Research Article

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Screening of polyphenolic plant extracts for anti-obesity properties in Wistar rats

Noemi Boqué,[a] Javier Campión,[a] Rocío de la Iglesia,[a] Ana L de la Garza,[a] Fermín I Milagro,[a] Belén San Román,[b] Óscar Bañuelos[b] and J Alfredo Martínez[a]*

Abstract

BACKGROUND: Polyphenols have been reported to prevent chronic diseases such as cardiovascular diseases, cancers, diabetes and neurodegenerative diseases. The objective of the study was to conduct a screening for potential anti-obesity polyphenolic plant extracts using a diet-induced animal model. Rats were fed a high-fat-sucrose (HFS) diet with or without supplementation of different polyphenolic plant extracts (almond, apple, cinnamon, orange blossom, hamamelis, lime blossom, grape vine, and birch) for 56–64 days.

RESULTS: Body weight gain was lower in rats supplemented with apple, cinnamon, hamamelis and birch extracts as compared to HFS non-supplemented group. Moreover, apple and cinnamon extracts prevented the increase in fat mass promoted by the HFS diet. Insulin resistance, estimated by the homostatic model assessment–insulin resistance (HOMA-IR) index, was reduced in rats fed apple, cinnamon, hamamelis and birch extracts. Apple extract also prevented the HFS-induced hyperglycaemia and hyperleptinaemia.

CONCLUSION: Only apple and cinnamon extracts were finally considered as potentially important anti-obesogenic extracts, due to their body fat-lowering effects, while the improvement of obesity-related metabolic complications by apple polyphenols highlights this extract as a promising functional food ingredient for the management of obesity and its metabolic complications.
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Supporting information may be found in the online version of this article.

Keywords: screening; plant extracts; polyphenols; high-fat-sucrose diet; anti-obesity; adipose tissue

INTRODUCTION

Obesity, defined by the World Health Organization (WHO) as an abnormal or excessive fat accumulation that may impair health, is becoming one of the greatest challenges to global health in this millennium, with more than 200 million men and nearly 300 million women obese globally in 2008.[1] Due to these increasing obesity rates, the WHO has considered it the epidemic of the 21st century and has promoted different strategies to prevent and control its progress.[2]

Plant extracts, defined as raw or refined products derived from plants or parts of plants (e.g. leaves, stems, buds, flowers, roots or tubers), are frequently used for the treatment of diseases.[3] Nowadays, there is scientific data supporting the evidence that some plant components have beneficial physiological effects.[4,5] Thus, research concerning new natural compounds addressing the treatment of obesity is increasing due to their low side-effects compared with conventional pharmacological agents as well as their potential applications for the development of new functional foods.

Here, we focused on the research of the potential effects of polyphenolic natural extracts on obesity prevention. Polyphenols possess multiple phenol sub-structures, consisting of one or more aromatic rings with one or more hydroxyl groups. These compounds are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, etc.) and partially responsible for their overall organoleptic properties.[6] Moreover, polyphenols are the most abundant antioxidants in the diet.[7]

Scientific research provides evidence that polyphenols could offer some protection against the development of chronic diseases such as cardiovascular diseases,[8] cancers,[9] diabetes[10,11] and neurodegenerative diseases.[12,13] In the past two decades, interest has been focused on the potential biological effects of polyphenols on the prevention of obesity and associated clinical manifestations. In this sense, it has been shown that dietary polyphenols supplementation reduces body weight gain and adipose tissue in rodents fed a hypercaloric diet, which prevents diet-induced hepatic steatosis.[14–16]

* *Correspondence to: Professor J Alfredo Martínez, Department of Nutrition, Food Science, Physiology and Toxicology, University of Navarra, C/Irunlarrea s/n, 31008, Pamplona, Spain. E-mail: jalfmtz@unav.es*

a *Department of Nutrition, Food Science, Physiology and Toxicology, University of Navarra, 31009, Pamplona, Spain*

b *Discovery Laboratory, Health and Nutrition Department, Biosearch S.A, 18004, Granada, Spain*

