Grape seed procyanidin supplementation to rats fed a high-fat diet during pregnancy and lactation increases the body fat content and modulates the inflammatory response and the adipose tissue metabolism of the male offspring in youth

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ABSTRACT

OBJECTIVE: Procyanidins are polyphenolic bioactive compounds that exert beneficial effects against obesity and its related diseases. The aim of this study was to evaluate whether supplementation with low doses of a grape seed procyanidin extract (GSPE) to rats during pre- and postnatal periods provides biological effects to their offspring in youth.

DESIGN: The metabolic programming effect of GSPE was evaluated in the 30-day-old male offspring of four groups of rats that were fed either a standard diet (STD) or a high-fat diet (HFD) and that were supplemented with either GSPE (25 mg kg^{-1} of body weight per day) or vehicle during pregnancy and lactation.

RESULTS: Significant increases in the adiposity index and in the weights of all the white adipose tissue depots studied (retroperitoneal, mesenteric, epididymal (EWAT) and inguinal) were observed in the offspring of rats that were fed a HFD and that were treated with GSPE (HFD-GSPE group) compared with the offspring of rats that were fed the same diet but that did not receive the procyanidins (HFD group). The HFD-GSPE animals also exhibited a higher number of cells in the EWAT, a sharp decrease in the circulating levels of monocyte chemoattractant protein-1 (MCP-1) and a moderate decrease in the plasma glycerol levels. The transcriptomic analysis performed in the EWAT showed 238 genes that were differentially expressed between the HFD and the HFD-GSPE animals, most of which were associated with the immune function and the inflammatory response, in addition to genes associated with adipose tissue remodeling and function, lipid and glucose homeostasis and the metabolism of methyl groups.

CONCLUSION: The GSPE treatment in rats that were fed an HFD during pregnancy and lactation induced a clear metabolic programming effect in the offspring, increasing adiposity, decreasing the circulating levels of MCP-1 and changing the gene expression in the EWAT toward a better inflammatory

profile.

INTRODUCTION

Recently, a growing body of literature has highlighted the relevance of maternal nutrition with regard to the health outcome of the offspring. Thus, dietary habits during pregnancy and lactation can influence fetuses or suckling newborns, leading to changes in the metabolic program with consequences in the health and the metabolism of the offspring even in later life.[1] To date, dietary patterns have shown a wide array of metabolic programming effects. For example, a high-fat diet (HFD) or being overweight has been associated with increased susceptibility to hypertension,[2] changes in the expression of hypothalamic genes related to feeding control,[3] expression of brain inflammation markers[4] or changes in the glycemic control of the offspring at different stages of life.[5] In contrast, moderate maternal caloric restriction during lactation has been shown to be protective against the development of obesity and/or insulin resistance in later life of the offspring fed with an HFD.[6]

Together with macronutrient composition, other dietary compounds, such as polyphenols, have been shown to interact with components of the DNA methylation machinery[7] and have been shown to reach the fetuses and placentas of pregnant rats.[8] Taken together, these properties suggest that polyphenols are bioactive compounds that are able to induce metabolic programming changes, some of them by DNA methylation-dependent mechanisms. Indeed, different studies have described the effects of maternal polyphenol intake in the offspring. For example, the adult offspring of dams fed an HFD presented increased susceptibility to obesity, hypertension and insulin resistance compared with the adult offspring of dams fed the same diet but supplemented with a grape skin extract that was rich in polyphenols.[9] Maternal Azuki bean polyphenol consumption has shown metabolic programming effects, promoting AMPK phosphorylation in the livers of the offspring.[10] Finally, the administration of quercetin to HFD-fed dams during gestation and lactation was shown to result in a reduced body weight gain of the offspring together with improved insulin sensitivity and lipid metabolism.[11]

Among polyphenols, the procyanidins, a family of flavonoids, have shown various beneficial properties against changes in lipid metabolism and inflammation. Thus, grape seed procyanidins might protect against dyslipidemia and atherosclerosis[12] by interacting with intracellular signaling pathways in tissues such as the liver.[13],[14] Together with these effects, it has been reported that procyanidins can lower adiposity gain and can modulate the expression of key genes that are involved in fatty acid and triglyceride homeostasis in the adipose tissue both in vivo[15] and in vitro.[16] Moreover, the actions of procyanidins in the adipose tissue can be extended to the pro-inflammatory status associated with obesity. In Zucker rats that were fed an HFD, procyanidin treatment reduced the expression of the inflammatory cytokines c-reactive protein (crp), interleukin-6 (il-6) and tumor necrosis factor alpha (tnf-a) in the mesenteric adipose tissue (MWAT) while enhancing the production of adiponectin at the transcriptional level. These effects were translated into a decreased level of plasma CRP, which correlated with its expression in the MWAT.[17] In a separate study,[18] procyanidins were effective in the prevention of the inflammation associated with the consumption of a cafeteria diet. Again, the effects on the MWAT included reduced crp, tnf-a and il-6 gene expression, together with a reduction in macrophage infiltration when evaluated as the expression of emr1.[18] Other works have highlighted the anti-inflammatory properties of these compounds in human macrophages in vitro[19] and at the systemic level in rats.[20] Altogether, dietary procyanidins have been suggested as useful anti-inflammatory molecules within the context of obesity and metabolic syndrome.

