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Apple Extracts Present Catabolic and Hipocolesterolemic Effect in Mice

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Abstract

Dyslipidemia is highly prevalent in the Chilean population: about 40% have total cholesterol levels over 200 mg/dl and 25% are obese (BMI > 30) which produces an increased risk of cardiovascular disease and deaths. Over consumption of foods rich in lipids and carbohydrates unchains these events, due to the high amount of lipid accumulation in adipocytes. These cells are capable of producing a large number of mediators of inflammation and adipokines which in large quantities can compromise the overall metabolism. Apple has been shown to stop these events. We used CF-1 mice that were fed on a high-fat diet which leads to a metabolic status similar to dyslipidemia. Different types of apple waste were direct from orchard and fruit industry. Extracts obtained were characterized and administered in drinking water. At the end of the 40-day experimental period, biochemical parameters in animals were measured and the weight of white adipose tissue (WAT) was quantified. The results were compared with the normal diet and fat diet controls. All apple extracts decrease total and LDL cholesterol to levels similar to normal control and decrease WAT. Apple extracts may be an effective protector against development of risk factors in cardiovascular disease in the Chilean population.

Keywords

Cholesterol, Apple, Antioxidants and Metabolic Syndrome

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1. Introduction

Chronic non communicable diseases (NCD), especially cardiovascular diseases (CVD), present a high rate of morbi-mortality in the world: 17.3 million people died in 2008 and it is forecast that about 23.3 million will die by 2030 [1]. According to the National Health Survey conducted in 2010 in Chile [2], the prevalence of high total cholesterol (over 200 mg/dl) corresponds to 38.5% of the population, high levels of LDL cholesterol and triglycerides can also be observed, mainly affecting men and people with low levels of education as shown in **Table 1**.

The excess of energy intake is mostly responsible for an inappropriate processing of two key components in human energetic metabolism: lipids and glucose. This energy overabundance is stored in adipocytes which released high levels of adipokines and cytokines (IL-6, TNF-α, VEGF, etc.) that affect the vascular system [3].

The main problem of increased blood total cholesterol is the development of atherosclerosis. This is the initial step that ends in a cardiovascular disease such as acute myocardial infarction, stroke and coronary artery disease. Cardiovascular disease remains today one of the leading causes of morbidity and mortality in western countries including Chile.

This disease has a multi-factorial pathogenesis: immune, oxidative stress, endotetial injury and increased total cholesterol. There is strong evidence that inflammation localized in arterial intima is key, caused by accumulation of low-density lipoprotein (LDL) in the arterial wall. Furthermore, another triggering event is the oxidation of LDL which activates the endothelial cells, resulting in the recruitment of various immune cells in the subendothelial space, such as peripheral monocytes and T and B lymphocytes. Then, infiltrating monocytes differentiate into dendritic cells that might form foam cells, generating in the interior of the *intima* a number of cytokines and chemoattractants that develop a cascade of events: recruitment of peripheral immune cells, increased migration and lipoprotein accumulation, and differentiation and proliferation of smooth muscle cells in an environment highly oxidative. This process ends in apoptosis and development of a necrotic core that is known as advanced lesion in the vascular endothelium [4].

At present there is strong scientific evidence that confirms that fruits and vegetables show a beneficial effect on the health of people, specifically for the treatment of obesity and dyslipidemia [5]. The importance of world apple production reached 76,000,000 tons in 2012 [6]. Besides, apple production represents 22% of phenols consumed in diet [7]. Apple skin, as a by-product of agro industry, and fruits discarded in the orchard, are the raw material that might be used as a source of phenolic antioxidants, to be added to diverse foods.

This availability of apple waste has led to the study of different fruit extracts to establish the probable causes of the beneficial effect. It has been found that extracts of grapes and apples reduce adiposity and improve the plasma lipid profile in different animal models [8]. In this context, apple bioactive compounds have shown a

Table 1. The prevalence of dyslipidemia and obesity that detailed in National Health Survey of Chile 2009-2010 (published with permission).

