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Expression analysis of genes related to lipid metabolism in peripheral blood lymphocytes of volunteers under diet intervention including beef with different fatty acid composition

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ABSTRACT. Beef fatty acid content and composition have an important effect on lipid metabolism; however, gene expression changes in response to their consumption have not been entirely explored. Thirteen candidate genes were selected according to its role in lipid metabolism and confirmed expression in PBMCs. They were studied to determine their gene expression effect in response to two types of beef: Wagyu-Cross beef characterized by a high concentration of total fat with higher concentration of monounsaturated fatty acids (MUFAs) especially oleic acid than Commercial beef. Both types of beef were consumed by two groups of volunteers (120 g commercial or Wagyu-Cross beef, three times per week) during a two-week clinical trial. The hypothesis of this work was: a dietary intervention involving the consumption of beef with different FA concentrations and compositions (Commercial and Wagyu-Cross beef) will lead to changes in the expression patterns of genes associated with total cholesterol, low-density lipoprotein (LDL), high-

density lipoprotein, and triglyceride concentrations in blood mononuclear cells obtained from both groups of consumers. Volunteers who consumed commercial beef showed a significant decrease in scavenger receptor class B member 1 (*SCARB1*), lactamase beta (*LACTB*), and sorting nexin 13 (*SNX13*). The expression of *SNX13* was inversely correlated with the change that occurred in the volunteers' LDL level (r= -0.398, P= 0.044) and had a significant predictive value for a decrease in LDL levels, with an area under the curve of 0.750 (P=0.009); thus, *SNX13* is proposed as a candidate gene for evaluating changes in LDL concentrations. It is inferred that Wagyu-Cross beef produced a lower expression effect and favorable lipid profile on this consumer- group, due to the PUFAs-mediated *SREBP* transcription factor-nuclear abundance suppression, resulting in a reduction in cholesterol and FAs synthesis.

Key words: Gene expression; Lipid metabolism; Food intervention; Beef; MUFAs

INTRODUCTION

Dietary fats are essential components of a healthy diet, and an imbalance in their consumption has been associated with an increased risk of a wide range of chronic diseases (Ruiz-Núñez et al., 2013).

Monounsaturated fatty acid (MUFA) consumption from meat sources such as beef has gained attention since the discovery of the lipid metabolism-regulating effect by significantly decreasing the concentrations of high-density lipoprotein (HDL), total cholesterol (TC), low-density lipoprotein (LDL), and different atherogenic indexes (Joo et al., 2017). Gilmore et al., (2011) reported a significant increase in the HDL concentration and a decrease in the LDL/HDL ratio in volunteers who consumed high-MUFA ground beef for five weeks. Recently, Vela-Vásquez et al., (2021) demonstrated that beef from Wagyu-Cross cattle is characterized by higher concentrations of MUFAs, (mainly oleic acid), polyunsaturated fatty acids (PUFAs) and lower saturated fatty acids (SFAs) than commercial beef, and in a two-week diet intervention, the authors found that Wagyu-Cross beef consumption generated a significant beneficial effect on the baseline values of lipid parameters (a reduction of LDL, TC, Non-HDL) and different atherogenic indexes (CT:HDL, LDL:HDL, Non-HDL:HDL) compared with increases in the observed baseline values of volunteers after commercial beef consumption. Their findings support the idea that the quality of fat is more important than the portion size consumed (Brown et al., 2007).

Fatty acids (FA) can regulate gene expression by controlling the activity of transcription factors or regulating their abundance (Jump, 2004) due to their importance, different studies have focused on determining the effect on gene expression of these bioactive compounds, mainly in animal products such as meat. Liver, adipose, and muscle tissues are the main targets to study the role of food and nutrients; however, Larsen et al., (2018) found that peripheral blood mononuclear cells (PBMCs) express genes involved in liver lipid metabolism and that gene expression is influenced by plasma FA levels, validating their use as a more affordable model to determine expression patterns in response

to FA consumption. Gene expression in PBMCs can be modified by the consumption of different meats; for example, fish meat FA composition can regulate the activity of transcription factors such as peroxisome proliferator activated receptors (*PPARs*), important regulators of genes involved in lipid metabolism (Larsen et al., 2018; Rundblad et al., 2018). Choi et al., (2018) found that the genes ATP binding cassette subfamily A member 1 (*ABCA1*) and ATP binding cassette subfamily G member 1 (*ABCG1*) had reduced expression in response to higher beef fat content consumption. Thus, changes in gene expression in response to beef consumption have been reported when comparing the fat content but not the FA composition. Therefore, the hypothesis of this work was: beef consumption will lead to changes in the expression patterns of genes associated with total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride concentrations in blood mononuclear cells obtained from two groups of volunteers under a dietary intervention involving the consumption of beef characterized by differences in their FA concentrations and compositions (commercial and Wagyu-Cross beef).

