Low doses of grape seed procyanidins reduce adiposity and improve the plasma lipid profile in hamsters

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ABSTRACT

OBJECTIVE: Procyanidins are polyphenolic compounds with beneficial effects on health in relation to cardiovascular disease and metabolic syndrome. In this study, we evaluated the potential beneficial effects of low doses of a grape seed procyanidin extract (GSPE) on body weight and fat deposition.

DESIGN: Four groups of hamsters were fed either a standard diet (STD) or a high-fat diet (HFD) for 30 days and supplemented with either GSPE at 25 mg per kg of body weight per day (STD-GSPE and HFD-GSPE groups) or vehicle (STD and HFD groups) during the last 15 days of the study.

RESULTS: A significant decrease in body weight gain was observed in both GSPE-treated animals at the end of the experiment. GSPE treatment significantly reduced the adiposity index and the weight of all the white adipose tissue depots studied (retroperitoneal (RWAT), mesenteric (MWAT), epididymal (EWAT) and inguinal (IWAT)) in both GSPE-treated groups. GSPE administration reversed the increase in plasma phospholipids induced by the HFD feeding. In the RWAT, GSPE treatment increased the mRNA

expression of genes related to β-oxidation and the glycerolipid/free fatty acid (GL/FFA) cycle, mainly in HFD-GSPE animals. In the MWAT, the effects of GSPE at the transcriptional level were not as evident as in the RWAT. Moreover, GSPE treatment induced heparin-releasable lipoprotein lipase activity in the RWAT and MWAT depots. The alterations in the lipid metabolic pathways induced by GSPE were accompanied by lower FFA levels in the plasma and decreased lipid and triglyceride accumulation in the MWAT.

CONCLUSION: The use of GSPE at low doses protects against fat accumulation and improves the plasma lipid profile in hamsters. We suggest that GSPE exerts these effects in part through the activation of both β-oxidation and the GL/FFA cycle, mainly in the RWAT.

Keywords: procyanidins; adiposity; body weight gain; β-oxidation; glycerolipid/free fatty acid cycle; plasma lipid profile

INTRODUCTION

It is well known that obesity and its derived complications, such as insulin resistance, dyslipidaemia, and cardiovascular and cerebrovascular events, represent a major health problem in developed and developing countries (reviewed in refs1, 2, 3, 4, 5). Several dietary and pharmacological strategies have been proposed for weight reduction and for ameliorating obesity-related pathologies, such as insulin resistance or metabolic syndrome (reviewed in Bray6). Among these approaches, the use of polyphenols of vegetable origin has gained interest during the past decades. Various in vitro and animal studies have shown beneficial effects of polyphenols in adipocyte-related pathologies and in dyslipidaemia-derived complications. Thus, polyphenolic compounds, such as resveratrol, epicatechin or epigallocatechingallate, and extracts rich in procyanidins obtained from different sources, such as tea, grape seed or cocoa, have been proposed as effective modulators of lipid metabolism and adiposity (reviewed in refs7, 8, 9, 10, 11, 12).

Various studies have shown that resveratrol, a polyphenol found in grapes and grape-derived beverages, protects against obesity, weight gain and dyslipidaemia.13, 14, 15, 16 Epigallocatechingallate, a catechin considered to be the main active compound in green tea, has been extensively studied in vitro and in animal models. As a result, there is currently a wide array of evidence pointing to a positive effect of this catechin against weight gain in either diet-induced or genetically obese animals. Nevertheless, different human trials have reported both positive and negative results (reviewed in refs11, 12). Therefore, it remains unclear whether the use of green tea catechins may be a useful strategy against body weight gain in humans.

Another known source of catechins, grape seed procyanidins, have shown different beneficial properties against obesity-associated alterations, such as dyslipidaemia,17, 18, 19, 20, 21, 22 inflammation23, 24 or insulin resistance,25, 26, 27 in different animal models. Nevertheless, unlike tea catechins, the effects of grape seed procyanidins on adiposity and obesity have not been extensively studied and, therefore, there are few data available on this subject. It has been shown that a Chardonnay grape seed procyanidin extract (GSPE)—at a dose of 35 mg per kg of body weight per day of catechins—protects hamsters subjected to a high-fat diet (HFD), inhibiting the increase of the abdominal fat pad.28 A grape seed and skin extract at high doses (500 mg per kg of body weight per day) protected rats against weight gain and dyslipidaemia-associated pathologies when the rats were fed an HFD for 6 weeks.29 In addition, the treatment of cafeteria-fed obese rats with a GSPE at 25 mg per kg of body weight reduced the amount of the visceral white adipose depot26 and protected against the weight gain.30 However, other authors20, 31, 22 showed that the treatment of rats with grape seed or red wine polyphenol extracts at low doses did not affect body weight. All in all, these data indicate that the beneficial effects of grape-derived polyphenols on body weight gain and adiposity are not completely defined.

In this work, we have used the Golden Syrian hamster fed with a standard diet (STD) or an HFD to study the effects on body weight and fat deposition of a GSPE at a dose (25 mg per kg of body weight per day) intended to mimic the average human consumption of procyanidins in the Spanish Mediterranean diet.[32, 33]

MATERIALS AND METHODS

Procyanidin extract

GSPE was kindly provided by Les Dérives Résiniques et Terpéniques (Dax, France). This extract contained essentially phenolic acids (1.63%), as well as monomeric (20.9%), dimeric (20.7%), trimeric (17.3%) and oligomeric (39.41%) procyanidins.

Animals

The Animal Ethics Committee of the University Rovira i Virgili (Tarragona, Spain) approved all of the procedures. The animals used were 3-month-old male Golden Syrian hamsters (Charles River Laboratories, Barcelona, Spain) weighing 130 g. The hamsters were housed singly at 22°C with a period of light/dark of 12h (lights on at 0900 h) and with free access to food and water. After an adaptation period of 4 days, the hamsters were randomly distributed into two experimental groups (n=16) and fed ad libitum with either an STD or an HFD for 15 days. At 4 days before the beginning of the treatment, the hamsters were trained to lick low-fat condensed milk (0.3 ml). Afterwards, the animals were divided into four groups (n=8) depending on the treatment received. Two groups were supplemented every day, at 0900 h, with 25 mg of GSPE per kg body weight dissolved in low-fat condensed milk (the STD-GSPE and HFD-GSPE groups). The other two groups received the same volume of low-fat condensed milk as the vehicle (the STD and HFD groups). Both treatments were administrated orally with a syringe of 1 ml in a volume of 0.3–0.4 ml. The dose of GSPE used in this study was calculated by extrapolating the mean daily intake of procyanidins in the Spanish diet to hamsters, using the formula proposed by Fuchs et al.: metabolic dose (mg per kg) = actual dose/weight^0.75 in kilograms. Thus, to obtain the metabolic doses,