				SEX		Educational level (according to year studying)		
	Disease	Standard	National total	Men	Women	<8	8 to 12	>12
Dislipimedia	Decreased HDL	<40 mg/dl in men, <50 mg/dl women	45.4%	37.6%	52.8%	48.5%	48.8%	37.2%
	Protected HDL	≥60 mg/dl	14.7%	8.3%	20.8%			
	High LDL	According to ATP III (fasting up ≥ 9 hour)	22.7%	27.2%	18.3%	35.3%	22.8%	14.2%
	High triglycerides	≥150 mg/dl (fasting up ≥ 9 hour)	31.2%	35.6%	27.1%	34.9%	33.0%	24.8%
	Total cholesterol high	>200 mg/dl	38.5%	39.0%	38.1%	43.6%	39.3%	33.8%
Obesity	Overweight 1	BMI ≥ 25	64.5%	64.6%	64.3%	76.4%	61.9%	61.2%
	Overweight 2	BMI ≥ 25, y < 30	39.3%	45.3%	33.6%	40.9%	37.2%	42.7%
	Obesity	BMI ≥ 30	25.1%	19.2%	30.7%	35.5%	24.7%	18.5%
	Extreme obesity	BMI ≥ 40	2.3%	1.3%	3.3%	5.3%	1.5%	2.1%

protective effect, especially for their antioxidant [9], hypolipidemic [10] and endothelium protective activity [11]. Different apple extracts have been reported to decrease the weight of visceral adipose tissue as compared to controls. Furthermore it has been found that diets supplemented with apple skin have a positive effect on the lipid profile [12] [13].

However, there is insufficient scientific evidence to determine if the source apple extracts such as traditionally or organically managed orchards, as well as green or red fruits and apple skin from by-product of agroindustry or discarded in the orchard have a better result in the lipid profile [14]-[16]. We studied possible beneficial effects of phenolic antioxidant-rich extracts from skin and small fruits discarded in the orchard in murine model of obesity.

2. Materials and Methods

2.1. Animals

Male CF1 mice weighing 25 g were purchased from the Institute of Public Health of Chile. Animals were quarantined for environmental adjustment, at standard conditions (22˚C ± 2˚C) and at a regular light-dark cycle (12 h - 12 h), with free access to food and water before testing. Thereafter they were divided into groups of 8 mice according to the experimental groups. Animals were kept in group during quarantine and experimental period (40 days) at the Biotery of Universidad de Talca and supervised daily by trained staff.

2.2. Extract Preparation

6 apple extract were obtained (organic Fuji (OF) and traditional Fuji (TF); and organic Granny Smith (OGS) and traditional Granny Smith (TGS), as well as apple skin at harvest time (Fuji (FS) and Granny Smith (GSS)) (detail of the extracts found in **Table 2**) using the following procedure: cutting the waste/peel into small pieces and homogenized with 80% aqueous EtOH for 6 hours.

In an early stage of patent procedure. The six extracts obtained were administered in the drinking water of the animals as performed by He [17].

2.3. Determination of Total Phenolics Concentration

Total phenolics were determined by the Folin-Ciocalteu method. Briefly, 0.1 ml of extract was mixed with 0.5 ml of the Folin-Ciocalteu phenol reagent (Merck, Darmstadt, Germany). The mixture was incubated for 5 min and then 0.5 ml of sodium carbonate (Na_2CO_3; 10%, w/v) was added and incubated for 15 min at room temperature. Absorbance was measured at 640 nm with the spectrophotometer. Total phenolic concentrations in the peel and diets were expressed as mg of chlorogenic acid equivalents (CAE) g^{-1} FW.

2.4. Diet Preparation

Two types of diet were used, normal (ND) and fat diet (FD). The increased calories of FD were supplemented

Table 2. Characteristics of each group in the study.

Abbreviation	Characteristic of each group
ND	Normal diet free demand
FD	Fat diet (FD)
FO	FD + apple extract from *cv Fuji* organic orchard waste 400 mg phenol/kg mice
FP	FD + apple extract from *cv Fuji* Fruit skin (by-product of agro industry) waste 400 mg phenol/kg mice
FT	FD + apple extract from *cv Fuji* traditional orchard waste 400 mg phenol/kg mice
GSO	FD + apple extract from *cv Granny Smith* organic orchard waste 400 mg phenol/kg mice
GSP	FD + apple extract from *cv Granny Smith* Fruit skin (by-product of agro industry) waste 400 mg phenol/kg mice
GST	FD + apple extract from *cv Granny Smith* traditional orchard waste 400 mg phenol/kg mice

with vegetable oil and animal fat. Vitamins and proteins (as peptone) were added to balance the amount of vitamins, minerals and proteins. FD was prepared once a week and kept frozen until use following Moore-Carrasco *et al.* [18].