Because dietary procyanidins prevent inflammation associated with obesity and modulate lipid metabolism and adipose tissue enlargement and considering that polyphenols can produce metabolic programming effects, it is plausible to speculate that the maternal intake of procyanidins could exert healthy metabolic properties to their offspring. Therefore, the aim of the present study was to evaluate the effects of a grape seed procyanidin extract (GSPE) that was administered to dams fed either an HFD or a standard chow diet (STD) during pregnancy and lactation on the metabolism of their offspring in youth.

MATERIALS AND METHODS

Procyanidin extract

The composition of GSPE, kindly provided by Les Dérives Résiniques et Terpéniques (Dax, France), was previously described by Arola-Arnal et al.[8]

Animals

The Animal Ethics Committee of the University Rovira i Virgili (Tarragona, Spain) approved all of the procedures. All the animals were housed at 22°C with a light/dark cycle of 12 h (lights on at 0900 h) and were given free access to food and water.

Thirty-two 11-week-old female virgin rats (Charles River Laboratories, Barcelona, Spain) were mated with males until impregnation, which was confirmed by the presence of sperm in their vaginal smears and which was designed as day 0 of pregnancy. Afterwards, the rats were housed individually and were divided into four groups (n = 8) depending on the diet and the treatment received during pregnancy and lactation. Two groups were fed with an STD (2.99 kcal g^{-1}; A04, Panlab, Barcelona, Spain) and were orally supplemented every day with either low-fat condensed milk (vehicle) or 25 mg of GSPE per kg body weight dissolved in low-fat condensed milk. The other two groups were fed with an HFD (5.24 kcal g^{-1}; D12492, Research Diets Inc., New Brunswick, NJ, USA) and were supplemented with either the vehicle or the 25 mg of GSPE per kg body weight. The dose of GSPE used in this study was calculated as previously described.[15] We obtained very similar metabolic doses between humans and rats (18.6 vs 17.7 mg kg^{-1}). The morning on which newborn litters were found was designated as day 0 of lactation, and the litter size was adjusted to 10 pups per dam. On day 21 of lactation, after weaning, the male pups were distributed into four experimental groups (n = 8) depending on the diet and the treatment received for their mothers: the STD group, corresponding to the offspring from the dams fed the STD and treated with the vehicle; the STD-GSPE group, corresponding to the offspring from the dams fed the STD and treated with GSPE; the HFD group, corresponding to the offspring from the dams fed the HFD and treated with the vehicle; and the HFD-GSPE group, corresponding to the offspring from the dams fed the HFD and treated with GSPE. All the experimental groups were fed the same diets as their mothers until their killing at the age of 30 days. The animals were killed in ad libitum conditions under anesthesia (pentobarbital sodium, 50 mg kg^{-1} 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), MWAT, epididymal (EWAT) and inguinal (IWAT)) were rapidly removed after death, weighed, frozen in liquid nitrogen and stored at -70°C until RNA analysis.

Adiposity index

The adiposity index was computed as previously described.[15]

Plasma analysis

Enzymatic colorimetric kits were used for the determination of glucose, plasma total cholesterol and triglycerides (QCA, Barcelona, Spain), phospholipids (phosphatidylcholine) (Spinreact, Girona, Spain), glycerol (Sigma, Madrid, Spain) and non-esterified free fatty acids (WAKO, Neuss, Germany). The plasma insulin, leptin, adiponectin and monocyte chemoattractant protein-1 (MCP-1) levels were measured using a mouse/rat insulin ELISA kit (Millipore, Barcelona, Spain), a rat leptin ELISA kit (Millipore), a rat adiponectin ELISA kit (Millipore) and a rat MCP-1 ELISA Kit (Thermo Scientific, Rockford, IL, USA), respectively.

Total DNA content and cell number and weight estimation

The DNA was isolated from the EWAT using the QiamP DNA Mini KIT (Izasa, Barcelona, Spain) and was quantified on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The estimations of the number and the weight of cells in the EWAT were calculated with the formulas of Enesco and Leblond.[21]

Total RNA isolation

EWAT total RNA was extracted as described previously.[15] The RNA yield was quantified on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies), and its integrity was measured on an Agilent 2100 Bioanalyzer using RNA 6000 Nano Chips (Agilent Technologies, South Queensferry, UK).

Microarray analysis

The microarray study was performed with the EWAT RNA samples of rats from the HFD and the HFD-GSPE groups (n = 8 animals each).