we considered a daily intake of 450 mg of procyanidins per day for a 70 kg human (equivalent to a dose of 6.43 mg per kg per day) and a dose of 25 mg per kg per day for a 130 g hamster. Taking into account these data, we obtained very similar metabolic doses between humans and hamsters (19 versus 16 mg per kg). The STD (3.9 kcal g^-1) contained 10% calories from fat, whereas the HFD (4.1 kcal g^-1) contained 21% calories from fat and 0.9 g per kg cholesterol. The STD contained 1.8% calories from coconut oil, 1.2% from flaxseed oil and 7.0% from sunflower oil; the HFD contained 1.9% calories from coconut oil, 1.3% from flaxseed oil, 7.3% from sunflower oil and 10.5% from lard. The diets were prepared in pelleted form by Research Diet Services BV (Wijk bij Duurstede, The Netherlands).

On day 15 of the GSPE treatment, all of the hamsters were deprived of food for 5 h (from 0900 to 1400 h) and killed at 1400 h under anaesthesia (pentobarbital sodium, 80 mg per kg body weight). The blood was collected by cardiac puncture and the plasma was obtained by centrifugation and stored at -20°C until analysis.

The liver and the different white adipose tissue depots (retroperitoneal (RWAT), mesenteric (MWAT), epididymal (EWAT) and inguinal (IWAT)) were rapidly removed after death, weighed, frozen in liquid nitrogen and stored -70°C until RNA analysis.

Adiposity index

The adiposity was determined by an adiposity index computed for each hamster as the sum of the EWAT, IWAT, MWAT and RWAT depot weights and expressed as a percentage of the total body weight.

Gene expression analysis

MWAT and RWAT total RNA was extracted using Tripure Reagent (Roche Diagnostic, Barcelona, Spain) and purified with Qiagen RNeasy Mini Kit spin columns (Izasa, Barcelona, Spain). The cDNA was synthesised using Moloney murine leukaemia virus reverse transcriptase (Applied Biosystems, Madrid, Spain), and was subjected to quantitative reverse transcriptase-polymerase chain reaction amplification using the Power SYBR Green PCR Master Mix (Applied Biosystems) in the ABI Prism 7300 SDS Real-Time PCR system (Applied Biosystems). The primers used for the different genes are

described in Supplementary Table 1 and were obtained from Biomers.net (Ulm, Germany). The relative expression of each mRNA was calculated as a percentage of the STD group, using the 2^-ΔΔCt method with β-actin as the reference gene.

Plasma analysis

Enzymatic colorimetric kits were used for the determination of plasma total cholesterol and triglycerides (QCA, Barcelona, Spain), phospholipids (phosphatidylcholine) (Spinreact, Girona, Spain), glycerol (Sigma, Madrid, Spain) and non-esterified free fatty acids (FFA) (WAKO, Neuss, Germany). The plasma insulin and leptin levels were measured using a mouse/rat insulin ELISA kit and a rat leptin ELISA kit, respectively (Millipore, Barcelona, Spain). The adiponectin levels were assayed using a mouse/rat adiponectin ELISA kit (B-Bridge International, Cupertino, CA, USA).

R-QUICKI analysis

The insulin sensitivity was assessed by the revised quantitative insulin sensitivity check index (R-QUICKI) using the following formula: 1/(log insulin (μU/ml) + log glucose (mg/dl) + log FFA (mmol/l)).

Lipoprotein lipase activity determination

RWAT and MWAT samples were incubated with gentle shaking in 1 ml of modified Krebs-Ringer solution (pH 7.4) containing 100 mM Tris, 150 mM NaCl, 4 mM KCl, 3 mM CaCl2·2H2O, 2 mM MgSO4·7H2O, 10 g l^-1 bovine serum albumin-FFA free and 10 000 IU l^-1 heparin at 37°C for 30 min and then centrifuged at 4400 g for 10 min at room temperature. Heparin-releasable lipoprotein lipase (LPL) activity was measured in 20 μl of the diluted supernatants (1/100) with an enzyme-fluorescent kit (LPL Activity Kit; Roar Biomedical, New York, NY, USA) according to the manufacturer's recommendations. The results were expressed as nanomole of substrate released per min per mg tissue.

Total lipid content extraction and quantification

Lipids were extracted from the MWAT and RWAT (30–35 mg), liver (80 mg) and oven-dried faeces (100 mg) using the methods described in Hara and Radin and Rodriguez-Sureda and Peinado-Onsurbe and with the modifications described in Caimari et al. The percentage of lipids was determined gravimetrically. The triacylglycerol content was analysed from the lipid extracts using the method

described in Rodriguez-Sureda and Peinado-Onsurbe with an enzymatic colorimetric kit (QCA).

Dietary lipid absorption

The dietary lipid absorption was measured during the last 24h of the experimental period as the difference between the amount of lipid consumed and the amount excreted. Faeces were collected and the amount of food consumed was measured. Aliquots of the diet and faeces were oven-dried overnight, and dietary and faecal lipids were extracted as commented above.

Statistical analysis

Data are expressed as means ± s.e.m. Data were analysed by testing the main effects of diet (STD or HFD) and GSPE (or) and their interaction (diet x GSPE) using two-way analysis of variance (ANOVA). When one or both main effects were statistically significant, one-way ANOVA followed by a Tukey's test was used to determine treatment differences between means. When only the interaction between diet and GSPE was statistically significant on the two-way ANOVA model, we compute all pairwise comparisons between diets and treatments by performing a Student's t-test adjusting the multiplicity by Holm's method. All statistical analyses were performed with SPSS Statistics 18 (SPSS Inc., Chicago, IL, USA) or SAS Release 9.2 (SAS Institute Inc., Cary, NC, USA), setting the level of statistical significance at bilateral 5%.

RESULTS

Food intake and body and tissue weights

No differences were found among groups in either food intake (data not shown) or body weight. However, at the end of the experiment, a significant decrease in body weight gain was observed in both GSPE-treated animals compared with the non-treated animals. Both GSPE-treated groups displayed lower adiposity indices and lower weights of all the white adipose tissue depots studied (the RWAT, MWAT, EWAT and IWAT). This effect was more evident in the RWAT than in the other adipose tissues. Thus, a significant decrease in the weight of this depot was found in the STD-GSPE group in comparison with the HFD group. Regarding the other adipose depots, the effect of the GSPE treatment was more clear in the

HFD-GSPE than in the STD-GSPE animals, in comparison with their respective non-treated controls (MWAT: STD-GSPE (9.8% lower), HFD-GSPE (18.8% lower); EWAT: STD-GSPE (8.8% lower), HFD-GSPE (16.6% lower); IWAT: STD-GSPE (10.5% lower), HFD-GSPE (16% lower)), although these differences among groups did not reach statistical significance.