MATERIAL AND METHODS

Biological samples

This study was part of a randomized controlled double-blinded two-arm parallel-group study designed to investigate the health effects on lipid metabolism of beef intake with different FA compositions (Vela-Vásquez et al., 2021). Differential expression analysis was considered an endpoint. Briefly, the intervention study took place at Centro de Biotecnología Genómica–Instituto Politécnico Nacional, located in Reynosa, México, in March 2019. Thirty-four healthy subjects (18 men and 16 women) were included in a diet intervention and were randomized into two different groups (commercial and Wagyu-Cross beef), of which 26 volunteers (13 volunteers per group) were included and analyzed for differential gene expression, and these are the subjects we refer to throughout this study. The Bioethics Committee of the Escuela Nacional de Medicina y Homeopatía approved the diet intervention study (CBE/013/2019). The clinical trial was registered as NUTRIRES: RPCEC00000302 in the Cuban Public Registry of Clinical Trials (RPCEC) Database. This work was conducted in accordance with the Declaration of Helsinki.

Database and written informed consent were obtained from all volunteers. A detailed description of the protocol, participant recruitment and enrollment, inclusion and exclusion criteria, and compliance are published in detail elsewhere (Vela-Vásquez et al., 2021).

At the beginning of the intervention, volunteers received a list of foods with the amount equivalent to a portion described by the equivalent system (Pérez-Lizaur et al., 2008) and a diet to follow with the number of portions per food group (fruits, vegetables, dairy, cereals, etc.) to consume according to their energy requirement (Vela-Vásquez et al., 2021). To standardize the diets, the energy requirement of each volunteer was averaged, resulting in the provision of diets containing 1400, 1600, 1800, 2000, and 2500 kilocalories, consisting of 55% carbohydrates, 15% proteins, and 30% fat.

Volunteers consumed 120 g of ground beef three days per week for two weeks. The clinical trial provided six meals served with 120 g of beef (from its respective group) along

with one serving of cereals and two servings of vegetables. The meal was cooked by a nutritionist following the standard sanitary care measurements and using different pans for cooking the two types of beef without any additional animal or vegetable fats. Volunteers were instructed to avoid ingestion of pork, fish, shellfish, other red meat, and consumed only what was recommended in the diet, such as panela or cottage cheese, 2% milk (reduced fat), plain yogurt, legumes, nuts, oats, bread, chicken, eggs, chicken, or turkey ham or sausage. Volunteers were asked to fill out a diet record four times a week during the intervention (including 1 day during the weekend) and were questioned regularly by phone call or in person to verify their adherence to the trial indications, description of dietary assessment is described in detail elsewhere (Vela-Vásquez et al., 2021).

Volunteers were randomized into two groups based on the FA composition profiles reported for the two types of consumed beef during the clinical trial, where the saturated fatty acid (SFA) content was significantly lower and the MUFA oleic acid (OA, 18:1 n-9) concentration was higher in Wagyu-Cross beef than in commercial beef (Vela-Vásquez et al., 2021).

During the intervention, fasting venous blood samples were collected after an overnight fast (≥ 12 h) at the beginning and end of the diet intervention (Vela-Vásquez et al., 2021). This blood sample bank was used in the current study to obtain RNA samples and to evaluate differential gene expression after the consumption of commercial and Wagyu-Cross beef.

PBMC and RNA isolation

PBMCs were isolated from the blood samples for RNA isolation. Pellets were stored in RNAlater™ Stabilization Solution (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA, AM7021) at -80 °C. RNA was extracted from PBMCs using a RiboPure™ RNA Purification Kit, Blood (Thermo Fisher Scientific, Waltham, MA, AM1928) according to the manufacturer's instructions. To optimize RNA extractions, an RNAse-free environment was created in the laboratory using laboratory material previously washed with 1% diethyl pyrocarbonate (DEPC) and baked at 100 °C for 12 h. To eliminate RNAse contamination, the table and work equipment were cleaned with RNAseZap™ RNAse Decontamination Wipes (Thermo Fisher Scientific, Waltham, MA, USA, AM9786).

The quality of the RNA was determined through capillary electrophoresis using an agarose gel (1.2%) and guanidinium thiocyanate (0.1 M). The concentration of total RNA was determined using quantification according to the fluorometric method using a Qubit® RNA HS Assay Kit (Thermo Fisher Scientific, MA, USA, Q32852) for a Qubit 2.0 (Thermo Fisher Scientific, MA, USA, Q32867). Some samples were concentrated and cleaned using a GeneJET RNA Cleanup and Concentration Micro Kit from Thermo Scientific (Thermo Fisher Scientific, MA, USA, K0842).

Differential gene expression

Based on their role in lipid metabolism and confirmed expression in PBMCs, a group of 13 genes (Table 1) was selected to design a TruSeq Targeted RNA Custom Panel Kit to determine their differential expression using the Illumina MiniSeq® system.

Reverse transcription was performed using RNA extracted from PBMCs from the 34 volunteers using ProtoScript® reverse transcriptase (New England Biolabs, MA, USA, M0368S).

Table 1. Genes included in the TruSeq Targeted RNA Custom Panel Kit and the functions and associations involved in lipid metabolism.