A total of 600 ng of Cy3-labeled cRNA was hybridized onto an 8x60 K SurePrint G3 Rat Gene Expression Microarray (ID 028279) (Agilent Technologies Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. The arrays were scanned with an Agilent Microarray Scanner, and the spot intensities were quantified using Feature Extraction 10.7 (Agilent Technologies Inc.). Quality control was performed for each microarray using both the Limma package in R from Bioconductor and Microsoft Excel. Background correction and interarray normalization were performed with the Limma package in R using normex't with offset 10 and Quantiles, respectively. The data were deposited into NCBI's Gene Expression Omnibus[22] and are accessible through GEO Series accession number GSE57005. Statistical analysis was performed with the non-parametric RANKPROD package in R. To control for false positives, we performed the Benjamini-Hochberg false discovery rate (FDR) test. Differentially expressed genes were defined by an FDR-derived P-value of < 0.05. Fold change values were calculated in Microsoft Excel. Fold change equals HFD-GSPE/HFD ratio in the case of increase or equals -1/ratio in the case of decrease. For the integration, visualization and analysis of the biological networks in the data set, we used the Cytoscape v3.0 plugins ClueGO[23] and CluePedia.[24] Since only about 40% of the genes on the whole genome array were recognized in this pathway program, we supplemented the significantly enriched biological processes with non-annotated genes from the selected gene-set using the scientific literature from biological databases (NCBI, IHOP). As processes overlap, we bundled some processes and renamed them.

Gene expression analysis

The cDNA was synthesized using MuLV reverse transcriptase (Applied Biosystems, Madrid, Spain) and was subjected to quantitative PCR (Q-PCR) using the LightCycler 480 II System with SYBR Green Master Mix (Roche Diagnostic, Barcelona, Spain). The primers 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 HFD group using the 2-ΔΔCt method[25] with Hprt, Ppia and Rplp0 as reference genes.

MCP-1 levels in adipose tissue

The MCP-1 protein content was quantified in the different white adipose tissue depots (EWAT, IWAT, MWAT and RWAT) according to the method described in Yu et al.[26] The levels of MCP-1 in the homogenate were measured using the rat MCP-1 ELISA Kit (Thermo Scientific). The amount of MCP-1 was normalized for protein content, which was determined using the Bradford protein assay.[27]

Total lipid content extraction and quantification

The total lipids were extracted from the EWAT (30–35 mg) using the methods described in Caimari et al.[15] The percentage of lipids was determined gravimetrically.

Statistical analysis

The data are expressed as means ± s.e.m. The differences between the groups were analyzed using two- or one-way ANOVA or Student's t-test using the statistical software SPSS Statistics 18 (SPSS Inc., Chicago, IL, USA), setting the level of statistical significance at bilateral 5%.

RESULTS

Body weight, tissue weights and plasma parameters

The HFD-GSPE animals displayed significantly higher adiposity indices and higher weights of the different white adipose tissue depots than the HFD animals, and the EWAT was the depot that showed the greatest enlargement (EWAT: 46.9% greater; RWAT: 30.4% greater; IWAT: 28.6% greater; MWAT: 21.1% greater). In addition, the plasma levels of MCP-1 and glycerol significantly decreased in the HFD-GSPE animals compared with those in the HFD group. These effects of GSPE were not observed in the STD animals. The intake of the HFD altered most of the biometric and plasma parameters similarly to what has previously been described.[28] However, the HFD produced a decrease in the circulating triglycerides, which could be explained as the result of a combination of a reduced very low-density lipoprotein production rate and an increase in triacylglycerol removal from the blood, and in the insulin plasma levels, which could be attributed to the much lower amount of carbohydrates provided by the HFD compared with the STD.[29]

DNA and lipid content in EWAT

The HFD-GSPE animals showed higher total lipid contents and total DNA amounts in the EWAT than those in the HFD animals. Moreover, there was a significant increase in the number of cells in the EWAT of the HFD-GSPE animals compared with that of the HFD group, suggesting increased hyperplasia. The weight of the cells and the ratio mg lipids per mg DNA, both used as indicators of hypertrophy, were slightly increased in the STD-GSPE and in the HFD-GSPE groups compared with their respective controls, although these differences among the groups did not reach statistical significance.

MCP-1 levels in adipose tissue

No changes were found in the MCP-1 content between the HFD-GSPE and the HFD groups in any of the adipose depots that were analyzed (Supplementary Table 2).

Differential gene expression and pathway analysis

A total of 238 unique genes were significantly changed ($P < 0.05$) in the EWAT of the HFD-GSPE animals compared with that of the HFD animals, most of which (81.9%) were upregulated.

Of these 238 genes, 38 were unknown, and they therefore could not be grouped into any pathway. The 200 remaining known genes were distributed into different metabolic pathways. The most significantly affected pathway covered an entire range of immune and defense processes, including cytokine-, chemokine- and complement-mediated immunity, response to stress, acute phase response, angiogenesis, wound healing and blood coagulation. Other frequently classified processes included cytoskeleton and cellular networks, lipid metabolism, control of adipose tissue mass and function, the metabolism of folate, polyamines and methyl groups and glucose and insulin homeostasis.