Plasma parameters

The FFA levels significantly decreased in response to GSPE treatment in both the STD-GSPE and the HFD-GSPE groups, and a similar pattern was observed for the glycerol levels, although it did not reach statistical significance. Treatment with GSPE produced a significant decrease in the circulating leptin levels only in the HFD-GSPE group, in comparison with the non-treated HFD animals. The GSPE treatment reversed the increase in phospholipids induced by the HFD feeding.

Lipid levels and dietary lipid absorption

The lipid and triglyceride concentrations (mg g^-1) were decreased by GSPE only in the MWAT depot of both the STD and the HFD groups. The dietary lipid absorption was not affected by the GSPE treatment in any of the GSPE-treated groups.

Gene expression in the RWAT and MWAT

In the RWAT, the GSPE treatment increased the mRNA levels of LPL and FABPpm, involved in fatty acid uptake and transport, in both the STD and the HFD animals, being this effect more clear in the HFD-GSPE group. The gene expression of FABP4 was higher in the HFD-GSPE animals than in the other three groups analysed. In this tissue, the mRNA levels of the β-oxidation-related genes ACADVL, CPT1B and PPARα was highly induced in response to the GSPE treatment in both the STD and the HFD animals, with this effect being more evident in the HFD-GSPE group than in the STD-GSPE group for the three genes analysed. The gene expression of two key lipolytic enzymes, HSL and ATGL, was mainly increased in the HFD-GSPE animals, in comparison with the other three groups. In this tissue, the GSPE treatment also affected the mRNA expression of some lipogenic genes, increasing the mRNA levels of GPAT and DGAT2 in both the GSPE-treated groups. Moreover, one gene involved in glycerol-3-phosphate metabolism, GYK, was also found to be overexpressed in response to the GSPE treatment in

the RWAT.

In the MWAT, in contrast to the RWAT, the GSPE treatment only produced slight changes in the mRNA expression of the genes involved in the different lipid metabolic pathways analysed.

Heparin-releasable LPL activity in the RWAT and MWAT

The GSPE treatment produced a significant induction of the heparin-releasable LPL activity in the RWAT of the HFD-GSPE animals compared with the HFD group and in the MWAT of both the GSPE-treated groups.

DISCUSSION

Various studies have shown that the intake of polyphenols, derived from many components of the human diet, prevents body weight gain and fat accumulation (reviewed in refs7, 8, 9, 11, 12). However, in most of the experiments performed with animals, the body weight-related effects of polyphenols have been observed after the administration of these compounds for a long time and/or at high doses.41, 42, 43, 44, 45 In this study, we show that the administration of GSPE during a short period of time (15 days) and at low doses, comparable to the average human consumption of procyanidins32, 33 when expressed as metabolic dose, significantly reduces the body weight gain and the adiposity of hamsters fed either an STD or an HFD. Our data demonstrate for the first time that GSPE significantly decreases the weight of all of the white adipose depots studied, both visceral and subcutaneous. Interestingly, this beneficial effect of GSPE on fat accumulation became more evident in the animals that were challenged with the HFD in most of the adipose depots. Moreover, in the HFD-GSPE hamsters, treatment with GSPE also produced a significant decrease in the circulating leptin levels, which could be attributed to the reduction of the fat depots. This higher adiposity-related response to GSPE observed in the HFD animals has been previously observed for other polyphenols16 and could be understood as an adaptive mechanism addressed to counteract the alteration of energy homoeostasis produced by the intake of an HFD.

Two proposed mechanisms to explain the antiobesity effects of polyphenols are, first, their inhibitory effects on energy intake and, second, the inhibition of fat absorption.42, 45, 46, 47, 48, 49 We did not find differences either in food intake or in faecal lipid content, thus indicating that this extract did not ameliorate the fat accumulation by limiting the dietary fat absorption.

In our experiment, the GSPE treatment did not prevent the hypertriglyceridaemia and hypercholesterolaemia produced as a consequence of the HFD feeding. Several works, including those performed with the same or a similar GSPE, have reported decreases in the plasma triglycerides and/or cholesterol-related parameters.17, 18, 19, 20, 21, 22 Nevertheless, several other works have found no effects of polyphenols (reviewed in Blade et al.10). These discrepancies may rely either on the dose of polyphenols, the differences among polyphenolic extracts, the length of treatment, the diets or the mammalian models used in the different studies. Therefore, it seems unlikely that the decreased adiposity observed in our GSPE-treated animals is due to diminished circulating triglycerides. On the other hand, the GSPE treatment reversed the increase in plasma phospholipids induced by the HFD. This is the first study reporting such an effect. Further studies focused on the analysis of lipoprotein composition and on the activity of plasma proteins involved in lipid exchange among lipoproteins could be of value to elucidate the mechanism by which GSPE exerts this effect.

Evidence from various studies indicates that the consumption of polyphenols prevents fat accumulation through the activation of lipid catabolism and the inhibition of lipogenic pathways in both the liver and the adipose tissue (reviewed in refs7, 8, 9, 11, 12). With regard to grape seed extracts, most of the in vivo studies addressed to elucidate the effects of these extracts on lipid metabolism have been focused on the liver. Del Bas et al.17 performed three experiments analysing the short-term effects of GSPE (at 250 mg per kg body weight) on gene expression patterns in the livers of rats and mice. Although they did not