Table 1. Composition and daily doses of polyphenolic plant extracts

Plant polyphenolic extract	Scientific name	Active principles	Daily dose (g kg^{-1} rat)
Almond	*Prunus dulcis* D.A. Webb	35% total polyphenols	0.82
Apple	*Malus domestica* Borkh	80% total polyphenols, 5% phloridizine	0.70
Cinnamon	*Cinnamomium zeylanicum* Blume	1% oligomeric proanthocyanidins	1.0
Orange blossom	*Citrus aurantium* L.	6% flavonoids	1.9
Hamamelis	*Hamamelis virgiana* L.	15% total polyphenols	0.96
Lime blossom	*Tilia tomentosa* Moench	1% flavonoids	0.49
Grape vine	*Vitis vinifera* L.	3% flavonoids	1.5
Birch	*Betula alba* L.	2% flavonoids	2.5

In this context, since new extraction technologies and procedures[17,18] have led to the identification and characterisation of many plant polyphenols, rapid and accurate screening for a large number of polyphenols is required. Therefore, the purpose of this study was to conduct a screening for potential anti-obesity polyphenolic plant extracts using a high-fat-sucrose (HFS) diet-induced animal model.

MATERIALS AND METHODS

Plant extracts

All plant extracts were provided by Biosearch S.A. (Granada, Spain). The polyphenolic composition of all the extracts tested in this study is given in Table 1, accompanied by the scientific name of each plant.

Determination of bioactive compounds

The determination of total phenols was performed with a spectrophotometric technique using Folin–Ciocalteu reagent.[19] Gallic acid was used as a standard for the calibration curve. All samples and standards were dissolved in water and the absorbance was measured at 760 nm. Total flavonoids content was determined by high-performance liquid chromatography (HPLC) using pure compounds diluted in ethanol as standards. Samples were dissolved in water–ethanol (1:1). Standard flavonoids used were hesperidin for orange blossom extract, rutin for birch extract, vitexin for grape vine extract and cumarin for cinnamon extract. Ultraviolet (UV) absorbance was measured at 270, 280 and 365 nm depending on the extract. Oligomeric procyanidins were quantified by HPLC using pure catechin diluted in water–ethanol (1:1) as standard. Samples were dissolved in water and the approximate retention times for trimers, tetramers and pentamers were 20, 27 and 30 min. UV absorption was determined at 278 nm. Finally, phloridzine was quantified by HPLC using pure compounds diluted in methanol as standards. Samples were dissolved in methanol and its absorbance quantified at 210 nm.

Animals

Assays were separately performed in two experimental sets. Thus, screening for almond and apple polyphenolic plant extracts was carried out during the 'Experimental Set 1' while screening for cinnamon, orange blossom, hamamelis, lime blossom, grape vine, and birch polyphenolic plant extracts was performed during the 'Experimental Set 2'. For this purpose, male Wistar rats from CIFA (Center for Applied Pharmacobiology Research) of the University of Navarra (Pamplona, Spain) with an initial average weight of 333 ± 30 g were kept in an isolated room with a constantly regulated temperature between 21 and 23°C and controlled humidity (50 ± 10%) under a 12 h/12 h artificial light/dark cycle. Animals were randomly assigned to four different groups (n = 12) for Experimental Set 1 and eight different groups (n = 12) for Experimental Set 2 and were fed a HFS diet with or without supplementation of different polyphenolic plant extracts during 56 and 64 days, respectively. Daily doses of each polyphenolic plant extract are shown in Table 1. Chow-fed rats were used as control group in both models and were fed a standard pelleted diet from Harlan Ibérica (2014; Teklad Global, Barcelona, Spain) containing 20% of energy as proteins, 67% as carbohydrates (5% sucrose, 62% starch) and 13% as fat by dry weight (290 Kcal 100 g^{-1}) (see Supporting information, Table S1). The composition of the HFS diet (473 Kcal 100 g^{-1}) from Research Diets (D12451; Research Diets, New Brunswick, NJ, USA) was 20% of energy as protein, 35% as carbohydrates (18% sucrose, 10% maltodextrin and 7% starch) and 45% as lipids by dry weight (see Supporting information, Table S2 and Table S3).

Rats had *ad libitum* water and food access, while body weight and food intake were recorded weekly. After finishing the experimental feeding period, rats were sacrificed, blood was collected from the trunk and serum stored at −20°C. Tissue samples of liver, muscles and retroperitoneal, mesenteric, epididymal and subcutaneous (inguinal) white adipose tissue (WAT) were isolated, weighed and immediately stored at −80°C. All the performed procedures agreed with the national and institutional guidelines of the Animal Care and Use Committee at the University of Navarra.