[HERE WOULD BE FIGURE 1]

The most representative genes of the different pathways regulated by the metabolic imprinting effect of the GSPE are shown in Table 3. The patterns of expression of many of these genes are analyzed in the Discussion section. In addition, the top 10 genes upregulated and downregulated in the HFD-GSPE group are presented as Supplementary information (Supplementary Table 3). Most of these top regulated genes are involved in immune and defense processes and are also included in Table 3.

Microarray data validation by Q-PCR

Q-PCR fully confirmed the microarray data, confirming the expression profiles of all the genes and obtaining very similar log fold change (FC) values to what was obtained with the microarray analysis. The P-value in the Q-PCR analysis reached significance ($P < 0.05$) for four out of the five analyzed genes. Nr4a3 had a P-value of < 0.1. The mcp1 mRNA levels in the EWAT were also measured by Q-PCR and, as happened with the microarray analysis, no significant changes were obtained.

DISCUSSION

In the present study, we show that the 30-day-old male offspring of rats fed with an HFD and that were supplemented with low doses of GSPE during pregnancy and lactation displayed increased weights of different white adipose tissue depots than the offspring of dams that were fed the same diet but that did not receive polyphenols. This increase in the fat mass content observed in the HFD-GSPE animals appeared mainly due to hyperplasia, at least in the EWAT, the depot that exhibited the highest percentage of enlargement. In contrast with our findings, Emiliano et al.[9] showed that a grape skin extract rich in anthocyanins that was administered at high doses during lactation to dams fed with an HFD prevented adipose tissue enlargement in the offspring. The two studies differ in the polyphenol composition (oligomeric procyanidins vs anthocyanins) and in the doses administered (25 vs 200 mg kg^{-1} per day), which could contribute to explain these contrasting results. However, although we cannot rule out this possibility, different studies have demonstrated the usefulness of GSPE in the prevention of weight gain and adipose tissue expansion.[15],[30] There is much evidence that shows a clear link between the prenatal and postnatal nutritional environment and adult health. Prenatal undernutrition has deleterious consequences on offspring health, increasing the propensity to develop obesity, particularly when exposed postnatally to an HFD. However, the offspring of dams submitted to moderate caloric restriction during lactation are protected against obesity and related pathologies associated with the intake of an HFD during adulthood.[6] These studies suggest that the nutritional interventions performed during pregnancy are more linked to deleterious effects in offspring in later life than those performed during lactation. Therefore, it is tempting to speculate that, in our study, the administration of GSPE during pregnancy could have contributed to provoking the adipose tissue enlargement in the HFD-fed offspring and that this effect was not observed by Emiliano et al. because the extract was exclusively administered to lactating rats. However, in the present study, energy intake of the dams during gestation and lactation was not affected by GSPE (Supplementary Figure 1). Therefore, the described effects on adipose tissue enlargement might not be attributed to maternal caloric restriction during pregnancy. In this sense, further studies focused on the metabolic programming action of GSPE administered only during lactation could be of great value.

MCP-1 is a chemokine that is strongly associated with obesity-induced inflammation and obesity-related pathologies, and it acts as a potent monocyte attractant in the adipose tissue.[31] Interestingly, we reported a clear metabolic imprinting effect of GSPE on the MCP-1 plasma levels, which sharply decreased in the HFD-GSPE animals. Although Bose et al.[32] demonstrated that treatment with epigallocatechin-3-gallate, a major tea polyphenol, decreased MCP-1 plasma levels in HFD-fed mice, to the best of our knowledge, this is the first study reporting such a metabolic programming effect of polyphenols. Therefore, our results suggest that although the HFD-GSPE animals possessed a higher adiposity compared with the HFD animals, they also possessed a better plasma inflammatory profile. However, the decrease in circulating MCP-1 levels observed in the HFD-GSPE animals cannot be explained by a lower production of this protein by the adipose tissue because no changes in its expression, either at the mRNA or at the protein levels, were observed in any of the adipose depots analyzed. This observation could be explained by a lower production of MCP-1 by other synthetizing cells, such as endothelial and liver cells.[33] Surprisingly, the imprinting effects of GSPE in adiposity and plasma glycerol and in MCP-1 levels were only found in the rats that were fed the HFD but not in the animals fed the STD. This result might indicate that the GSPE imprinting targets signaling pathways or processes that are involved in the maintenance of adipose tissue and/or lipid homeostasis. Nevertheless, further research is necessary to elucidate the cause of the diet-dependent differential response.

