–18]. Administering the PE treatment partially mitigated the increase exerted by the HFD, although this effect was not enough to reach statistical significance when comparing the HFD and HFD + PE groups. It also appears that the effect of the PE was more effective when administered as part of the SD, instead of the HFD. As with TAGs, all groups had increased total cholesterol, as compared to initial values. Increased total cholesterol is a common finding in various organisms that consume comparable diets with unbalanced macronutrient profiles (HFD, Western diets, high fructose diets, among others), since these promote exogenous lipid accumulation and/or endogenous lipid synthesis, although such alterations may be countered by plant-based diets in order to promote overall health [19]. The SD and SD + PE groups had a 14% and 21% increase, respectively, while the HFD and HFD + PE both had a significantly higher increase of 48%. It is apparent that the PE treatment had no effect on total cholesterol concentration, independent of the diet in which it was administered. The lipid-normalizing effects of phenolics from different vegetable sources have been reported across multiple studies [20]. For example, in rodents fed HFDs, cinnamon [21] and strawberries [22] have shown such effects, with mechanisms of action involving regulating the gene and protein expression of various key mediators of lipid metabolism.

HDL increased in most groups, as compared to initial values. Increases of 17% and 21% were found in the SD and SD + PE groups, respectively, while the HFD + PE group had only an 11% increase, indicating that the bioactivity of the PE is not hindered when the animal's diet has an unbalanced macronutrient profile and may counter some of its negative effects. In contrast, the HFD group had a 13% decrease. It is therefore apparent that the HFD exerted a markedly negative effect on this lipoprotein's concentration, according to decreased values, which the PE treatment was able to counter by maintaining statistically similar values to those of animals fed the SD or SD + PE, thereby impeding this decrease. Thus, the negative effect of consuming the HFD on HDL was apparently mitigated by the PE treatment. LDL remained unchanged in the animals fed the SD and SD + PE, as compared to that of initial values. Its concentration significantly increased in animals fed the HFD (+106%), and although animals fed the HFD + PE also had a statistically significant increase, it was less than that of untreated animals (+62%), suggesting a modest, but non-significant effect. Changes to the concentrations of HDL and LDL have been reported in response to consuming HFDs in animal models [23] while, in humans, this has been observed after only 3 weeks of consuming a low-carbohydrate and HFD [24]. A similar pattern was also observed in a review of various randomized controlled clinical trials [25], suggesting that an HFD can induce deleterious changes to the consumer after only a few weeks of consumption.

According to these data, it is apparent that the negative effects of the HFD were exerted on all variables analyzed, and a non-significant ($p > 0.05$) tendency of the PE treatment to mitigate them was observed. However, the most relevant and statistically significant change was documented on HDL, which prevented the decrease exerted by the HFD, while also normalizing its value to a statistically similar one to that of the SD and SD + PE groups.

mRNA Expression

Previous studies have reported that various PCs can significantly modulate the mRNA expression of LDLR, for example, Choi et al. [26] administered a Welsh onion extract to HepG2 cells, with a similar composition of the PE extract administered herein (kaempferol, quercetin, ferulic acid, and *p*-coumaric acid). They determined that their extract contributed to the maintenance of LDLR levels, with kaempferol and *p*-coumaric acid being associated with PCSK9 inhibition (which promotes recycling of the LDLR and decreases its concentration), thereby preventing its depletion.

According to these findings, it appears that the PE treatment exerted no effect on the relevant lipoproteins. However, it induced an increase on the relative expression of SCARB1, regardless of the composition of the diet (SD or HFD), but was only able to increase it for the LDLR when administered as part of the SD + PE diet. In other words, the HFD appears to have countered the possible effects of the PE treatment. SCARB1 regulates reverse cholesterol transport by catalyzing its transfer from mature