Gene	Gene ID	Location	Function	Association	References
Lysine acetyltransferase 5 (*KAT5*)	10524	11q13	Positive regulator of the transcription of the *PPARG* gene involved in adipogenesis	HDL, TG and TC, differential expression in individuals with high and low FA levels	(Willer et al., 2013; Larsen et al., 2018)
Kelch like family member 8 (*KLHL8*)	57563	4q22.1	The substrate-specific adapter of a *BCR* E3 ubiquitin ligase complex. Mediates the ubiquitination and degradation of *RAPSN*	TG, BMI, differential expression between individuals with high and low percentage levels of SFAs to PUFAs and FA levels	(Willer et al., 2013; Larsen et al., 2018)
Selenoprotein S (*SELENOS*)	55829	15q26.3	Putative receptor for serum amyloid A. Involved in the process of misfolded protein degradation in the endoplasmic reticulum and may also have a role in inflammation control	Type 2 diabetes mellitus, inflammation. LDL, HDL, BMI, TG, insulin, differential expression in individuals with high and low FA levels	(Larsen et al., 2018)
Insulin induced gene 2 (*INSIG2*)	51141	2q14.1-q14.2	A strong candidate gene for cholesterol regulation, given the key roles of *INSIG* proteins in lipid metabolism	TC, LDL, differential expression between individuals with high and low percentage levels of SFA to PUFA	(Willer et al., 2013; Larsen et al., 2018)
SPT2 chromatin protein domain containing 1 (*SPTY2D1*)	144108	11p15.1	Binds DNA and histones and promotes nucleosome assembly. A candidate gene for dyslipidemias	TC, LDL, TG	(Willer et al., 2013)
ER lipid raft associated 2 (*ERLIN2*)	11160	8p11.23	Participates in the regulation of cellular cholesterol homeostasis by regulating the *SREBP* signaling pathway	BMI, differential expression between individuals with high and low percentage levels of SFA to PUFA	(Larsen et al., 2018)
Diacylglycerol lipase beta (*DAGLB*)	221955	7p22.1	Catalyzes the hydrolysis of arachidonic acid (AA)-esterified diacylglycerols (DAGs) to 2-arachidonoylglycerol (2-AG)	HDL, differential expression in individuals with high and low FA levels, TG	(Willer et al., 2013; Larsen et al., 2018)
Family with sequence similarity 117 member B (*FAM117B*)	150864	2q33.2	An uncharacterized protein. Nearby *BMPR2* encodes a bone morphogenetic protein receptor. Defects in *BMPR2* cause primary pulmonary hypertension	TC, differential expression between individuals with high and low levels of the percentage of SFA to PUFA	(Willer et al., 2013; Larsen et al., 2018)
Lactamase beta (*LACTB*)	114294	15q22.2	Mitochondrial serine protease that acts as a regulator of mitochondrial lipid metabolism	Obesity, diabetes, and atherosclerosis in mice. BMI, differential expression of FA, HDL	(Chen et al., 2008; Willer et al., 2013; Larsen et al., 2018)
Sorting nexin 13 (*SNX13*)	23161	7p21.1	Encodes a protein containing the *PHOX* domain and the *RGS* domain that belongs to the nexin sorting family and the regulator of G protein signaling (*RGS*)	BMI, differential expression between individuals with high and low levels of the percentage of SFA to PUFA, HDL	(Willer et al., 2013; Larsen et al., 2018)
Glycogen synthase kinase 3 beta (*GSK3B*)	2932	3q13.33	Role in energy metabolism and in the regulation of lipid levels in the blood	HDL, BMI, TG, differential expression between individuals with high and low levels of the percentage of SFA to PUFA	(Willer et al., 2013; Larsen et al., 2018)
LDL receptor related protein 5 (*LRP5*)	4041	11q13.2	Important in the metabolism of glucose and cholesterol	TC, LDL, differential expression between individuals with high and low levels of the percentage of FA	(Larsen et al., 2018)
Scavenger receptor class B member 1 (*SCARB1*)	949	12q24.31	Works as a receptor for different ligands such as phospholipids, cholesterol esters, lipoproteins, phosphatidylserine, and apoptotic cells. It is a key component in the reverse cholesterol transport pathway	HDL, TG, LDL, VLDL, BMI	(Rundblad et al., 2018)

HDL: high-density lipoprotein; TG: triglyceride; TC: total cholesterol; FA: fatty acids; RAPSN: receptor-associated protein of the synapse; BMI: body mass index; SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; LDL: low-density lipoprotein; *INSIG*: insulin induced gene; *SREBP*: sterol regulatory element binding protein; *BMPR2*: bone morphogenetic protein receptor type 2; *RGS*: regulation of G-protein signaling; VLDL: very-low-density lipoprotein.

Library preparation for sequencing was achieved using the TruSeq Targeted RNA Expression protocol from Illumina (RT-101-1001). After this step, 26 out of 34 libraries

reached the required quality for sequencing and were used to complete the differential expression analysis (Figure 1).