Our transcriptomic analysis in the EWAT of the HFD-fed offspring also shows a clear effect of maternal GSPE consumption on the mRNA expression of many genes involved in immune and inflammatory responses. For example, cxcl1, cxcl2, ccl11 and ccl3, four genes coding for pro-inflammatory chemokines that are increased in the adipose tissue[34],[35] and plasma[36],[37] during obesity, were strongly downregulated in the HFD-GSPE animals. Moreover, we found a significant overexpression of ptgs and a reduced expression of pla2g2, which are involved in the synthesis of the key anti-inflammatory mediator 15-deoxy-delta12-14-prostaglandin J2[38] and the pro-inflammatory molecule prostaglandin E2,[39] respectively, suggesting a modulation of the release of prostaglandin-derived metabolites dispatched to improve the inflammatory state in the adipose tissue. In addition, the observed overexpression of cish, which negatively regulates cytokine signaling by inhibiting JAK2/STAT5 activity,[40] and zc3h12d, which negatively regulates Toll-like receptor signaling and macrophage activation,[41] suggest that the HFD-GSPE animals possessed a lower grade of inflammation in their EWAT depots than the HFD animals. However, in contrast to the above explained results, certain genes encoding for chemokines and cytokines (ccl5, spp1), complement components (cfi, c5), procoagulant factors (f2, fgb), acute phase proteins (ashg, saa4, afp), arachidonic acid-processing enzymes (ltc4s, alox15) and response-to-stress proteins (cyp2d1, gsta2) were significantly upregulated in the HFD-GSPE animals.

One interesting finding in our study is that the GSPE treatment also produced changes in the expression of genes that are involved in angiogenesis and in the remodeling of the extracellular matrix, such as serpina3k, tmprss2 and elf3, which code for the transcription factor ESE-1, a transcriptional mediator of angiogenesis during inflammation through the induction of angiopoietin-1.[42] In addition, the mRNA levels of genes coding for cytoskeletal filaments (actb, tuba3a), intercellular junctions proteins (cldn23, gjb2, gjb6) and cell adhesion molecules (cdh1, cdh16) were also modulated in the EWAT of the HFD-GSPE animals. Interestingly, we also observed an increased expression of the gene coding for ESR1, an estrogen receptor that has been described as a key transcription factor mediating adipocyte remodeling in response to fasting through the upregulation of genes involved in cytoskeleton organization and cell matrix morphogenesis.[43] In summary, the modulation of genes involved in the different processes required for the enlargement of adipose tissue[34],[43] suggests a clear effect of maternal GSPE consumption on the morphology and structure of offspring adipocytes.

Our results also revealed a marked downregulation in the EWAT of the HFD-GSPE animals of genes coding for key transcription factors that have important roles in the adipose tissue function. For example, decreases in the mRNA levels of nr4a1, nr4a3, atf3 and egr1, which are involved in the inhibition of the differentiation of both murine[44],[45] and human[46] preadipocytes, could suggest an activation of the adipogenic program. Moreover, we found highly increased mRNA levels of the gene coding for ALDH1A1, one of the major retinoic acid-producing enzymes, which has an important role in the activation of adipogenesis.[47] In addition, the mRNA levels of sfrp4, increased in the EWAT of the HFD-GSPE animals, have been shown to be overexpressed in obese humans[48] and to activate adipogenesis.[49] However, we found a decreased expression of egr2, which codes for a transcription factor that potently stimulates adipogenesis,[44] and we could not find increased expressions of adipogenic marker genes such as pparg, cebpa and cebpβ. It is worth highlighting that the genes coding for transcription factors that were repressed in the EWAT of the HFD-GSPE animals (nr4a1, nr4a3, atf3, egr1, egr2, c-fos and junb) with the exception of egr2 are overexpressed during the obese state.[46],[50–52] Interestingly, the inhibition of the transcriptional activity of egr1 in the EWAT of BKs db/db mice has been shown to produce a decrease in the levels of TNF-a and IL-6 levels in plasma and in EWAT.[52] In humans, the fat loss is associated with a strong downregulation of nr4a1, nr4a3, c-fos and junb expression in adipose tissue.[46],[51] We also observed an overexpression of azgp1, which codes for the adipokine ZAG, which has a key role in the control of adipose tissue mass and function, most likely through its activation of the production of adiponectin.[53] ZAG mRNA and protein levels are downregulated in adipose tissue of both obese mice and humans, and inverse associations between ZAG, body fat mass and parameters related with insulin resistance have previously been described.[53] In addition, an inverse relationship between ZAG mRNA and plasma MCP-1 has also been reported, suggesting that this protein could be involved in mitigating inflammation in adipose tissue.[53] In summary, although our transcriptomics results in the EWAT do not fully support a higher activation of adipogenesis in the HFD-GSPE animals, we hypothesize that there is a better inflammatory programming of the adipose tissue in the offspring of rats that were fed with an HFD and supplemented with GSPE than in their HFD counterparts.