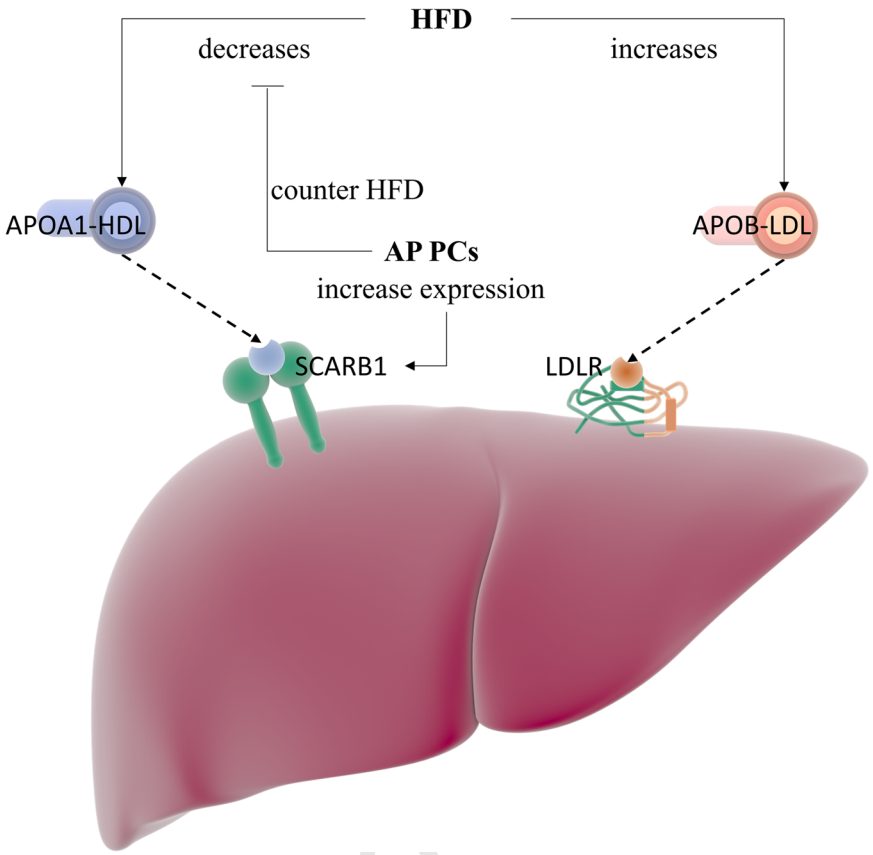

Fig. 3 Simplified mechanism of action by which avocado paste (AP) phenolics (PCs) counter some effects on the plasma lipid profile exerted by a high-fat diet (HFD). A HFD decreases HDL and increases LDL; AP PCs increase mRNA expression of hepatic HDL receptor (SCARB1, scavenger receptor class B type 1), thereby indirectly countering these effects, without directly acting on the main apolipoproteins themselves (APOA1 on HDL and APOB on LDL)

HDL particles to the liver, for subsequent processing into bile acids/salts and their biliary excretion [27]. An increased mRNA expression of SCARB1 may be indicative of improvements in cholesterol levels [28], however, this was not seen under the experimental conditions of the present work. Previous studies have mentioned the ability of PCs (ferulic acid and caffeic acid in particular) to reverse or improve dyslipidemia by increasing the expression of SCARB1, suggesting that reverse cholesterol transport may be among their anti-dyslipidemia mechanisms [17]. Specifically, ferulic acid, which is present in the PE extract administered herein, has been reported as potentially anti-atherogenic due to its ability to dose-dependently improve reverse cholesterol transport via HDL and an increased expression of SCARB1 [29].

Data for plasma lipoprotein concentration and relative gene expression are congruent with each other. Regarding LDL, the treatment was unable to mitigate the deleterious effects of the HFD, but was able to do so for HDL. This suggests that the mechanism of action of the PE treatment administered for modulating the lipid profile, is based on targeting the hepatic receptors, and can do so for SCARB1 independent of dietary composition. This suggests high preference and strong modulatory action on the regulatory expression elements that promote or inhibit its expression. In contrast, the treatment is only effective for LDLR when administered as part of a regular diet (non-HFD), suggesting that the effects of this diet outweigh those exerted by the treatment at the mRNA expression level. The association of SCARB1 with anti-atherosclerotic and anti-inflammatory effects has also been reported, thus, the AP-derived PE treatment administered could further contribute to mitigating comorbidities found in diseases related to lipid metabolism and plasma lipid profile [30].

The mechanism of action of AP PCs is graphically summarized in Fig. 3. Others have also shown that avocado products (such as its oil) and byproducts (such as its peel) contain various compounds with significant bioactive potential, in fact, some molecular species are only found in the byproducts, which supports the premise of using them as sources of health-promoting molecules [31–33]. The data reported herein provides further evidence regarding their potential on in vivo models, thus, integral use of avocado should be considered.

Conclusions

Industrially-generated avocado paste (AP) was used as a source of phenolic compounds (PCs). The present study showed their bioactivity, regarding their lipid-modulating effects in male Wistar rats that consumed them as part of

their diet (standard or high-fat). Results showed that the phenolic extract (PE) treatment was able to mitigate a high-fat diet (HFD)-induced decrease in HDL. This effect was apparently related to increased mRNA expression of the hepatic HDL receptor (SCARB1), although other mechanisms of action could also be simultaneously taking place. These results argue in favor of the use of vegetable byproducts as sources of bioactive PCs, that would otherwise be underutilized or entirely discarded. Additional studies are required to validate their effects in human consumers.

Data Availability

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

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12013-023-01190-9.

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Author Contributions G.A.G.-A.: conceptualization, funding acquisition, investigation, project administration, resources, supervision, conceptualization, methodology, writing—original draft; J.A.D.-A.: conceptualization, data curation, formal analysis, funding acquisition, methodology, project administration, writing—original draft, writing—review and editing; N.J.S.-L. and M.M.-H.: formal analysis, investigation, methodology, writing—review and editing; D.A.C.-S. and H.A.-G.: formal analysis, investigation, writing—original draft, writing—review and editing; J.R.-G. and M.A.V.-O.: investigation, methodology, project administration, supervision.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

Ethical Approval The experimental protocol was reviewed and approved by the Bioethics Committee of the Research Centre for Food and Development (CIAD) (CE/014_1/2019), and followed national and international guidelines applicable for animal experimentation.

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