observe changes in the expression of genes related to fatty acid synthesis and oxidation in the livers of rats,17 in mice, the authors reported a downregulation of several hepatic lipogenic genes and an upregulation of genes involved in fatty acid oxidation.18, 19 In rats, GSPE treatment at 25 mg per kg body weight for 10 days did not change the hepatic expression of CPT1A, but counteracted the overexpression of several lipogenic genes induced by a cafeteria HFD.20, 50 To the best of our knowledge, there are scarce data about the effect of GSPE at low doses on the lipid metabolic pathways in the adipose tissue. Our results show that, in the RWAT, the GSPE treatment highly increased the mRNA levels of several key genes involved in β-oxidation (ACADVL, CPT1B and PPARα) in both the GSPE-treated groups, strongly suggesting that GSPE could prevent fat accumulation through the activation of this lipid catabolic pathway. Interestingly, the mRNA expression of some lipolytic (HSL and ATGL) and lipogenic genes (GPAT and DGAT2) was simultaneously increased in response to GSPE treatment, mainly in the animals that were challenged with the HFD. This simultaneous activation of both catabolic and anabolic lipid pathways induced by GSPE was previously reported in different in vitro experiments.51, 52, 53 In these studies, the authors showed that the treatment of 3T3-L1 adipocytes with GSPE increased the lipolytic process51 and the release of glycerol into the medium without changing their triglyceride content.52 Paradoxically, in the same cell line, GSPE also enhanced the glucose uptake for glycerolipid (GL) synthesis.53 Although the authors nicely argued that the increase in glycerol synthesis from glucose could explain how the triglyceride content of the cells could be maintained simultaneously with a greater glycerol release,53 the metabolic meaning of the apparently futile cycle induced by GSPE remained unclear (reviewed in Pinent et al.7). This cycle, named the GL/FFA cycle, refers to the cyclic process of simultaneous lipolysis and re-esterification that can take place in all cells (reviewed in Prentki and Madiraju54). Boosting the GL/FFA cycle requires the production of glycerol-3-phosphate as a substrate for fatty acid re-esterification, and it is of vital importance for reducing the release of fatty acids into the plasma, contributing, for example, to protect against the development of type 2 diabetes (reviewed in Prentki and Madiraju54). Related with this, Tordjman et al. 55 demonstrated that the thiazolidinediones, a family of antidiabetic drugs, block fatty acid release from adipose tissue by

the induction of glyceroneogenesis (through the increase of the mRNA expression and activity of phosphoenolpyruvate carboxykinase) and glycerol phosphorylation (by increasing GLYK activity and expression). In our experiment, the GSPE treatment triggered the GLYK mRNA expression in the RWAT of both STD and HFD groups. In parallel, many genes involved in the GL/FFA cycle (GPAT, DGAT2 and HSL) were found to be overexpressed in these animals, mainly in the HFD-GSPE group. These changes at mRNA level were slight (<50% increase), but followed a very similar pattern, suggesting that the cycle could be magnified. In addition to these observations, GSPE also triggered the expression of genes related to fatty acid transport and uptake (FABP4, FABPpm, LPL). This finding is consistent with the idea that an increased GL/FFA cycle needs raw material. Thus, more glycerol-3-phosphate is needed for the synthesis of triglycerides, as discussed above, but also more FFA are demanded to maintain the cycle. To supply those FFA, an increase in the expression and activity of fatty acid transporters is needed, together with enhanced LPL activity (reviewed in Prentki and Madiraju54), which is essential in the adipose tissue for the release of fatty acids associated with circulating triglyceride-rich lipoproteins.56 In fact, our results show that the LPL activity was increased by GSPE in the RWAT of the HFD animals. Altogether, these findings could point to an activation of the GL/FFA cycle by GSPE in the HFD animals. Nevertheless, in the STD-GSPE animals, the activation of the GL/FFA cycle at the transcriptional level was not as evident. Interestingly, the GSPE treatment clearly decreased the FFA levels and trended to decrease the glycerol levels in the plasma of both the GSPE-treated groups. All in all, these findings would suggest that, together with the increased β-oxidation programme, GSPE could drop the plasma FFA levels through the activation of the GL/FFA cycle in the RWAT, mainly in HFD hamsters.

In the MWAT, although the weight of this tissue was reduced by the GSPE treatment, we did not find any convincing evidence at the gene expression level pointing to an increase in the β-oxidation programme or in the GL/FFA cycle. Very similar results were obtained when the gene expression of EWAT was analysed (data not shown). These contrasting observations in the different white adipose tissues could

suggest that, differently to that observed in the RWAT, GSPE does not directly affect the metabolism of the MWAT and the EWAT and that the changes observed in these tissues would be an indirect consequence of the treatment with GSPE. Because previous works have shown that different adipose depots show different sensitivity to nutritional conditions or challenges,[40, 57, 58, 59] it could be speculated that GSPE affects the RWAT, the MWAT and the EWAT differently. In the MWAT, we observed a marked decrease in the lipid and triglyceride content and a clear increase of the LPL activity in those animals treated with GSPE. At first glance, this result may seem paradoxical and it is tempting to speculate that the increase in MWAT LPL activity could be a compensatory mechanism responding to the decreased adiposity of the tissue. A second hypothesis could also be proposed, which is that the changes observed in the RWAT do also take place in the MWAT and EWAT depots, but with a different timing. Thus, the MWAT and the EWAT would show the physiological final result of the processes that are ongoing in the RWAT at the end point of our experiment. One limitation of the gene expression data is the fact that they only represent the end point of the study. Further studies of gene expression performed at an earlier time point, when the changes in the size of the MWAT and EWAT depots were not so evident, could contribute to understand the mechanisms by which GSPE decreases the mesenteric and the epididymal fat masses.

In conclusion, in this study, we show that the oral administration of GSPE, at low doses and for a short period of time, has a remarkable body fat-lowering effect in hamsters by reducing the sizes of the different white adipose tissue depots. This phenomenon is associated with an improvement of the lipid profile in the plasma and with a reduction of the lipid content in the MWAT. We propose the activation of both β-oxidation and the GL/FFA cycle in the adipose tissue (mainly in the RWAT) as putative mechanisms by which GSPE exerts part of these effects. Further studies would be necessary to evaluate the potential of GSPE as a therapy to decrease the risk of developing obesity.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from ACCIO (TECCT11-1-0012) and from the European Union's Seventh Framework Programme FP7 2007-2013 under Grant agreement no. 244995 (BIOCLAIMS Project). We gratefully acknowledge the aid of Mr David Moriñas for the statistical support, and also Dr Isabel Baiges, Dr Anna Arola and Vanessa Grifoll, the laboratory technician.

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FIGURE LEGENDS

Figure 1. The evolution of body weight and body weight gain of hamsters fed with an STD or an HFD for 30 days and that received a daily oral dose of GSPE (25 mg per kg body weight) or the vehicle during the last 15 days. The body weight was recorded twice a week. The data are given as the mean ± s.e.m. (n=7–8). T, the effect of GSPE treatment (two-way ANOVA, $P<0.05$). ab Mean values with unlike letters were significantly different among groups (one-way ANOVA and Tukey's post hoc test, $P<0.05$).