Maternal GSPE intake also affected the expression of genes that are involved in different pathways related to lipid homeostasis in the offspring, such as fatty acid metabolism (fabp1, acsm2a, acot5), cholesterol metabolism (pdzk1, ch25h, apoc2) and glycerol transport (aqp3, aqp9). Rodríguez et al.[54] showed that AQP9 is located in the plasma membrane of the adipocytes and that it facilitates the glycerol uptake in adipocytes for the synthesis of triglycerides.[54] The same authors also observed increased levels of AQP3 in adipocytes in response to leptin and an isopreoterenol-induced translocation of AQP3 from the lipid droplets to the plasma membrane, suggesting that AQP3 promotes glycerol efflux in adipose tissue in response to lipolytic stimuli.[54] In addition, they reported positive correlations between the transcript levels of aqp3 and aqp9 and body mass index and an upregulation of AQP3 in response to insulin treatment during adipogenesis.[54] Therefore, it is tempting to speculate that the increased mRNA levels of aqp3 and aqp9 observed in the EWAT of HFD-GSPE animals could contribute to promote the glycerol uptake in adipose tissue, which would subsequently decrease the plasma glycerol levels and lead to adipose tissue expansion as observed in the HFD-GSPE animals. Moreover, the observed downregulation of the gene coding for ACOT5, involved in the conversion of medium chain acyl-CoAs to free fatty acids in peroxisomes for their eventual oxidation in mitochondria,[55] and the overexpression of npr3, which may abrogate the lipolytic effect of active atrial natriuretic peptide that contributes to fat deposition,[56] could contribute to the EWAT expansion.

Together with the above mentioned changes, the offspring of the HFD-GSPE dams also exhibited a significant modulation of the expression of genes involved in the metabolism of SAMe (S-adenosylmethionine), the major biological methyl donor.[57] For example, mat1 was upregulated, and gnmt was repressed. Both are the primary genes involved in the SAMe synthesis and degradation, respectively.[58] Separately, the levels of the genes coding for the folate receptor and choline dehydrogenase, FOLR1 and CHDH, were increased. All these changes point to enhanced SAMe production via activation of the folate/b12-dependent and independent remethylation of homocysteine pathways.[59] Though the implications of our findings are still unknown, the changes in the metabolism of SAMe, which is required by DNA methyltransferase 1 to add methyl groups onto the DNA,[57] could be related to epigenetic mechanisms underlying the effects described in the offspring of the GSPE-treated dams. Nevertheless, more research is still needed to clarify whether the effects described herein are a consequence of metabolic programing by DNA methylation-dependent or other mechanisms and, therefore, their impact on the offspring in later life.

In conclusion, we report a clear metabolic programming effect of procyanidins on the adipose tissue metabolism of the offspring of dams that were fed with an HFD and that were treated with low doses of GSPE during pregnancy and lactation. The decrease in the MCP-1 plasma levels and in the gene expression profile found in the EWAT of the HFD-GSPE animals suggests that, although these animals showed an increase in adiposity, they possessed a more favorable adipose tissue development than the HFD animals. Further studies addressed to evaluate the epigenetic mechanisms involved in the developmental programing effects of the procyanidins are warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE CAPTIONS

Figure 1. Classification into biological processes of the genes affected by the metabolic imprinting effect of GSPE. All known genes (200) expressed twofold over background and differentially expressed between the HFD-GSPE and HFD animals (FDR-derived P-value < 0.05) were selected and classified using the Cytoscape v3.0 plugins ClueGO and CluePedia, biological databases (NCBI, IHOP) and the scientific literature.

TABLES

Table 1. Biometric and plasma parameters in 30-day-old male offspring of rats that were fed a chow diet (STD) or a high-fat diet (HFD) and that were supplemented with GSPE or vehicle during the gestation and lactation periods

Biometric parameters	**STD**	**STD-GSPE**	**HFD**	**HFD-GSPE**	**P-value**
Body weight (g)	101 ± 2^{a}	101 ± 3^{a}	108 ± 3^{a,b}	112 ± 2^{b}	D
RWAT (g)	0.48 ± 0.04^{a}	0.46 ± 0.04^{a}	0.79 ± 0.08^{b}	1.03 ± 0.09^{c}	D
MWAT (g)	0.68 ± 0.05^{a}	0.62 ± 0.05^{a}	0.71 ± 0.04^{a}	0.86 ± 0.06^{b}	D, DxP
EWAT (g)	0.41 ± 0.02^{a}	0.41 ± 0.03^{a}	0.81 ± 0.07^{b}	1.19 ± 0.09^{c}	D, P, DxP
IWAT (g)	2.64 ± 0.16^{a,b}	2.49 ± 0.16^{a}	3.18 ± 0.27^{b}	4.09 ± 0.25^{c}	D, DxP
Adiposity index (%)	4.13 ± 0.18^{a}	3.93 ± 0.18^{a}	5.06 ± 0.32^{b}	6.41 ± 0.36^{c}	D, P, DxP
Liver (g)	4.58 ± 0.12^{a}	4.53 ± 0.20^{a}	4.96 ± 0.24^{a,b}	5.24 ± 0.18^{b}	D
Liver (%)	4.54 ± 0.09	4.49 ± 0.07	4.60 ± 0.13	4.69 ± 0.15	
Plasma					