Serum measurements

Serum glucose was measured with the HK-CP kit (ABX Pentra, Montpellier, France), total cholesterol was determined with the Cholesterol-CP kit (ABX Pentra) and triglycerides with the RANDOX triglycerides kit (Randox Laboratories, Crumlin, UK), adapted for the COBAS MIRA equipment (Rochel, Basel, Switzerland). Leptin and insulin quantification was performed by specific ELISA kits (Linco Research, St. Charles, MO, USA, and Mercodia AB, Uppsala, Sweden) in a TRITURUS automated ELISA immunoassay analyser (Grifols International S.A., Barcelona, Spain). Finally, the homeostatic model assessment–insulin resistance (HOMA-IR) was calculated using the formula: [fasting insulin (μU mL^{-1}) × fasting glucose (mmol L^{-1})/22.5] as described elsewhere.[20]

Quantification of hepatic fat

The fat content of liver samples was measured by quantitative nuclear magnetic resonance spectroscopy using the EchoMRI™ system (Echo Medical Systems, Houston, TX, USA).

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Statistics

All results are expressed as the mean ± standard error. The effects of dietary HFS intake were not significantly different between both sets; therefore, when comparing the HFS group with the control group, the mean of both experimental sets was used. Statistical analyses were performed using the ANOVA test while the Dunnet's test post-hoc was used to test differences between each group against HFS group. A level of probability of $P < 0.05$ was set up as statistically significant and $P < 0.01$ as statistically very significant. For the statistical tests, SPSS software for Windows 15.0 was applied (SPSS, Chicago, IL, USA).

RESULTS

Rats fed a HFS diet exhibited higher body weight gain as compared with chow-fed animals (163%, $P < 0.01$), which was accompanied by an increase in energy food intake (135%, $P < 0.05$). As expected, subcutaneous and visceral fat mass weight was markedly increased by HFS feeding (163%, $P < 0.01$; 155%, $P < 0.01$). In addition, glucose levels raised significantly (115%, $P < 0.05$) in animals fed a HFS diet in comparison with the control group while the intake of this diet induced a clear hyperinsulinaemia and hyperleptinaemia in these animals (273%, $P < 0.01$; 273%, $P < 0.01$). Our data provide evidence that HFS-diet feeding resulted in a well-established model of diet-induced obesity in male Wistar rats.

Regarding the screening of polyphenolic plant extracts for the prevention of obesity, body weight gain was significantly lower in rats supplemented with apple, cinnamon, hamamelis and birch extracts as compared with HFS non-supplemented animals (Table 2 and Table 3), despite a similar daily food intake in all groups (from 92 to 97 Kcal rat^{-1} day^{-1} in Experimental Set 1 and from 94 to 100 Kcal rat^{-1} day^{-1} in Experimental Set 2). Moreover, cinnamon-fed animals had attenuated food efficiency, while birch-fed animals presented a statistical tendency towards lower food efficiency (Table 2). Importantly, apple and cinnamon plant extracts prevented the increase in total WAT promoted by the HFS diet (Table 2 and Table 3). This fact was accompanied by a significant reduction of visceral WAT in animals whose diet was supplemented with apple, while it tended to be lower in cinnamon-fed animals against HFS non-supplemented animals (Table 2 and Table 3). As shown in Fig. 1, different effects of polyphenolic plant extracts on subcutaneous, retroperitoneal, epididymal and mesenetric fat pads revealed a depot-specific action. Thus, cinnamon extract intake led to significant reduction of subcutaneous fat mass, without changes in the other fat depots weights, whereas apple extract intake led to significant reduction of epididymal and mesenteric fat mass, with a tendency to decrease subcutaneous fat pad weight.

On the other hand, in order to determine whether body weight loss could be due at least in part to changes in liver or muscle weights, these values were analysed. Liver weight was unchanged in all groups except in animals fed birch extract that presented an increased hepatic mass (Table 3). Likewise, regarding effects of polyphenolic extracts on muscle mass, total muscle weight was unchanged in supplemented rats. Even so, hamamelis extract was able to increase soleus muscle weight (Table 3), while apple extract consumption led to a slightly but not significant increase in gastrocnemius muscle weight as compared to HFS-fed rats (Table 2).