Random samples of the diet were taken to the Instituto de Química de los Recursos Naturales de la Universidad de Talca for proximal chemical analysis (percentages of humidity, ashes, lipids or fats, proteins, fibre and elements free of nitrogen) using standardized methods for AOAC [19].

2.5. Study Groups

Animals were randomized and divided into groups of 8 animals per experimental group (FO, FP, FT, GSO, GSP, GST), normal diet (ND) control and fat diet (FD) control. Each experimental group received FD plus water with the corresponding extract in drinking water as explained in **Table 2**. The extract was standardized 400 mg phenols per kilogram of animal weight (400 mg/kg), according to Hogan [20] and toxicology recommendation from Shoji [21] (see antioxidant consumption).

2.6. Anaesthesia and Sacrifice

Water and food intake was quantified daily and the mice weight was controlled weekly. The study length was 40 days; at the end of this period animals were anesthetized in fasting condition with an intraperitoneal injection containing a mixture of *Ketamine* (Richmond, Argentina), *Acetopromacine* and *Xylazine* (Centrovet, Chile). Then, blood obtained with 1% heparin from aorta was centrifuged, separated and frozen at −70˚C until analysis to determine plasma: glycemia (GLI), total cholesterol (T-CHOL), cholesterol low density lipoprotein (CHOL LDL) and cholesterol high density lipoprotein (CHOL HDL) by commercial kit ROCHE following manufacturer's instruction in autoanalyzer ROCHE, Elecsys 1010. Organs were also obtained: heart, liver, white adipose tissue (WAT), kidney and gastrocnemius, which were weighed on a precision scale (Shimadzu UX-UW) and frozen at −70˚C.

Animal handling met all the rules established by the National Commission for Scientific and Technological Research of Chile (CONICYT). All protocols carried out in this work have the approval of the bioethics and bio security committee of Universidad de Talca.

2.7. Statistical Analysis

Statgraphics centurion was used for the analysis of the results with an ANOVA statistical test. $p < 0.05$ was considered statistically meaningful.

3. Results and Discussion

3.1. Characterization of Extracts and Diet

We characterized extract and diet. **Table 3** shows the results for each extract obtained, as well as its characterization according to phenol content in each of them. Additionally, characterization of diets to which animals were subjected is shown in **Table 3**. Difference between both diets was based on lipid contents in ND was 3% and in FD was 28%. The lipid content of the diets are within the values obtained in accordance previously reported by Moore [18].

3.2. Results in Animal Model of Dyslipidemia

Evolution of the weight was expressed in mean % of weight gain in relation to mean % initial weight. There is a significant difference between mean % of weight gained in the FD group (32.8%) and the rest of the study groups and including ND (**Figure 1**).

In heart, liver, kidney and gastrocnemius there are no significant difference (**Table 4**); only visceral white adipose tissue (WAT) showed a significant difference of weight (**Figure 2**) expressed in mean % of weight gain of WAT in relation to mean % initial weight of WAT. Mean weight of WAT in relation to the mean of initial weight shows the difference between the group with FD and ND. Statistical analysis shows that WAT weight decreased considerably in all study groups reaching similar levels to ND group (**Figure 2**) it means that all groups are statistically different compared to ND group.