Figure 2. The mRNA expression levels of genes related to fatty acid transport and uptake (a), fatty acid synthesis and β-oxidation (b), and glycerolipid synthesis and lipolysis (c) in the RWAT of hamsters fed with an STD or an HFD for 30 days and that received a daily oral dose of GSPE (25 mg per kg body weight) or the vehicle during the last 15 days. The data are given as the mean ± s.e.m. (n=7–8). D, the effect of the type of diet; T, the effect of GSPE treatment (two-way ANOVA, $P<0.05$). ab Mean values with unlike letters were significantly different among groups (one-way ANOVA and Tukey's post hoc test, $P<0.05$). ACADVL, acyl-CoA dehydrogenase, very long chain; ACC, acetyl-coenzyme A carboxylase; ATGL, adipose triglyceride lipase; CD36, fatty acid translocase, homologue of CD36; CPT1B, carnitine palmitoyltransferase IB; DGAT2, diacylglycerol acyltransferase-2; FABP4, fatty acid binding protein 4; FABPpm, fatty acid binding protein plasma membrane; FAS, fatty acid synthase; GYK, glycerol kinase; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PEPCK-C, phosphoenolpyruvate carboxykinase; PPARα, peroxisome proliferator-activated receptor α; PPARγ, peroxisome proliferator-activated receptor γ; SCD1, stearoyl-CoA desaturase-1; SREBP1-1C, sterol regulatory element-binding protein-1C.

Figure 3. The mRNA expression levels of genes related to fatty acid transport and uptake (a), fatty acid synthesis and β-oxidation (b), and glycerolipid synthesis and lipolysis (c) in the MWAT. The experimental design, the genes analysed and the statistical analysis have already been described in Figure 2.

Figure 4. Heparin-releasable LPL activity in the RWAT (a) and in the MWAT (b) of hamsters fed with an STD or an HFD for 30 days and that received a daily oral dose of GSPE (25 mg per kg body weight) or the vehicle during the last 15 days. The LPL activity is expressed as nanomole of substrate released per min per mg tissue. The data are given as the mean ± s.e.m. (n=7–8). D, the effect of the type of diet; T, the effect of GSPE treatment; D x T, the interaction of the type of diet and GSPE treatment (two-way ANOVA, $P<0.05$). ab Mean values with unlike letters were significantly different among groups (one-way ANOVA and Tukey's post hoc test, $P<0.05$). # The effect of GSPE treatment in the HFD groups (Student's t-test adjusted by Holm's method, $P<0.05$). * The effect of diet in the vehicle or GSPE-treated groups (Student's t-test adjusted by Holm's method, $P<0.05$).

TABLES

Table 1. Adipose tissue weights, adiposity index, liver weight and plasma concentrations of metabolites in hamsters fed with an STD or an HFD diet and supplemented with GSPE or vehicle

	STD	**STD-GSPE**	**HFD**	**HFD-GSPE**	**ANOVA**
Tissue weight					
RWAT (g)	2.30 ± 0.14 ab	1.97 ± 0.11 a	2.64 ± 0.10 b	2.29 ± 0.11 ab	D, T
MWAT (g)	2.95 ± 0.17 a	2.66 ± 0.19 a	3.21 ± 0.21 a	2.61 ± 0.15 a	T
EWAT (g)	3.60 ± 0.29 a	3.28 ± 0.20 a	3.90 ± 0.21 a	3.25 ± 0.17 a	T
IWAT (g)	5.40 ± 0.43 a	4.83 ± 0.16 a	5.87 ± 0.40 a	4.93 ± 0.38 a	T

Adiposity index (%)	9.39 ± 0.44 a	8.66 ± 0.36 a	10.09 ± 0.39 a	8.77 ± 0.37 a	T
Liver (g)	6.10 ± 0.31	6.18 ± 0.27	7.01 ± 0.37	6.27 ± 0.16	
Plasma parameters					
Glucose (mmol l^-1)	8.47 ± 0.84	8.51 ± 1.14	8.37 ± 0.80	8.55 ± 0.32	
Insulin (ng ml^-1)	7.06 ± 0.98	7.46 ± 0.61	7.58 ± 0.77	8.15 ± 0.77	
Leptin (ng ml^-1)	0.57 ± 0.07	0.69 ± 0.12	0.78 ± 0.10	0.39 ± 0.07 #	D x T
Adiponectin (µg ml^-1)	8.37 ± 0.78	8.90 ± 1.21	9.28 ± 1.07	6.65 ± 0.86	
Free fatty acids (mmol l^-1)	3.56 ± 0.24 a	2.21 ± 0.43 b	3.60 ± 0.63 a	2.93 ± 0.35 ab	T
Glycerol (mmol l^-1)	0.93 ± 0.06	0.73 ± 0.09	1.12 ± 0.18	0.99 ± 0.11	D
Triglycerides (mmol l^-1)	1.37 ± 0.10 a	1.36 ± 0.15 a	2.53 ± 0.28 b	2.42 ± 0.36 b	D

Phospholipids (mmol l^-1)	4.22 ± 0.13 b	4.39 ± 0.10 a	4.93 ± 0.12 b	4.23 ± 0.18 a	D, T, D x T
Total cholesterol (mmol l^-1)	5.20 ± 0.20 a	5.67 ± 0.13 ab	6.66 ± 0.28 c	6.20 ± 0.32 b	D
R-QUICKI	0.21 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	

Abbreviations: ANOVA, analysis of variance; EWAT, epididymal white adipose tissue; GSPE, grape seed procyanidin extract; HFD, high-fat diet; IWAT, inguinal white adipose tissue; MWAT, mesenteric white adipose tissue; R-QUICKI, revised quantitative insulin sensitivity check index; RWAT, retroperitoneal white adipose tissue; STD, standard diet. Hamsters were fed with an STD or an HFD for 30 days and received a daily oral dose of GSPE (25 mg per kg body weight) or the vehicle during the last 15 days. The table shows the weight of the RWAT, MWAT, EWAT and IWAT depots, adiposity index and liver weight. The weight of the adipose tissues is expressed in grams, and the adiposity index was computed as the sum of the RWAT, MWAT, EWAT and IWAT depot weights and expressed as a percentage of the total body weight. The plasma concentrations of different metabolites were determined at the end of the study after 5 h of fasting and from blood samples collected by cardiac puncture. The R-QUICKI was computed as stated in the Materials and methods section. The data are given as the mean ± s.e.m. (n=7–8). D, the effect of the type of diet; T, the effect of GSPE treatment; D x T, the interaction of the type of diet and GSPE treatment (two-way ANOVA, $P<0.05$). ab Mean values with unlike letters were significantly different among groups (one-way ANOVA and Tukey's post hoc test, $P<0.05$). # The effect of GSPE treatment in the HFD groups (Student's t-test adjusted by Holm's method, $P<0.05$).