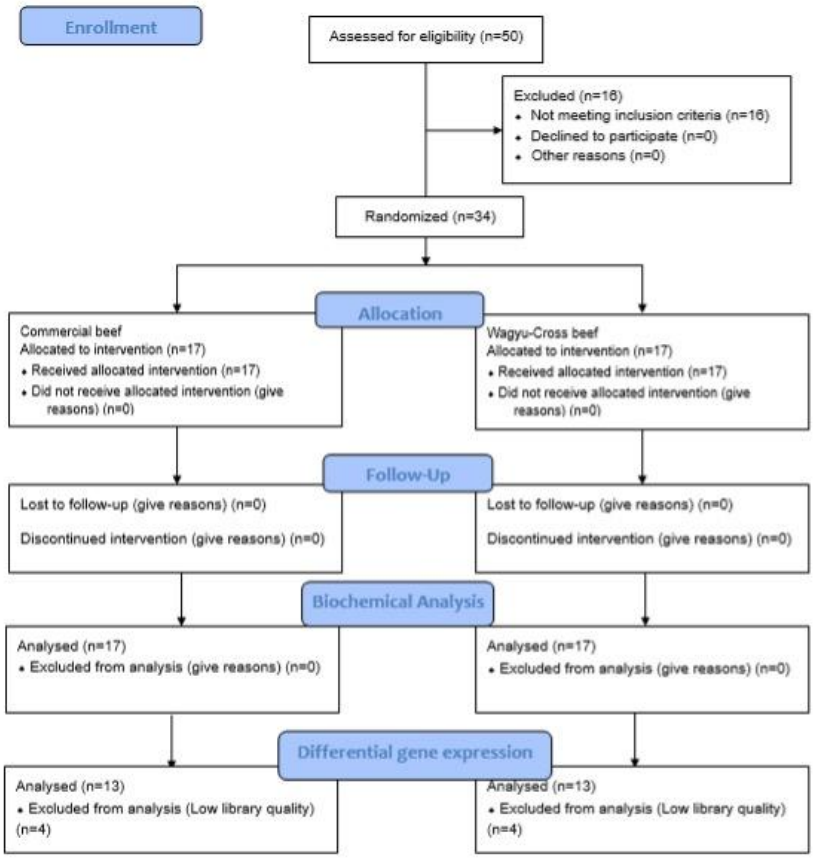

Figure 1. Consort 2010 flow diagram with the number of volunteers screened, included, eliminated, and analyzed in NUTRIRES: RPCEC00000302 study.

Data obtained from targeted RNA sequencing were uploaded and analyzed using the Galaxy web platform. We used the public server at usegalaxy.org to analyze the data (Afgan et al., 2018) using the following tools: FastQC for quality revision, Trimmomatic for removing adaptors and poor-quality nucleotides, STAR for read mapping, and featureCounts to estimate expression levels.

The collected data were analyzed using Degust: interactive RNA-seq analysis (Powell, 2015). According to the type of beef consumed (commercial or Wagyu-Cross beef), the groups were analyzed based on the total counts before and after the intervention using the voom/limma method. The groups consisted of 13 volunteers in the commercial beef group (8 men and 5 women) and 13 volunteers in the Wagyu-Cross beef group (7 men and 6 women). A multiple-testing false discovery rate (FDR)-corrected p value ≤ 0.05 was considered significant. Additionally, the Mann–Whitney U test was applied to compare the gene expression between the groups after diet intervention.

Statistical analysis

The statistical analysis included the data following the methodology previously described (Vela-Vásquez et al., 2021). Because most of the variables of the study groups had a nonnormal distribution (determined using the Kolmogorov–Smirnov test) and had a small sample size (less than 30 per group), the statistical analysis was performed with nonparametric tests. A detailed description of the statistical analysis is published in detail elsewhere (Vela-Vásquez et al., 2021).

The change from the baseline was used to examine the absolute differences between the evaluation periods, calculated with the score after intervention minus the score at baseline for each variable in each volunteer variable, including gene expression, as recommended by Vickers (2001) when using baseline and posttreatment measurements (Vickers et al., 2001). Pearson's correlation coefficients (r) were calculated for bivariate correlations between the absolute values of gene expression changes and serum lipid parameters. To evaluate the predictive value of gene expression changes, receiver operating characteristic (ROC) curve and area under the curve (AUC) analyses were performed.

RESULTS

Volunteer lipid profile before and after diet intervention.

A total of 26 volunteers (13 from the commercial beef group and 13 from the Wagyu-Cross beef group) were included in the expression analysis (Figure 1), and the subjects were young and middle-aged adults (32.5 ± 8.1 years). No significant differences were found in the age or baseline biochemical serum parameters between the groups, with no significant difference in the sex ratios (compared using Fisher's exact test). As shown in Table 2, a greater decrease in the baseline change values for most of the parameters analyzed was observed at the end of the diet intervention in volunteers from the Wagyu-Cross beef group than those observed in the volunteers from the commercial beef group. These reductions in the lipidic values did not show statistical significance, as previously reported (Vela-Vásquez et al., 2021), probably due to the small sample analyzed.

Table 2. Comparison of the changes in the lipid profiles after diet intervention[1].

Clinical parameter	Commercial beef	Wagyu-Cross
TC*	-2.1 (± 7.4)	-4.1 (± 6.9)
TG*	-16.2 (± 22.2)	-21.9 (± 33.4)
HDL*	-3.0 (± 11.2)	-3.7 (± 8.7)
LDL*	-0.6 (± 16.0)	-1.2 (± 8.2)
VLDL*	-15.9 (± 22.2)	-26.8 (± 37.1)
Non-HDL*	-2.7 (± 10.0)	-4.5 (± 8.6)
TC/HDL	0.0 (± 10.9)	-0.8 (± 8.2)
LDL/HDL	3.8 (± 11.6)	0.3 (± 4.0)
Non-HDL/HDL	-1.0 (± 16.1)	- 1.4(± 11.2)

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; VLDL, very low-density. [1]All values are means and the standard deviation. *mg/dL.

Intergroup comparison of the gene expression patterns before and after diet intervention.