Given that biochemical measurements are important to assess possible benefits of plant polyphenols on obesity and metabolic syndrome, we evaluated whether the ingestion of the polyphenolic extracts tested in this study together with a HFS diet could revert the effects of this obesogenic diet on serum values. Therefore, we selected the most effective extracts (as evaluated by the results previously described) and we measured biochemical parameters in the supplemented groups with apple, cinnamon, hamamelis and birch extracts. These results show that supplementation with apple extract was able to prevent the hyperglycaemia caused by the HFS diet (Table 4). Moreover, although cholesterol levels were not affected by HFS feeding, supplementation of this diet with apple extract decreased cholesterol serum levels, whereas supplementation with birch extract resulted in an unexpected increase in cholesterol levels in comparison to HFS group (Table 4 and Table 5). Interestingly, apple extract prevented the HFS-induced hyperleptinaemia (Table 4). Rats supplemented with hamamelis extract displayed lower insulin levels than HFS-fed animals, and the same occurred with rats supplemented with apple, although it did not reach statistical significance (Table 4 and Table 5). Even so, the four analysed plant extracts were able to improve insulin resistance as determined by HOMA-IR index (Table 4 and Table 5). Finally, when liver fat content was analysed we could not find any significant differences between groups (Experimental Set 1: control 4.77 ± 0.74, HFS 6.42 ± 0.83, apple 6.84 ± 0.81%, NS; Experimental Set 2: control 3.81 ± 1.06, HFS 6.98 ± 0.63, cinnamon 7.59 ± 1.70, hamamelis 7.31 ± 0.71, birch 5.88 ± 0.69%, NS).

DISCUSSION

Recent evidence concerning the protective effects of polyphenols administration against chronic diseases[21,22] has become of great interest to the food industry regarding research on new polyphenol-rich ingredients for the development of functional foods or nutritional supplements. Since obesity is one of the key targets for new functional foods,[23] the aim of this study was to screen different polyphenolic plant extracts for the prevention of obesity using a diet-induced obesity rodent model.

The establishment of an appropriate obesity model is essential for the study of natural compounds with anti-obesity properties. Thus, different animal models have been developed for this purpose. Further, many models rely on genetic changes to induce symptoms even though the human disease is usually diet induced. Indeed, the combination of simple carbohydrate and saturated fat-rich dietary components, such as a 'cafeteria diet', have been used in rodents to mimic human obesity.[24,25] This is considered probably the best model to test pharmaceutical and nutraceutical preparations for the treatment or prevention of diet-induced human obesity and metabolic syndrome, as concluded in some reviews. Therefore, the first objective of this work was to establish a diet-induced obesity model in male Wistar rats. As described before by our research group,[24,25] long-term HFS feeding resulted in increased body weight, food efficiency and adiposity as well as development of marked hyperleptinaemia and insulin resistance, leading to commonly associated undesirable Western diet features.

The next step, and the main objective of this study, was to test different polyphenolic plant extracts in diet-induced obesity animals and to select those that could exhibit some promising anti-obesity properties, as assessed by the effects observed on body weight gain and tissue weights. Taking into account that apple, cinnamon, hamamelis and birch plant extracts prevented the increase in body weight gain induced by the HFS diet, while cinnamon and birch extracts also reduced food efficiency,

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Table 2. Body-related and tissue measurements of male Wistar rats fed with HFS diet with or without supplementation of different plant extracts[a]