parameters					
Glucose (mmol l^{-1})	6.95 ± 0.29	6.99 ± 0.21	7.18 ± 0.40	6.94 ± 0.34	
Insulin (ng ml^{-1})	1.54 ± 0.22^{a}	2.07 ± 0.28^{a}	0.74 ± 0.19^{b}	0.88 ± 0.24^{b}	D
Leptin (ng ml^{-1})	3.53 ± 0.30^{a}	4.12 ± 0.30^{a}	6.50 ± 0.40^{b}	6.01 ± 0.52^{b}	D
Adiponectin (µg ml^{-1})	25.1 ± 1.6^{a}	27.8 ± 1.4^{a}	15.7 ± 1.7^{b}	15.3 ± 1.2^{b}	D
Free fatty acids (mmol l^{-1})	0.16 ± 0.02^{a}	0.14 ± 0.01^{a}	0.32 ± 0.04^{b}	0.29 ± 0.03^{b}	D
Glycerol (mmol l^{-1})	0.065 ± 0.002^{a}	0.060 ± 0.003^{a}	0.12 ± 0.02^{b}	0.092 ± 0.007^{c}	D
Triglycerides (mmol l^{-1})	0.86 ± 0.06^{a}	0.88 ± 0.06^{a}	0.69 ± 0.07^{b}	0.57 ± 0.02^{b}	D
Phospholipids (mmol l^{-1})	2.13 ± 0.11	2.15 ± 0.07	2.22 ± 0.06	2.15 ± 0.07	
Total cholesterol	2.47 ± 0.10	2.53 ± 0.11	2.74 ± 0.12	2.52 ± 0.11	

(mmol l^{-1})					
MCP-1 (ng ml^{-1})	14.8 ± 1.0[a]	15.4 ± 0.4[a]	13.4 ± 1.1[a]	9.35 ± 0.85[b]	D, DxP

The male offspring of the rats fed with chow diet (STD) or an HFD and that received a daily oral dose of grape seed procyanidin extract (GSPE) (25 mg per kg body weight) or the vehicle during the gestation and lactation periods (43 days). Weaning was conducted at 21 days of life, and afterwards the animals were maintained with the same diets of their mothers until killing (day 30). The table shows the body weights, the weights of the retroperitoneal, mesenteric, epididymal and inguinal white adipose tissue depots (RWAT, MWAT, EWAT and IWAT, respectively), the adiposity indices and the liver weights. The adiposity index was computed as the sum of the RWAT, MWAT, EWAT and IWAT depot weights and was expressed as a percentage of the total body weight. The liver (%) was calculated as the ratio of liver wet weight/body weight and was expressed as a percentage of the total body weight. The plasma concentrations of different metabolites are also shown and were determined at the end of the study under ad libitum conditions from blood samples collected by cardiac puncture. The data are expressed as the mean ± s.e.m. (n=8). D: effect of the type of diet, P: effect of metabolic programming of GSPE, DxP: the interaction of the type of diet and metabolic programming ($P < 0.05$, two-way ANOVA), [a],[b],[c] Mean values with unlike letters were significantly different among groups (one-way ANOVA and LSD post hoc comparison, $P < 0.05$).

Table 2. EWAT DNA and lipid content in 30-day-old male offspring of rats that were fed a chow diet (STD) or a high-fat diet (HFD) and that were supplemented with GSPE or vehicle during the gestation and lactation periods.

	STD	**STD-GSPE**	**HFD**	**HFD-GSPE**	**P-value**
Lipids (mg g^{-1})	584 ± 20	598 ± 24	607 ± 31	642 ± 28	
Total lipid content (mg)	240 ± 17^{a}	258 ± 29^{a}	495 ± 58^{b}	760 ± 63^{c}	D, P
DNA (mg g^{-1} tissue)	0.38 ± 0.04^{a}	0.34 ± 0.03^{a,b}	0.30 ± 0.02^{a,b}	0.27 ± 0.02^{b}	D
Total DNA (mg)	0.15 ± 0.02^{a}	0.13 ± 0.01^{a}	0.24 ± 0.02^{b}	0.32 ± 0.03^{b}	D, DxP
Number of cells (millions)	24.7 ± 2.4^{a}	21.7 ± 2.0^{a}	38.3 ± 2.4^{b}	51.0 ± 4.5^{c}	D, DxP
Weight per nucleus (ng)	17.3 ± 1.4^{a}	19.3 ± 1.5^{a}	21.2 ± 1.6^{a,b}	24.2 ± 2.1^{b}	D
mg lipids per mg DNA	1625 ± 137	2068 ± 314	2084 ± 202	2521 ± 267	

The lipid levels and DNA content in the epididymal white adipose tissue depot (EWAT) of the male offspring of rats that were fed a chow diet (STD) or an HFD and that received a daily oral dose of grape seed procyanidin extract (GSPE) (25 mg per kg body weight) or the vehicle during the gestation and lactation periods (43 days). Weaning was conducted at 21 days of life, and afterwards the animals were maintained with the same diets of their mothers until killing (day 30). Lipid and DNA determinations were computed as stated in the Materials and methods section. The data are expressed as the mean ± s.e.m. (n=8). D: effect of the type of diet, P: effect of metabolic programming of GSPE, DxP: the interaction of the type of diet and metabolic programming (P < 0.05, two-way ANOVA), [a],[b] Mean values with unlike letters were significantly different among groups (one-way ANOVA and LSD post hoc comparison, P < 0.05).