Using the Degust web tool for RNA-seq, we compared the read counts for the expression of the analyzed genes in volunteers before and after the diet intervention on the basis of treatment group (commercial and Wagyu-Cross beef). The *KAT5* gene was not included in the analysis because no counts were obtained for this gene in either group. The average gene expression in both groups was highly similar and correlated, suggesting that the measurements were consistent among the groups. Significant expression differences were observed in the commercial beef group after the diet intervention in 3 genes, namely, scavenger receptor class B member 1 (*SCARB1*), lactamasa beta (*LACTB*), and *SNX13* (Table 3), which are related to lipoproteins, body mass index (BMI), and obesity (Table 1). No significant expression differences were observed in the Wagyu-Cross beef group after the diet intervention (Table 4).

Table 3. Differential expression in volunteers from the commercial beef group after the diet intervention.

Name	Average Expression	Average CPM	Commercial beef [1]	Raw p value	FDR[2]
LACTB	15.986	49596.22	-0.809	0.013	0.046
SCARB1	15.304	30537.14	-0.73	0.003	0.042
SNX13	15.616	35971.74	-0.65	0.01	0.046
FAM117B	18.351	304090.53	-0.353	0.138	0.241
GSK3B	14.122	17787.47	-0.127	0.704	0.896
KLHL8	15.556	42612.10	-0.004	0.985	0.985
INSIG2	16.427	111088.27	0.026	0.92	0.985
SPTY2D1	15.93	66311.02	0.047	0.894	0.985
SELS	15.832	82792.34	0.132	0.546	0.765
ERLIN2	16.429	125230.54	0.377	0.134	0.241
DAGLB	16.778	126997.79	0.398	0.075	0.174
LRP5	11.172	2853.23	0.563	0.385	0.599

[1] log2-fold change; [2] FDR: *false discovery rate* adjusted p value. CPM: Counts per million

Table 4. Differential expression in volunteers from the Wagyu-Cross beef group after the diet intervention.

Name	Average Expression	Average CPM	Wagyu-Cross beef[1]	Raw p value	FDR[2]
SPTY2D1	15.75	59840.09	-0.252	0.473	0.904
SNX13	15.71	55179.35	-0.119	0.695	0.904
FAM117B	18.414	347605.06	-0.066	0.439	0.904
LRP5	11.256	1571.92	-0.064	0.897	0.904
LACTB	15.573	48910.78	-0.041	0.868	0.904
INSIG2	16.329	84217.74	0.016	0.904	0.904
GSK3B	13.942	17889.66	0.061	0.861	0.904
SCARB1	15.164	38054.01	0.207	0.323	0.904
DAGLB	16.775	118020.90	0.323	0.009	0.132
KLHL8	15.571	49380.22	0.329	0.119	0.418
SELS	15.742	58007.88	0.416	0.026	0.184
ERLIN2	16.651	118698.81	0.541	0.066	0.308

[1] log2 fold change; [2] FDR: *false discovery rate.* is an adjusted P value. CPM: Counts per million.

Changes in gene expression due to different beef FA compositions.

Comparison of the average gene expression changes between groups during the diet intervention (commercial and Wagyu-Cross beef groups) showed a significant difference in the *SCARB1*, *LACTB* and *SNX13* genes (Table 5). The commercial beef group had a major decrease in the expression of these genes, while the Wagyu-Cross beef group had lesser changes.

Table 5. Comparison of the expression changes of the *SCARB1*, *LACTB*, and *SNX13* genes between the Commercial and Wagyu-Cross beef groups.

Name	Group	Average[2]	Standard deviation	P value*
SCARB1[1] change	Commercial beef	-15,225.33	17,438.05	0.007
	Wagyu-Cross beef	1,520.20	14,984.61	
LACTB [1] change	Commercial beef	-45,125. 46	53,907.15	0.018
	Wagyu-Cross beef	-6,426.34	33,042.70	
SNX13[1] change	Commercial beef	-25,993.12	17,809.91	0.034
	Wagyu-Cross beef	-6,845.39	31,612.40	

[1] Score expression after intervention minus the score expression at baseline; [2] Mean of the change expression. * Student's t test, one tail.

The change in *SNX13* expression was inversely correlated with the change that occurred in the volunteers' LDL level (r= -0.398, P= 0.044). No correlations between the expression changes of the *SCARB1* and *LACTB* genes (r= -0.002, P= 0.991; r= 0.020, P=0.922, respectively) and lipid biochemical parameters were observed.

A ROC curve analysis was performed to calculate whether *SCARB1*, *LACTB* and *SNX13* expression could be a predictor of changes in the volunteers' LDL concentrations. Only the expression of *SNX13* had a significant predictive value for a reduction in LDL levels, with an area under the curve (AUC) of 0.750 (95% CI 0.561-0.939, P= 0.009, cutoff -22663.85, specificity = 71%, sensitivity = 25%). *SCARB1* and *LACTB* were not statistically significant in this analysis (P= 0.801 and 0.496, respectively).

DISCUSSION

The consumption of red meat has been associated with different diseases, including atherosclerosis, colon cancer, and cardiovascular disease. However, these associations are still controversial (Smith et al., 2020), and more studies are necessary to demonstrate the impact of this important source of protein on human health. Fatty acids are important bioactive compounds that have been a focus of interest in beef improvement strategies due to their importance in health and the organoleptic properties of beef (Ladeira et al., 2016).