Measurements	Group (HFS + plant extract), Experimental Set 1			
	Control ($n = 12$)	HFS ($n = 12$)	Almond ($n = 12$)	Apple ($n = 12$)
Body weight measurements (g)				
Final body weight	$434.7 \pm 8.79^{**}$	503.8 ± 11.1	488.7 ± 10.4	479.5 ± 12.7
Weight gain	$88.7 \pm 4.73^{*}$	151.8 ± 6.38	134.7 ± 5.12	$125.5 \pm 7.08^{**}$
Food efficiency	$1.79 \pm 0.09^{*}$	2.56 ± 0.1	2.21 ± 0.08	2.00 ± 0.11
Tissue weights (g kg^{-1} body weight)				
Total WAT weight	$75.4 \pm 5.82^{**}$	110.2 ± 5.75	109.1 ± 4.29	$90.1 \pm 4.22^{*}$
Visceral WAT weight	$64.5 \pm 4.53^{**}$	95.6 ± 6.25	93.6 ± 5.03	$77.4 \pm 3.81^{*}$
Liver weight	22.5 ± 0.44	22.3 ± 0.65	21.4 ± 0.68	22.3 ± 0.66
Soleus muscle weight	0.40 ± 0.01	0.37 ± 0.01	0.34 ± 0.01	0.39 ± 0.01
EDL muscle weight	2.10 ± 0.03	1.92 ± 0.04	1.93 ± 0.05	2.00 ± 0.04
Gastrocnemius muscle weight	5.69 ± 0.09	5.06 ± 0.09	5.06 ± 0.10	$5.40 \pm 0.12^{\#}$
Total muscle weight	8.20 ± 0.14	7.36 ± 0.13	7.33 ± 0.16	7.80 ± 0.17

[a] All the results are expressed as the mean ± SE.
Food efficiency was calculated as grams of the body weight gain per 100 Kcal of food intake.
Statistical analyses were performed using the ANOVA test, and Dunnet's test was used to test differences between each group against HFS group.
Total WAT weight indicates the sum of the subcutaneous, retroperitoenal, mesenteric and epidydimal fat pad weights.
Visceral WAT weight indicates the sum of the retroperitoenal, mesenteric and epidydimal fat pad weights.
Total muscle weight indicates the sum of the soleus, EDL and gastocnemius muscle weight.
HFS, high-fat-sucrose; WAT, white adipose tissue; EDL, extensor digitorum longus.
$^{*}P < 0.05$; [b] $^{**}P < 0.01$; [c] $^{\#}P < 0.1$.

Table 3. Body-related and tissue measurements of male Wistar rats fed with HFS diet with or without supplementation of different plant extracts[a]

Measurements	Group (HFS + plant extract), Experimental Set 2							
	Control ($n = 12$)	HFS ($n = 12$)	Cinnamon ($n = 12$)	Orange blossom ($n = 12$)	Hamamelis ($n = 12$)	Lime blossom ($n = 12$)	Grape vine ($n = 12$)	Birch ($n = 12$)
Body weight measurements (g)								
Final body weight	$435.7 \pm 15.4^{**}$	510.7 ± 17.8	464.9 ± 11.4	488 ± 14.4	473.4 ± 13.1	479.2 ± 19.5	500.7 ± 12.2	471.9 ± 15.0
Weight gain	$125.5 \pm 4.75^{**}$	194.3 ± 13.0	$146.3 \pm 7.7^{**}$	168.8 ± 10.1	$156 \pm 6.9^{*}$	163.8 ± 12.2	179.9 ± 7.8	$156.2 \pm 10.4^{*}$
Food efficiency	$2.00 \pm 0.11^{*}$	3.06 ± 0.20	$2.36 \pm 0.13^{*}$	2.72 ± 0.16	2.64 ± 0.12	2.71 ± 0.20	2.86 ± 0.12	$2.52 \pm 0.17^{\#}$
Tissue weights (g kg^{-1} body weight)								
Total WAT weight	$64.7 \pm 3.63^{**}$	115.6 ± 6.32	$93.6 \pm 4.63^{*}$	114.9 ± 4.23	100.3 ± 4.93	115.3 ± 6.20	115.8 ± 6.18	109.5 ± 4.16
Visceral WAT weight	$50.9 \pm 1.44^{**}$	82.0 ± 4.45	$68.8 \pm 3.47^{\#}$	86.4 ± 2.66	72.6 ± 3.55	82.6 ± 4.75	86.1 ± 4.15	80.6 ± 3.41
Liver weight	$24.9 \pm 0.63^{**}$	21.5 ± 0.61	22.5 ± 0.50	21.5 ± 0.69	23.3 ± 0.55	22.3 ± 0.86	22.1 ± 0.43	$23.9 \pm 0.42^{*}$
Soleus muscle weight	0.39 ± 0.02	0.33 ± 0.01	0.35 ± 0.01	0.33 ± 0.01	$0.39 \pm 0.01^{*}$	0.33 ± 0.03	0.37 ± 0.01	0.34 ± 0.01
EDL muscle weight	2.09 ± 0.12	1.83 ± 0.07	1.92 ± 0.08	1.86 ± 0.09	2.04 ± 0.09	1.88 ± 0.06	1.84 ± 0.10	1.72 ± 0.09
Gastrocnemius muscle weight	6.05 ± 0.08	5.05 ± 0.20	5.51 ± 0.11	5.09 ± 0.16	5.41 ± 0.16	5.15 ± 0.14	5.17 ± 0.17	5.19 ± 0.11
Total muscle weight	8.55 ± 0.15	7.20 ± 0.26	7.80 ± 0.17	7.28 ± 0.24	7.83 ± 0.23	7.36 ± 0.20	7.39 ± 0.26	7.24 ± 0.19