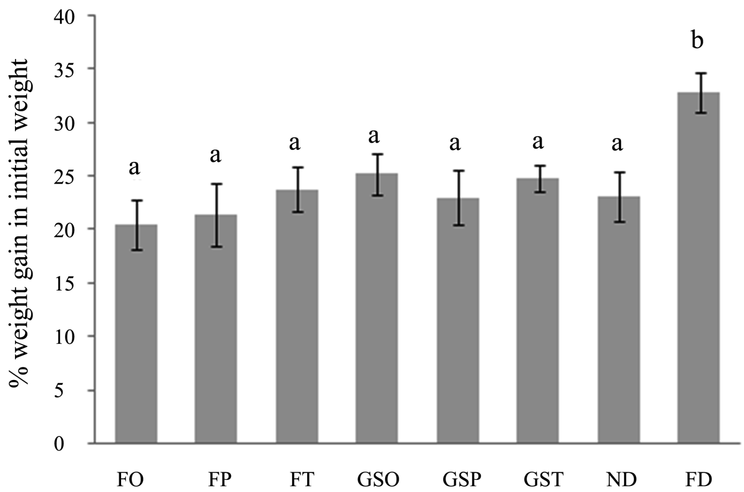

Figure 1. Mean % of weight gain in relation to mean % initial weight. Organic Fuji (FO). Fuji skin (FP). Traditional Fuji (TF). Organic Granny Smith (GSO). Granny Smith skin (GSS). Traditional Granny Smith (GST). Normal diet (ND). Fat diet (FD). *Different letters indicate higher significant difference with $p > 0.05$. Results are found with mean error (a.e).

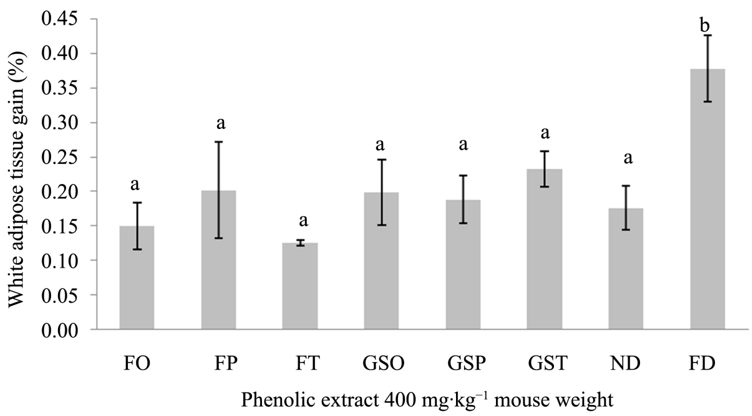

Figure 2. Percentage of WAT gain in 400 mg·kg^{-1} assay phenolic extract. Weight gain of soft adipose tissue (WAT) according to the percentage of initial weight of the mice. in the trial 400 mg/kg. Organic Fuji (FO). Fuji skin (FP). Traditional Fuji (TF). Organic Granny Smith (GSO). Granny Smith skin (GSS). Traditional Granny Smith (GST). Normal diet (ND). Fat diet (FD). *Different letters indicate higher significant difference with $p > 0.05$. Results are found with mean error (a.e).

There have been studies showing that apple extracts are one of the few fruits that have demonstrated anti-obesity effect. *Boque et al.* conducted a screening of different plants (almond, apple, cinnamon, orange, witch hazel, lime blossom, grape, birch) activities anti-obesity seeking in *Wistar* rats [22], finding that only extracts of apple and cinnamon were considered anti-obesity because of their effects in reducing body fat (body fat-lowering) in the animals.

Studies have confirmed this trend in animal models, Cho [12] testing pome apple (PA) and apple juice concentrate (AJC) on different groups with a high fat diet. They found that the groups which were supplied with PA and AJC in the presence of high-fat diet had a lower weight, lower total CHOL, lower CHOL LDL, while CHOL HDL was significantly higher in the PA group compared with AJC. The results suggest that products supplemented with PA and AJC can help suppress the increase in WAT and improve the lipid profile in obese rats, supporting our results.

Table 3. Proximal chemistry and phenol quantification for each obtained extracts and diets administered.

	% water	% proteins	% lipids	% ashes	% fiber	% NFE	Total mass extract (gr)	Total phenols present in the extract (gr)	% total phenols
FO	1.8	0.6	3.8	4.6	0.5	88.7	22.1	6.7	30
FP	1.8	2.6	12.3	11.4	1.1	70.8	104.7	4.3	5
FT	2.5	1.2	2.5	3.7	0.3	89.8	23.6	7.5	32
GSO	1.9	0.4	1.2	1.8	0.2	94.5	19.4	7.4	38
GSP	2.1	1.1	2.5	6.7	1.3	86.4	28.1	5.9	21
GST	2.3	0.8	2.9	5.7	1.3	87.0	42.1	7.4	18
ND	10	20	3	3	1	63	-	-	-
FD	7	17	28	6	2	40	-	-	-

NFE: nitrogen free elements. FO: organic Fuji. FP: Fuji skin. FT: traditional Fuji. GSO: organic Granny Smith. GSS: Granny Smith skin. GST: traditional Granny Smith. ND: normal diet. FD: fat diet.