Gene expression in response to meat FA consumption has been studied mainly with fish or fish oil (Rundblad et al., 2018). However, less information is known about the effect of these bioactive compounds from beef, where only a single study has been reported (Choi et al., 2018) analyzing the expression of five genes (liver X receptor (*LXR*), sterol regulatory element binding transcription factor 1 (*SREBF1*), ATP binding cassette subfamily A member 1 (*ABCA1*), ATP binding cassette subfamily G member 1 (*ABCG1*), and stearoyl-CoA desaturase (*SCD*) in PBMCs in response to consumption of SFAs in the form of high-fat ground beef (Choi et al., 2018). Recently, we found that consumption of

Wagyu-Cross beef generated a beneficial effect significantly lowering LDL-C and the LDL-C:HDL-C ratio whereas commercial ground beef increased the LDL-C:HDL-C ratio. Both ground beef types, depressed TG and VLDL-C concentrations after a diet intervention (Vela-Vásquez et al., 2021).

In this research, the differential expression of thirteen genes related to lipid metabolism was studied. The expression of three genes (*SCARB1*, *LACTB*, and *SNX13*) involved in the metabolism of lipoproteins, BMI, and obesity was found to decrease in the group of volunteers after the consumption of commercial beef, and a significant difference between the change in gene expression of the *SCARB1*, *LACTB*, and *SNX13* genes between the groups after the consumption of commercial and Wagyu-Cross beef was observed. The relationship between *SCARB1*, *LACTB*, and *SNX13* and the differential expression associated with plasma FA levels and the SFA-to-polyunsaturated fatty acid (PUFA) ratio has been reported (Larsen et al., 2018).

Comparison of the commercial and Wagyu-Cross voluntary groups before and after the intervention and between the groups using the change in gene expression led to an expression reduction in the *LACTB*, *SCARB1*, and *SNX13* genes after the consumption of the commercial beef group and between the commercial group and the Wagyu-Cross group. *LACTB* is a mitochondrial protein that is ubiquitously expressed in mammals and acts as a regulator of mitochondrial lipid metabolism, and it has been suggested that it acts by regulating complex I of the mitochondrial electron transport chain by participating in the organization of the intramitochondrial membrane (Polianskyte et al., 2009). Mitochondria play an important role in lipid metabolism, including β oxidation of FAs; the biosynthesis of phospholipids, FAs, and steroid hormones; and the initial steps for cholesterol degradation (Mayr, 2015). Xue et al., (2018) made predictions in cell lines and found that *LACTB* had an association with key enzymes and genes of lipid metabolism, and it is thus believed that this gene could play an important role in this metabolic pathway (Xue et al., 2018). In addition, studies in transgenic mice (Yang et al., 2009) have associated *LACTB* with obesity. Chen et al., (2008) reported that transgenic mice displayed an increased fat mass/lean mass ratio by 20% compared to that of control mice when studying the relationship of *LACTB* with obesity and the changes in weight, fat and lean mass in *LACTB* transgenic and control mice for 7 weeks, thereby validating in vivo that *LACTB* acts as an obesity gene (Chen et al., 2008). Likewise, Yang et al., (2009) confirmed the increase in adiposity in mice of the *LACTB*tg line; however, this effect was observed only in female mice, and none of the mice in the study presented an increase in lipid concentrations (Yang et al., 2009).

Given the demonstrated role in the activity of this gene, it is interesting that after the diet intervention, the volunteers in the commercial beef group had a significant reduction in the expression of the *LACTB* gene (FDR = 0.0464). Chatterjee et al., (2009) found that *LACTB* acts as a suppressor gene in the sterol regulatory element binding protein (SREBP) pathway (Chatterjee et al., 2009). This family of transcription factors acts as regulators of the expression of genes involved in the synthesis of cholesterol, triglycerides, and FAs (Polianskyte et al., 2009). Specifically, when the cell is deprived of sterols, *SREBF* chaperone (*SCAP*) escorts SREBPs from the endoplasmic reticulum of the Golgi apparatus to the nucleus, where they exert their activity by activating genes involved in the synthesis and absorption of cholesterol (Larsen et al., 2018). On the other hand, when cells are enriched with cholesterol, *SCAP*-SREBP migration fails, and *SREBP* remains intact in the endoplasmic reticulum, resulting in reduced transcription of *SREBP* target genes (Larsen et

al., 2018). Further studies increasing the sample number could confirm that decreasing the expression of the *LACTB* gene is mediated by commercial beef consumption.

A significant decrease in the expression of *SCARB1* (FDR = 0.04) after the diet intervention was also observed in the commercial group and between the commercial group and the Wagyu-Cross group. *SCARB1* has been described as an important component in the reverse cholesterol transport pathway, thus playing an important role in lipid metabolism (Liu et al., 2008); likewise, *SCARB1* is an HDL receptor and has also been shown to regulate LDL and VLDL concentrations (Chiba-Falek et al., 2010). *SCARB1* encodes human scavenger receptor class B type I (*SR-BI*), one of the lipid receptors involved in lipid metabolism, which is a key regulator of systemic cholesterol levels. *SCARB1* deficiency in humans and mice (West et al., 2009) has been reported to result in hypercholesterolemia.