[a] All the results are expressed as the mean ± SE.
Food efficiency was calculated as grams of body weight gain per 100 Kcal of food intake.
Statistical analyses were performed using the ANOVA test, and Dunnet's test was used to test differences between each group against HFS group.
Total WAT weight indicates the sum of the subcutaneous, retroperitoenal, mesenteric and epidydimal fat pad weights.
Visceral WAT weight indicates the sum of the retroperitoenal, mesenteric and epidydimal fat pad weights.
Total muscle weight indicates the sum of the soleus, EDL and gastocnemius muscle weight.
HFS, high-fat-sucrose; WAT, white adipose tissue; EDL, extensor digitorum longus.
$^{*}P < 0.05$; [b] $^{**}P < 0.01$; [c] $^{\#}P < 0.1$.

this was considered as an important issue for the identification of potential anti-obesity polyphenolic plant extracts. Notably, apple and cinnamon were the only plant extracts that reduced total and visceral WAT weight increase in Wistar rats. It is well known that a key issue in the prevention of obesity is to impede diet-induced body fat accumulation. Furthermore, fat depot-specific effects of these plant extracts revealed that apple anti-adiposity effects were more pronounced in epididymal and retroperitoneal fat depots (both visceral fat pads) whereas cinnamon effects were more important at the subcutaneous level.

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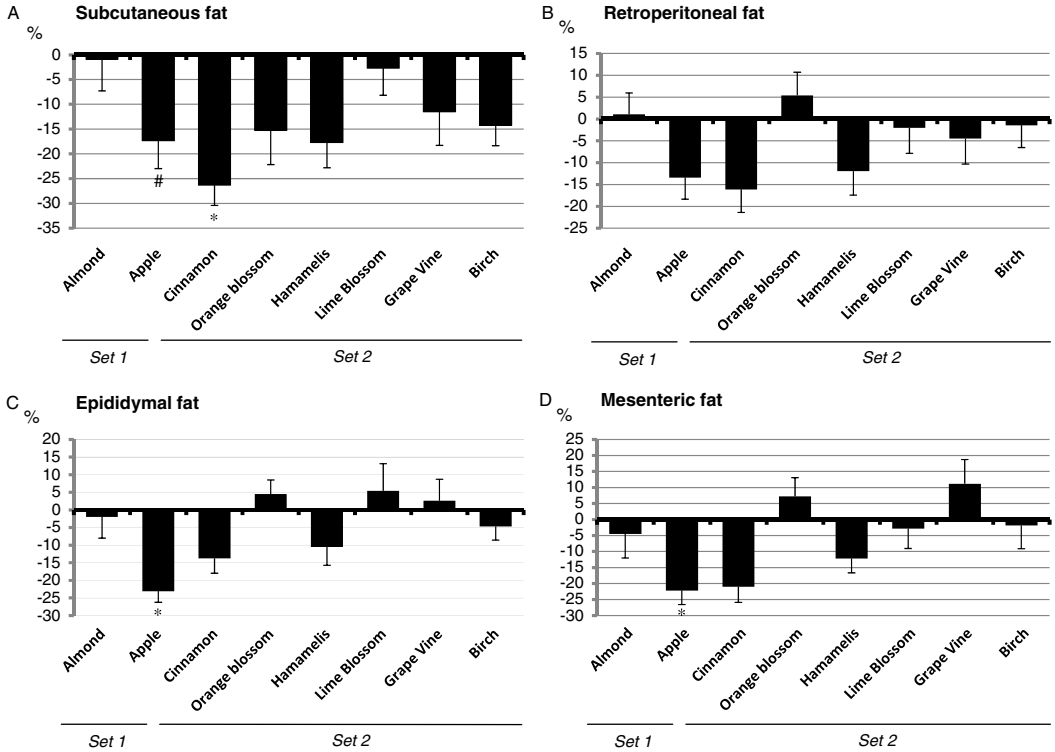