Table 2. Lipid levels in adipose tissue, liver and faeces, and dietary lipid absorption of hamsters fed

524 **with an STD or an HFD and supplemented with GSPE or vehicle**

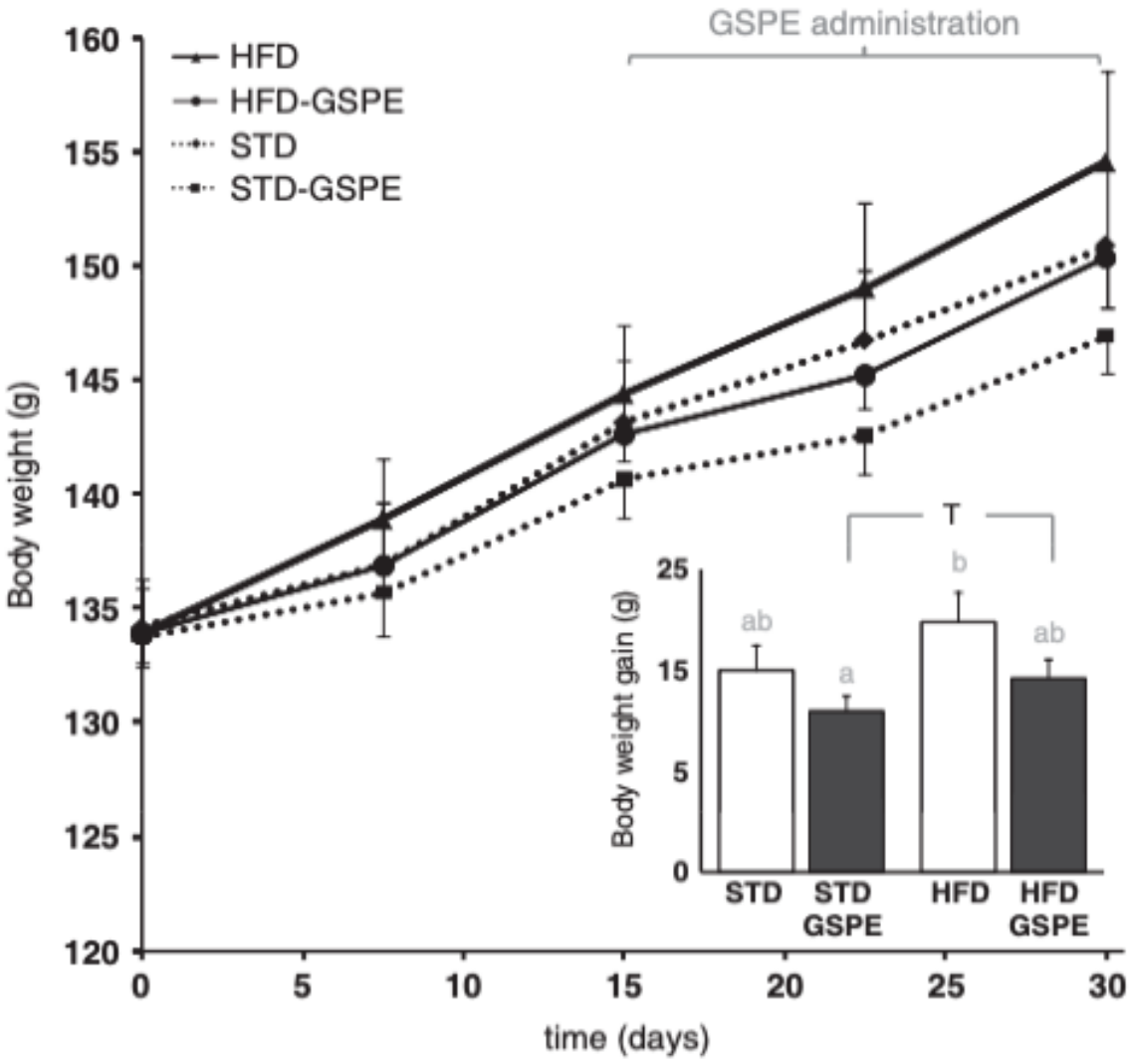
	STD	**STD-GSPE**	**HFD**	**HFD-GSPE**	**ANOVA**
RWAT					
Lipids (mg g^-1)	734 ± 28	687 ± 46	747 ± 37	764 ± 14	
Triglycerides (mg g^-1)	486 ± 29	442 ± 34	479 ± 26	502 ± 13	
MWAT					
Lipids (mg g^-1)	740 ± 16 b	689 ± 18 ab	691 ± 24 ab	659 ± 18 a	T
Triglycerides (mg g^-1)	632 ± 21 b	540 ± 40 a	675 ± 76 b	576 ± 29 a	T
Liver					
Lipids (mg g^-1)	38.23 ± 0.97 ab	37.09 ± 1.87 b	42.88 ± 2.57 ab	46.07 ± 2.37 a	D
Faeces					
Lipids (mg g^-1)	13.21 ± 1.17 ab	11.54 ± 1.42 a	16.77 ± 1.04 b	16.25 ± 1.83 ab	D

Dietary lipid absorption (mg per day)	350 ± 13 a	357 ± 20 a	627 ± 30 b	599 ± 36 b	D

Abbreviations: ANOVA, analysis of variance; GSPE, grape seed procyanidin extract; HFD, high-fat diet; MWAT, mesenteric white adipose tissue; RWAT, retroperitoneal white adipose tissue; STD, standard diet. The lipid levels in the RWAT and MWAT, liver and faeces, and dietary lipid absorption of hamsters that were fed with an STD or an HFD for 30 days and that received a daily oral dose of GSPE (25 mg per kg body weight) or the vehicle during the last 15 days. Lipid, triglyceride determinations and dietary lipid absorption were computed as stated in the Materials and methods section. The data are given as the mean ± s.e.m. (n=7–8). D, the effect of the type of diet; T, the effect of GSPE treatment (two-way ANOVA, P<0.05). ab Mean values with unlike letters were significantly different among groups (one-way ANOVA and Tukey's post hoc test, P<0.05).