Table 4. Weight from different organs.

	Heart	Liver	Kidney	Gastrocnemius
FO	0.17 ± 0.02 a	5.25 ± 0.04 ab	1.83 ± 0.02 ab	0.57 ± 0.03 a
FP	0.22 ± 0.02 bc	5.81 ± 0.09 c	1.78 ± 0.01 a	0.64 ± 0.07 abc
FT	0.22 ± 0.01 bc	5.22 ± 0.05 ab	1.86 ± 0.01 abc	0.63 0.01 bc
GSO	0.22 ± 0.01 bc	5.29 ± 0.07 a	1.95 ± 0.01 ab	0.67 ± 0.01 bc
GSP	0.19 ± 0.02 ab	5.52 ± 0.04 bc	1.90 ± 0.01 abc	0.72 ± 0.03 c
GST	0.22 ± 0.01 abc	6.33 ± 0.07 d	1.66 ± 0.01 a	0.64 ± 0.05 abc
ND	0.65 ± 0.05 bc	5.80 ± 0.33 cd	1.97 ± 0.08 c	0.67 ± 0.06 bc
FD	0.67 ± 0.04 c	5.79 ± 0.13 d	1.81 ± 0.03 bc	0.64 ± 0.03 bc

NFE: nitrogen free elements. FO: organic Fuji. FP: Fuji skin. FT: traditional Fuji. GSO: organic Granny Smith. GSS: Granny Smith skin. GST: traditional Granny Smith. ND: normal diet. FD: fat diet.

3.3. Phenolics of Apple Peel/Discard Consumption and Feeding

The administration of phenols of apple peel/discard to animals was 400 mg/kg mouse weight according to their initial weight. The amount of food that was consumed during the assay per group was between 1.311 gr (FP and GSP) and 1.451 gr (FO).

3.4. Biochemical Parameters

Table 5 shows biochemical results obtained in plasma of test mice dosed 400 mg of phenol/kg. In mice subjected to FD and the contribution of different apple extracts, a decrease of CHOL TOT, CHOL LDL and an increase of CHOL HDL was observed with all extracts studied, at similar level to the ND group. Levels of CHOL LDL and CHOL HDL are in **Table 5**. No difference was found between the extract from discarded fruit or apple skin. Our obtained results allow inferring that the extract consumed by animals in the study (400 mg phenols/kg) decreases the content of total cholesterol and LDL cholesterol, and increases HDL cholesterol, with WAT decrease playing a key role. Results confirm the findings of other researchers, in which cholesterol accumulation in rabbits in the ascending aorta decreased with the administration of *quercetin glucoside* [23]. Also, in obese *Zucker* rats that consumed apples, cholesterol level decreased [24]. Another study which confirms apple use for plasma cholesterol decrease in model animals was carried out by Leontowicz *et al.* [13] in which skin and pulp

Table 5. Summary of biochemical mean results for experiment 400 mg·kg^{-1}.

	CHOL HDL (mg/dl)*	CHOL LDL (mg/dl)*	CHOL TOT (mg/dl)
FO	92 ± 8.4 b	95 ± 12.7 d	152 ± 9.4 c
FP	98 ± 5 b	40 ± 11.4 d	90 ± 26.1 a
FT	90 ± 10.1 b	62 ± 19.9 bc	111 ± 36.4 ab
GSO	96 ± 9.4 b	53 ± 5.6 ab	131 ± 36.6 bc
GSP	97 ±5.6 b	53 ± 18.4 ab	115 ± 19.6 ab
GST	86 ± 7.5 b	77 ± 15 cd	114 ± 18.7 ab
ND	80 ± 8.8 b	33 ± 3.1 a	119 ± 23.1 abc
FD	13 ± 6.2 a	217 ± 33 e	252 ± 37.2 d