Cerda et al., (2011) reported a lesser expression of the *SCARB1* gene in volunteers with a higher concentration of LDL (>160 mg/dL) compared to that in volunteers with a lower concentration of LDL (<100 mg/dL) (P = 0.031) (Cerda et al., 2011). In our previous study, the volunteers in the commercial beef group showed an increase in LDL concentrations. In contrast, the volunteers in the Wagyu-Cross beef group showed decreased concentrations after the diet intervention with significant differences between groups (Vela-Vásquez et al., 2021). Comparing only the 23 volunteers included in the expression analysis, a nonsignificant reduction was observed in both groups. However, the Wagyu-Cross beef group had a higher decrease than that of the commercial beef group. This is an important result because when comparing gene expression between the groups, *SCARB1* had a significant difference, meaning that this LDL-lowering effect could be due to the reduction in *SCARB1* expression, as Cerda et al., (2011) reported. In addition, Retterstøl et al., (2018) reported a decrease in the expression of *SCARB1* (almost significant, 0.05) and a significant increase in the concentration of LDL after a 3-week low-carbohydrate/high-fat diet, which was 29% SFAs. In our study, we observed the effect of the consumption of commercial beef at the molecular level and its effect on the lipid profile.

Finally, the *SNX13* gene showed significant differences between the commercial beef group volunteers after the diet intervention (FDR= 0.046) and between groups after diet intervention. The *SNX13* gene could be involved in intracellular trafficking (Willer et al., 2013). *SNX13* has been reported to participate in the preservation of cardiomyocyte survival and participates in the degradative sorting of apoptosis repressors (Li et al., 2014). An association between coronary artery disease and *SNX*s has been reported, although the mechanisms are still unclear (Li et al., 2014). To our knowledge, there are no reports of changes in gene expression of this gene in response to the consumption of FAs; however, as shown in the correlation and prediction analyses, it is a valued candidate gene that can be used as a predictor of important changes in LDL concentrations. It has been reported that *sorting nexins* can regulate the serum lipid level, probably due to the interaction of *SNX*s (sorting nexin 1 (*SNX1)*, sorting nexin 2 (*SNX2*) and sorting nexin 4 (*SNX4*)) with the leptin receptor. In addition, there are studies that suggest that *SNX* functions to regulate lipid metabolism and may contribute to coronary artery disease (Yang et al., 2019). Moreover, an association between abnormal *SNX* expression and heart failure has been reported; specifically, a reduction in *SNX13* expression is observed in failing hearts of humans and mice (Li et al., 2014). More studies are needed to confirm its role in the expression in response to the consumption of FAs and how its expression is related to an LDL decrease,

which is an important finding since a high concentration of LDL is a prevalent risk factor for coronary artery disease.

The Wagyu-Cross beef group did not cause changes in the gene expression of the studied gene panel; since most of these genes play an important role in the regulation of cholesterol and LDL concentrations, it is possible that the higher MUFA and PUFA consumption in this group could be an important factor in obtaining a more favorable lipid profile, as was reported previously (Vela-Vásquez et al., 2021), because it has been reported that PUFAs suppress the nuclear abundance of the *SREBP* transcription factor (Georgiadi et al., 2012); therefore, with a lower amount of this transcription factor, the SREBP-activated synthesis of cholesterol and FAs is reduced (Afman and Müller, 2012). In a recent clinical trial Vela-Vásquez et al. 2022, showed that the consumption of Wagyu-Cross beef maintained a decrease in lipid profiles while commercial beef led to an increase (TC, LDL) in a four weeks-clinical trial, however, further studies with a larger number of volunteers will confirm the findings and broaden the knowledge in this area, which is important for supporting the consumption of beef and breeding strategies aimed at obtaining beef with high nutritional quality.

These results highlight the importance of developing well-controlled nutrigenomic studies to better understand how beef FAs regulate gene transcription in humans and their health effects.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Afgan E, Baker D, Batut B, Van Den Beek M, et al. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res.* 46: W537-W544.

Afman LA and Müller M (2012). Human nutrigenomics of gene regulation by dietary fatty acids. *Prog. Lipid Res.* 51: 63-70.

Cerda Á, Genvigir FDV, Rodrigues AC, Willrich MAV, et al. (2011). Influence of polymorphisms and cholesterol-lowering treatment on *SCARB1* mRNA expression. *J. Atheroscler. Thromb.* 1104200366-1104200366.

Chatterjee S, Szustakowski JD, Nanguneri NR, Mickanin C, et al. (2009). Identification of novel genes and pathways regulating *SREBP* transcriptional activity. *Plos one.* 4: e5197.

Chen Y, Zhu J, Lum PY, Yang X, et al. (2008). Variations in DNA elucidate molecular networks that cause disease. *Nature*. 452: 429-435.

Chiba-Falek O, Nichols M, Suchindran S, Guyton J, et al. (2010). Impact of gene variants on sex-specific regulation of human Scavenger receptor class B type 1 (SR-BI) expression in liver and association with lipid levels in a population-based study. *BMC Med. Genet*. 11: 9. doi: 10.1186/1471-2350-11-9.

Choi SH, Gharahmany G, Walzem RL, Meade TH, et al. (2018). Ground beef high in total fat and saturated fatty acids decreases X receptor signaling targets in peripheral blood mononuclear cells of men and women. *Lipids*. 53: 279-290.

Georgiadi A and Kersten S (2012). Mechanisms of gene regulation by fatty acids. *Adv. Nutr*. 3: 127-134.