Figure 1. Percentage increase/decrease of fat depot weights (g kg^{-1} body weight) from male Wistar rats fed a high-fat-sucrose (HFS) diet supplemented with different polyphenolic plant extracts during 56–64 days compared with HFS-fed rats (considered as 0). (A) Subcutaneous fat depot; (B) retroperitoneal fat depot; (C) epipidymal fat depot; (D) mesenteric fat depot. $^{*}P < 0.05$; $^{\#}P < 0.1$.

Table 4. Biochemical measurements of male Wistar rats fed with HFS diet with or without supplementation of different plant extracts[a]

	Group (HFS + plant extract), Experimental Set 1		
Biochemical measurement	Control ($n = 12$)	HFS ($n = 12$)	Apple ($n = 12$)
Glucose (mmol L^{-1})	$5.45 \pm 0.23^{*}$	6.36 ± 0.28	$5.50 \pm 0.22^{*}$
Total cholesterol (mg dL^{-1})	64.0 ± 6.44	74.3 ± 3.54	$63.5 \pm 3.67^{*}$
Triglycerides (mg dL^{-1})	136.3 ± 15.0	100.6 ± 5.63	82.5 ± 4.91
Leptin (ng mL^{-1})	$7.59 \pm 0.99^{**}$	16.0 ± 1.38	$11.8 \pm 1.27^{\#}$
Insulin (μU mL^{-1})	$9.12 \pm 2.28^{**}$	22.9 ± 3.69	$14.6 \pm 2.60^{*}$
HOMA-IR	$2.18 \pm 0.55^{**}$	6.65 ± 1.19	$3.45 \pm 0.60^{*}$

[a] All the results are expressed as the mean ± SE.
Statistical analyses were performed using the ANOVA test and Dunnet's test post-hoc.
HFS, high-fat-sucrose; HOMA-IR, homeostatic model assessment of insulin resistance.
$^{*}P < 0.05$; [b] $^{**}P < 0.01$; [c] $^{\#}P < 0.1$.

This finding has to be considered, inasmuch as obesity-associated complications are particularly related to visceral adipose tissue, which is more metabolically active and produces larger amounts of adipocytokines.[26] Moreover, the anti-adipogenic effects of apple polyphenols were previously described in a chow-fed rat model, although there were no changes in body weight gain.[27]

On the other hand, birch extract supplementation led to higher hepatic mass. Surprisingly, this was not associated with an increase in liver fat deposition. Other polyphenolic plant extracts with anti-diabetic and anti-obesity properties, such as *Salacia obolonga*, have been found to induce hepatic hypertrophy in male rats via the peroxisome proliferator-activated receptor-α pathway, without increasing hepatic lipid accumulation and transaminase activities.[28] Furthermore, lack of muscle mass loss in polyphenol-supplemented groups is an important consideration, as sometimes lower body weight is due to reduced lean mass instead of reduced body fat.[29] Accordingly, results from this preliminary screening revealed that, of the eight polyphenolic extracts tested, apple, cinnamon, hamamelis and birch presented significant properties against the obesogenic effects of HFS intake.

With the purpose of determining the polyphenolic plant extracts that could enhance the biochemical profile aggravated by HFS feeding, biochemical measurements were analysed in these extracts that were selected as the most promising anti-obesity extracts in the preliminary screening. Reduction in HOMA-IR, an insulin resistance index, in the four analysed groups showed that apple, cinnamon, hamamelis and birch polyphenols could improve the insulin resistance state associated with obesity. These findings agree with the results presented in several articles[30,31] pointing out that different plant polyphenolic extracts present

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Table 5. Biochemical measurements of male Wistar rats fed with HFS diet with or without supplementation of different plant extracts[a]