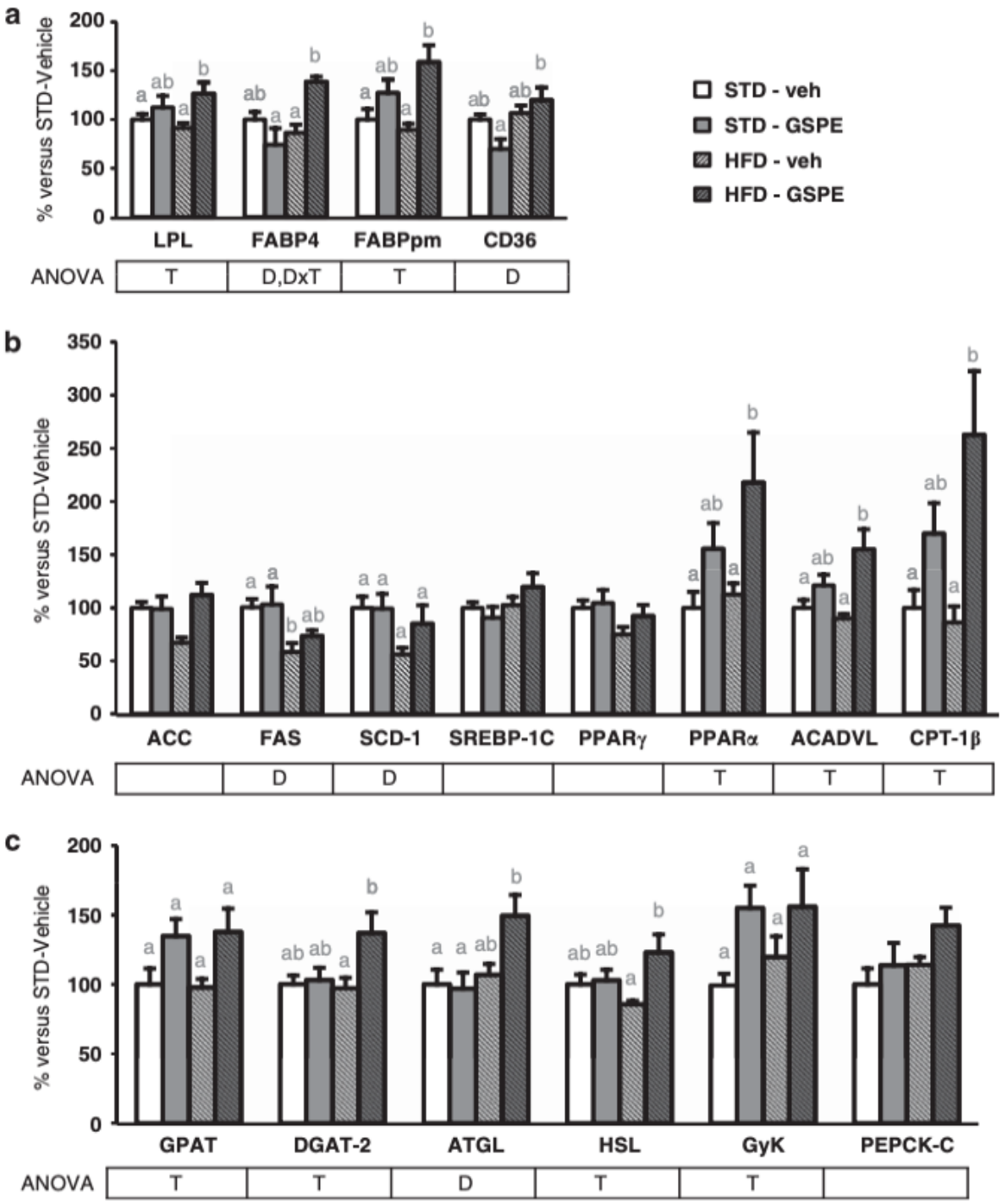
FIGURES

Figure 1



536

537 Figure 2



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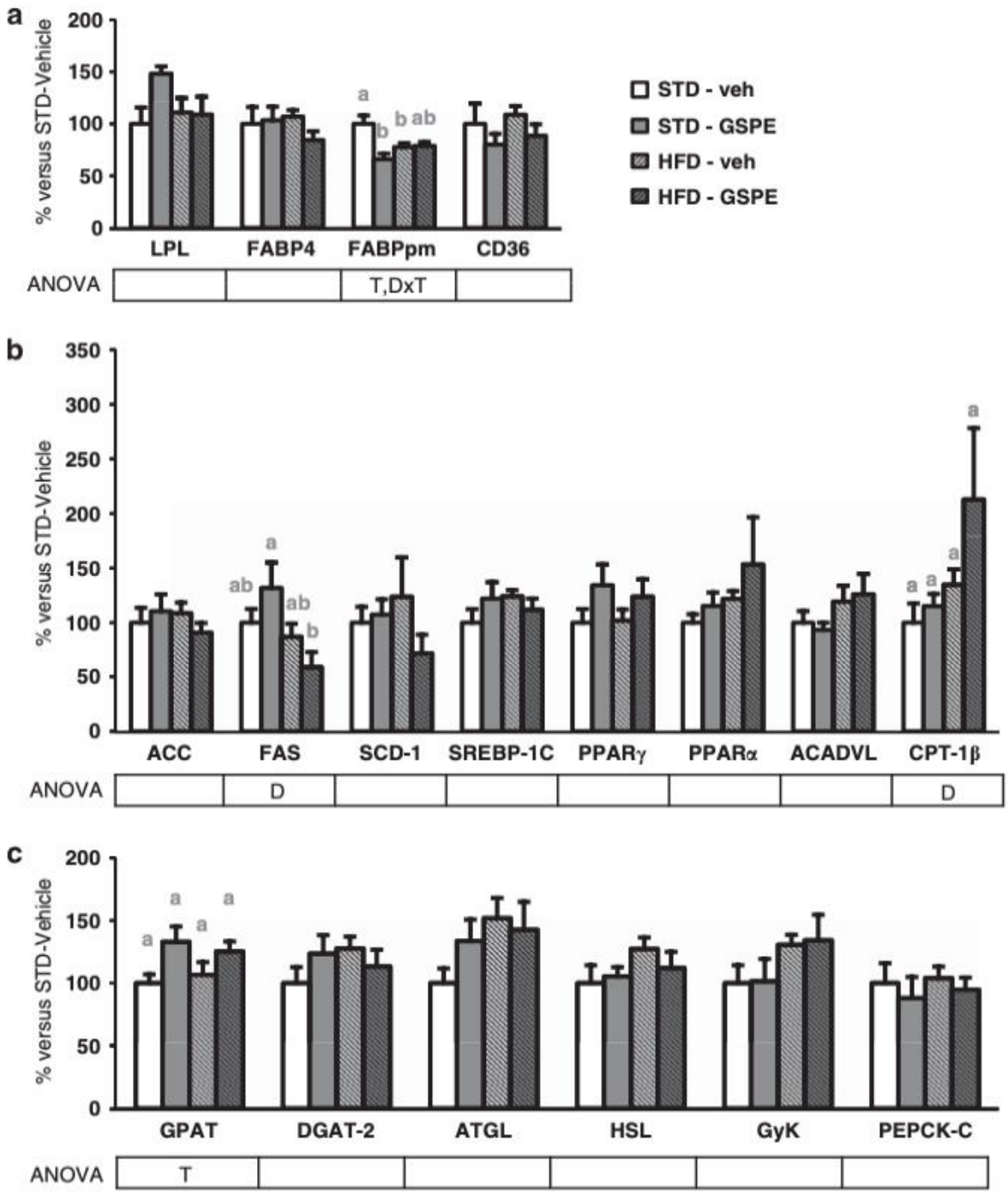
539 Figure 3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b ab
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STD - GSPE
HFD - veh
HFD - GSPE
b
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FAS
SCD-1
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PPARγ
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ACADVL
CPT-1β
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% versus STD-Vehicle
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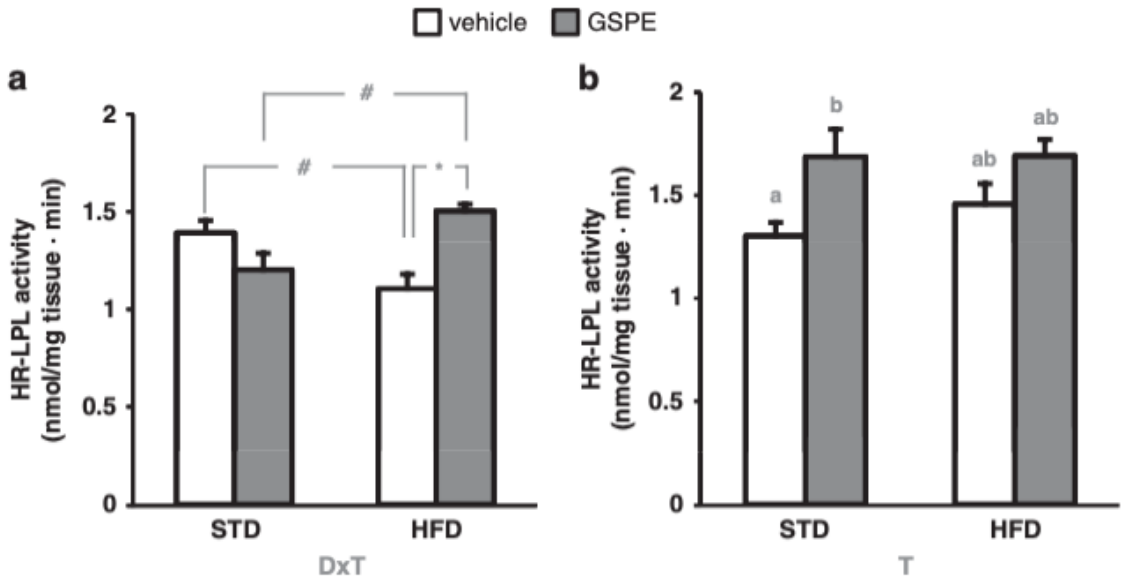
Figure 4