Gilmore LA, Walzem RL, Crouse SF, Smith DR, et al. (2011). Consumption of high-oleic acid ground beef increases HDL-cholesterol concentration but both high-and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. *J. Nutr*. 141: 1188-1194.

Joo ST, Hwang YH and Frank D (2017). Characteristics of Hanwoo cattle and health implications of consuming highly marbled Hanwoo beef. *Meat Sci.* 132: 45-51.

Jump DB (2004). Fatty acid regulation of gene transcription. *Crit. Rev. Clin. Lab. Sci.* 41: 41-78.

Ladeira MM, Schoonmaker JP, Gionbelli MP, Dias JC, et al. (2016). Nutrigenomics and beef quality: a review about lipogenesis. *Int. J. Mol.* 17: 918.

Larsen SV, Holven KB, Ottestad I, Dagsland KN, et al. (2018). Plasma fatty acid levels and gene expression related to lipid metabolism in peripheral blood mononuclear cells: a cross-sectional study in healthy subjects. *Genes Nutr*. 13: 9. doi: 10.1186/s12263-018-0600-z.

Li J, Li C, Zhang D, Shi D, et al. (2014). *SNX13* reduction mediates heart failure through degradative sorting of apoptosis repressor with caspase recruitment domain. *Nat. Commun*. 5: 5177. https://doi.org/10.1038/ncomms6177

Liu Y, Ordovas JM, Gao G, Province M, et al. (2008). The *SCARB1* gene is associated with lipid response to dietary and pharmacological interventions. *J. Hum. Genet.* 53: 709-717.

Mark Brown J, Shelness GS and Rudel LL (2007). Monounsaturated fatty acids and atherosclerosis: opposing views from epidemiology and experimental animal models. *Curr. Atheroscler. Rep.* 9: 494-500.

Mayr JA. (2015). Lipid metabolism in mitochondrial membranes. *J. Inherit. Metab. Dis*. 38: 137-144.

Pérez-Lizaur AB, Palacios G, Castro BAL and Flores-Galicia I (2008). Sistema mexicano de alimentos equivalentes. México: Fomento de Nutricion y Salud AC, Ogali.

Polianskyte Z, Peitsaro N, Dapkunas A, Liobikas J, et al. (2009). *LACTB* is a filament-forming protein localized in mitochondria. *Proc. Natl. Acad. Sci*. 106: 18960-18965.

Powell D (2015). Degust: Visualize, explore and appreciate RNA-seq differential gene-expression data. In COMBINE RNA-seq workshop.

Retterstøl K, Svendsen M, Narverud I and Holven KB (2018). Effect of low carbohydrate high fat diet on LDL cholesterol and gene expression in normal-weight, young adults: A randomized controlled study. *Atherosclerosis.* 279: 52-61.

Ruiz-Núñez B, Pruimboom L, Dijck-Brouwer DJ and Muskiet FA (2013). Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. *J. Nutr. Biochem.* 24: 1183-1201.

Rundblad A, Holven KB, Bruheim I, Myhrstad MC, et al. (2018). Effects of fish and krill oil on gene expression in peripheral blood mononuclear cells and circulating markers of inflammation: a randomised controlled trial. *J. Nutr. Sci.* 7.

Smith SB, Lunt DK, Smith DR and Walzem RL (2020). Producing high-oleic acid beef and the impact of ground beef consumption on risk factors for cardiovascular disease: A review. *Meat Sci.* 163: 108076.

Vela-Vásquez DA, Sifuentes-Rincón AM, Delgado-Enciso I, Delgado-Enciso OG, et al. (2021). Improvement of serum lipid parameters in consumers of Mexican Wagyu-Cross beef: A randomized controlled trial. *J. Food Sci.* 86: 2713-2726.

Vela-Vásquez DA, Sifuentes-Rincón AM, Delgado-Enciso I, Ordaz-Pichardo C, et al. (2022). Effect of Consuming Beef with Varying Fatty Acid Compositions as a Major Source of Protein in Volunteers under a Personalized Nutritional Program. *Nutrients*. 14(18): 3711. https://doi.org/10.3390/nu14183711.

Vickers AJ (2001). The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. *BMC Med. Res. Methodol.* 1: 6 https://doi.org/10.1186/1471-2288-1-6.

West M, Greason E, Kolmakova A, Jahangiri A, et al. (2009). Scavenger receptor class B type I protein as an independent predictor of high-density lipoprotein cholesterol levels in subjects with hyperalphalipoproteinemia. *J Clin. Endocrinol. Metab*. 94: 1451-1457.

Willer CJ, et al. (2013). Discovery and refinement of loci associated with lipid levels. *Nat. Genet.* 45: 1274-1283.

Xue C, He Y, Zhu W, Chen X, et al. (2018). Low expression of *LACTB* promotes tumor progression and predicts poor prognosis in hepatocellular carcinoma. *Am. J. Transl. Res*. 10: 4152.

Yang J, Villar VAM, Rozyyev S, Jose PA, et al. (2019). The emerging role of sorting nexins in cardiovascular diseases. *Clin. Sci.* 133: 723-737.

Yang X, Deignan JL, Qi H, Zhu J, et al. (2009). Validation of candidate causal genes for obesity that affect shared metabolic pathways and networks. *Nat. Genet.* 41: 415-423.