	Group (HFS + plant extract), Experimental Set 2				
Biochemical parameter	Control ($n = 12$)	HFS ($n = 12$)	Cinnamon ($n = 12$)	Hamamelis ($n = 12$)	Birch ($n = 12$)
Glucose (mmol L^{-1})	$6.61 \pm 0.21^{*}$	7.45 ± 0.25	7.39 ± 0.35	6.88 ± 0.28	7.21 ± 0.22
Total cholesterol (mg dL^{-1})	66.7 ± 3.68	64.2 ± 2.26	61.8 ± 3.22	64.0 ± 3.08	$78.1 \pm 1.98^{*}$
Triglycerides (mg dL^{-1})	119.8 ± 10.4	102.3 ± 3.59	112.2 ± 15.3	96.3 ± 4.87	120.3 ± 12.8
Leptin (ng mL^{-1})	$6.37 \pm 0.83^{**}$	17.4 ± 1.80	13.5 ± 2.27	12.5 ± 1.25	15.4 ± 1.67
Insulin (μU mL^{-1})	$9.47 \pm 1.31^{**}$	27.9 ± 2.67	20.3 ± 4.01	$15.2 \pm 2.23^{**}$	19.8 ± 3.69
HOMA-IR	$2.84 \pm 0.44^{**}$	8.12 ± 0.64	$5.42 \pm 0.63^{*}$	$4.61 \pm 0.66^{**}$	$5.45 \pm 0.84^{*}$

[a] All the results are expressed as the mean ± SE.
Statistical analyses were performed using the ANOVA test and Dunnet's test post-hoc.
HFS, high-fat-sucrose; HOMA-IR, homeostatic model assessment of insulin resistance.
[b] $^{*}P < 0.05$; $^{**}P < 0.01$.

beneficial actions on insulin resistance. However, only apple extract could prevent HFS-diet induced hyperglycaemia. In line with this glucose-lowering effect, Johnston *et al*.[32] described a substantial reduction of glycaemia in humans by naturally occurring phenolic compounds in apple juice. Although we did not find any changes in serum glucose levels of cinnamon-supplemented diet-induced obese rats, Jia *et al*.[33] reported that polyphenolic oligomers found in cinnamon reduced glucose levels in Wistar and streptozotocin-induced diabetic rats fed a standard laboratory diet. Moreover, cinnamon polyphenols have been described to reduce fasting serum glucose in subjects with type 2 diabetes or metabolic syndrome.[34]

On the other hand, prevention of HFS-diet induced hyperleptinaemia by apple extract supplementation was consistent with the reduced total WAT weight, as this adipokine is secreted in direct proportion to body fat amount.[35] Moreover, apple extract was the only one that was able to inhibit diet-induced hypercholesterolaemia. This effect has been previously shown in overweight humans supplemented with apple polyphenols[36] but it has not been shown in supplemented chow-fed animals.[27] Surprisingly, birch extract supplementation resulted in increased levels of serum cholesterol. This fact could possibly be explained by the increased hepatic mass seen in this group.

Regarding the possible mechanisms implicated on the anti-obesogenic effects of polyphenols, *in vitro* studies demonstrated that they could act as inhibitors of adipocyte differentiation,[37,38] lipogenesis,[39] lipolysis[40] or intestinal lipid absorption[41] as well as inducers of fatty acid oxidation[39] or antagonist at cannabinoid receptors.[42] Moreover, the effects of polyphenols on lipogenesis, lipid absorption and energy homeostasis have been validated *in vivo*.[14–16,43] Another mechanism that could explain their anti-obesity properties is the attenuation of inflammatory changes, evidenced in co-culture of adipocytes and macrophages.[44,45]

CONCLUSIONS

The screening performed in this work led to the identification of four polyphenolic plant extracts with potential anti-obesity properties (apple, cinnamon, hamamelis and birch extracts) as they presented some beneficial effects on different weight parameters. Even so, only apple and cinnamon extracts were finally considered as significant anti-obesogenic extracts, due to their body fat-lowering effects. Actually, apple polyphenolic extract displayed the most outstanding properties, highlighting this extract as the most promising functional food ingredient for the prevention of obesity, as reflected by the improvement of obesity-related metabolic complications, such as hyperglycaemia and hyperleptinaemia.

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Supporting Information

Supporting information may be found in the online version of this article.

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