vehicle
GSPE
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HR-LPL activity (nmol/mg tissue · min)
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1
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STD
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STD
HFD
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542

Supplementary table 1. Nucleotide sequences of primers used for PCR amplification in retroperitoneal and mesenteric white adipose tissue depots (RWAT and MWAT).

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Ref. or Acc. No.
ACADVL	AGATGAGTGCATCCAGATAATGG	AATGTCATTTGTCCCTTCAAAGA	(1)
ACC	ACACTGGCTGGCTGGACAG	CACACAACTCCCAACATGGTG	(2)
ATGL	CACTTTAGCTCCAAGGATGA	TGGTTCAGTAGGCCATTCCT	(3)
β-ACTIN	ACGTCGACATCCGCAAAGACCTC	TGATCTCCTTCTGCATCCGGTCA	(4)
CD36	TCCCACCTTTGAGAAG	TGATGTCCAGCCGTAT	*
CPT1B	CCCACAGACCCAGGAACTT	GAAGGCGAACACAGATAGCC	AY762567.1
DGAT2	TACAAGCAGGTGATCTTTGAGG	GGGCGAAACCAATATACTTCT	(1)
FABP4	CCGAACAGAGAGCACATTCA	GACTTCCCGTCCCACTTCT	AY762570.1
FABPpm	GGAGCCAGTTGCAAAG	ACGGCTCCTGGTCAAA	*
FAS	AGCCCCTCAAGTGCACAGTG	TGCCAATGTGTTTTCCCTGA	(1)
GYK	CCTCTCTACAATGCCGTGGT	TCAATGGTCCCAAAAAGAGC	*
GPAT	GCAGACATCTGCTTCACCAA	GCAGGATGATGGGGTTTAGA	(5)
HSL	GGTGACACTCGCAGAAGACAATA	GCCGCCGTGCTGTCTCT	(3)
LPL	CAGCTGGGCCTAACTTTGAG	CCTCTCTGCAATCACACGAA	(2)
PEPCK-C	ATACCTAGCCCACTGGCTGA	TCGATCCTGTTGAACATCCA	*
PPARγ	CTCACGAAGAGCCTTCCAAC	GGATCCGGCAGTTAAGATCA	AB525757.1
PPARα	GTGGCTGCTATAATTTGCTGTG	AGCTTCGGGAAGAGAAAGGTAT	(1)
SCD1	ACATGTCTGACCTGAAAGCTGA	GTACCTCTGGAACATCAC	(1)
SREBP1C	GCGGACGCAGTCTGGG	ATGAGCTGGAGCATGTCTTCAAA	(1)

Hamsters were fed with a standard diet (STD) or a high fat diet (HFD) diet during 30 days and received a daily oral dose of GSPE (25 mg per kg body weight) or the vehicle during the last 15 days. The table shows the nucleotide sequences of primers used for PCR amplification in RWAT and MWAT. ACADVL, acyl-CoA dehydrogenase, very long chain; ACC, acetyl-Coenzyme A carboxylase; ATGL, adipose triglyceride lipase; β-ACTIN, beta-actin; CD36: fatty acid tranlocase, homolog of CD36; CPT1B, carnitine palmitoyltransferase I beta; DGAT2, diacylglycerol acyltransferase-2; FABP4: fatty acid binding protein 4; FABPpm: fatty acid bibnding protein plasma membrane; FAS, fatty acid synthase; GYK: glycerol kinase; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LPL: Lipoprotein lipase; PEPCK-C: phosphoenolpyruvate carboxykinase; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ, peroxisome proliferator-activated receptor gamma; SCD1, stearoyl-CoA desaturase-1; SREBP1-1C, sterol regulatory element-binding protein-1c. Primer pairs for PCR were designed using Primer3 software and sequence information obtained from Genbank. *No hamster sequence available. In these cases primers were designed to highly conserved regions determined by multiple sequence alignments performed on mice, rats and humans.

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