CHOL TOT: total cholesterol. CHOL HDL: HDL cholesterol. CHOL LDL: LDL cholesterol. NFE: nitrogen free elements. FO: organic Fuji. FP: Fuji skin. FT: traditional Fuji. GSO: organic Granny Smith. GSS: Granny Smith skin. GST: traditional Granny Smith. ND: normal diet. FD: fat diet. *Different letters indicate higher significant difference with $p > 0.05$. Results are found with the mean error.

was used at the ripeness peak. In this report we used fruits discarded in orchard and skin waste from fruit processing. Components that are present in apple presenting the beneficial effect in reducing CHOL are complex polysaccharides such as pectin and different polyphenol. Aprikian [24] separated these compounds and demonstrated that they produce a greater decrease in the lipid profile in Wistar rats acting together than separately. A high correlation has been found between anti-hypercholesterolemic effects and certain compounds present in apple like *catechin*, *epicatechin*, *procyanidin* B1 and *β-carotene* which would be primarily responsible phytocompounds by lowering cholesterol by this fruit [25]. It is necessary to explain the mechanisms by which metabolites present in apples as well as in its extracts produce a decrease in plasmatic cholesterol levels in mammals. In this respect, two hypotheses have been proposed: 1) The component or components present in apples as well as in its extracts can inhibit cholesterol absorption, as pointed out by Koo *et al.* [26], who details decrease in plasma cholesterol for the effect of *catechin* present in green tea. This effect would be due to the inhibition of lipid absorption in the intestine and the inhibition of lipid hydrolysis, mostly *pancreatic lipase* and *pancreatic phospholipase A*2. These findings have been reaffirmed by meta-analysis reviews [27]. In future trials, lipid concentrations in the faeces of animals during the experiment might be quantified, and studies might focus on transporters and enzymes in the intestines, either in animal models or cellular cultures that are related to cholesterol absorption.

2) According to another hypothesis, there is evidence that compounds present in apples and their extracts interfere in cholesterol metabolism; this was demonstrated by Osada *et al.* [28]. It has been proposed that *Ursolic acid*, present in fruit, is capable of modulating cholesterol catabolism, producing increase of muscle mass and brown adipose tissue (BAT), decreasing obesity, glucose intolerance and fatty liver [29]. This work would explain, in turn, WAT decrease in studied mice [30]. This effect has also been confirmed in another animal model such as the Wistar rats, showing improved glucose tolerance to a diet high in fructose and improved lipolytic response of adipocytes, proposing that the anti-obesity effects are achieved through the regulation of genes included in adipogenesis, lipolysis and oxidation of fatty acids, reducing the expression of mRNA of SREBF1 and *increasing aquaporin* 7 (AQP7), *adipocyte binding enhancer protein* 1 (Aebp1) and *peroxisome proliferator activator receptor gamma co*-1 *alpha* (PPARGC1A) [31].

Important efforts have been made in the search of functional ingredients from apple pulp and skin, in order to incorporate them in healthier food for the population [32]. In this study, supplies to prepare the fruit extracts obtained from fruit discarded in orchard and the apple skin waste from fruit processing, both considered discards of low commercial value.

The contribution of mediterranean diet to the health of people consuming it has been widely studied and associated to a decrease of cardiovascular diseases [33]-[35]. Of foods that are consumed, one of the main candidate compounds are the phenols which have high antioxidant capacity [36].

4. Conclusion

Obtaining an extract with a high content of antioxidants, in this case of apples, should improve parameters which are altered in plasmatic cholesterol. This work shows that important metabolites can be obtained from unused items in apple production, such as discard from orchard fruit and apple skin waste, and that these metabolites can decrease plasma cholesterol levels and the WAT weight in studied mice. In addition, there are no differences between red or green variety or if they come from a traditional or organic agronomic management: all experimental groups present decreased total cholesterol and increased HDL cholesterol which suggest the potential beneficial effects of regular consumption of these products favouring protection to cardiovascular disease. These findings need further studies to explain the mechanisms of action.

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Disclosure Policy

The author(s) declare(s) that there is no conflict of interests regarding the publication of this paper.

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