Table 3. Functional categorization of differentially expressed genes in the EWAT of HFD-GSPE rats

Biological process	**Specific pathway**	**Gene name**	**Gene symbol**	**Sequence ID**	**FC**	**P-value**
Immune and defense	**Cytokine/ chemokine Mediated immunity**	Chemokine (C-C motif) ligand 3	Ccl3	NM_013025	-1.56	0.0097
		Chemokine (C-C motif) ligand 5	Ccl5	NM_031116	+1.41	0.0367
		Chemokine (C-C motif)	Ccl11	NM_019205	-1.74	0.0368

		ligand 11				
		Chemokine (C-X-C motif) ligand 1	Cxcl1	NM_030845	-3.75	0.0000
		Chemokine (C-X-C motif) ligand 2	Cxcl2	NM_053647	-2.31	0.0000
		Cytokine inducible SH2-containing protein	Cish	NM_031804	+1.35	0.0423
		Secreted phosphoprotein 1	Spp1	NM_012881	+1.43	0.0352
	Eicosanoid pathway mediated inflamma	Arachidonate 15-lipoxygenase	Alox15	NM_031010	+1.45	0.0129

	tion					
		Leukotriene C4 synthase	Ltc4s	NM_053639	+1.53	0.0036
		Phospholipase A2, group IIA	Pla2g2a	NM_031598	-1.50	0.0289
		Prostaglandin D2 synthase	Ptgds	NM_013015	+2.71	0.0000
	Complement mediated immunity	Complement component 5	C5	XM_345342	+1.94	0.0026
		Complement factor I	Cfi	NM_024157	+1.72	0.0096
	Response to stress	Cytochrome P450, family 2, subfamily d, polypeptid	Cyp2d1	NM_153313	+2.96	0.0000

		e 1				
		Glutathione S-transferase A2	Gsta2	NM_017013	+1.52	0.0214
		Heat shock 70kD protein 1B	Hspa1b	NM_212504	-2.89	0.0000
	Angiogenesis, wound healing and blood coagulation	Coagulation factor II	F2	NM_022924	+1.81	0.0011
		E74-like factor 3	Elf3	NM_001024768	+1.78	0.0133
		Fibrinogen beta chain	Fgb	NM_020071	+1.85	0.0014
		Serine (or cysteine)	Serpina3k	NM_012657	+1.52	0.0125

		peptidase inhibitor, clade A, member 3K				
		Transmembrane protease, serine 2	Tmprss2	NM_130424	+1.93	0.0036
	Acute phase response	Alpha-fetoprotein	Afp	NM_012493	+3.02	0.0000
		Alpha-2-HS-glycoprotein	Ahsg	NM_012898	+1.67	0.0063
		Serum amyloid A4, constitutive	Saa4	NM_001009478	+1.57	0.0075
Cytoskele	**Regulatio**	Caspase	Card9	NM_0223	+1.44	0.0241

ton and cellular network	**n**	recruitment domain family, member 9		03		
		G protein-coupled receptor 35	Gpr35	NM_001037359	+1.54	0.0034
		Zinc finger CCCH type containing 12D	Zc3h12d	NM_001107469	+1.39	0.0285
	Cytoskeletal filaments	Actin, beta	Actb	NM_031144	-3.26	0.0375
		Tubulin, alpha 3A	Tuba3a	NM_001040008	+1.59	0.0210
	Intercellular junctions	Claudin 23	Cldn23	NM_001033062	-2.00	0.0001

		Gap junction protein, beta 2	Gjb2	NM_001004099	+2.31	0.0012
		Gap junction protein, beta 6	Gjb6	NM_053388	+1.51	0.0260
	Cell adhesion	Cadherin 1	Cdh1	NM_031334	+1.95	0.0025
		Cadherin 16	Cdh16	NM_001012055	+2.59	0.0000
Lipid metabolism	**Fatty acid metabolism**	Acyl-CoA synthetase medium-chain family member 2A	Acsm2a	NM_144748	+1.87	0.0148
		Acyl-CoA thioesterase 5	Acot5	NM_001079709	-3.12	0.0000

		Fatty acid binding protein 1, liver	Fabp1	NM_012556	+1.69	0.0026
		Apolipoprotein C-II	Apoc2	NM_001085352	+1.63	0.0049
	Cholesterol metabolism	Cholesterol 25-hydroxylase	Ch25h	NM_001025415	-1.56	0.0435
		PDZ domain containing 1	Pdzk1	NM_031712	+1.92	0.0198
	Glycerol transport	Aquaporin 3	Aqp3	NM_031703	+1.86	0.0146
		Aquaporin 9	Aqp9	NM_022960	+1.56	0.0042
Control of adipose tissue mass and	**Regulation**	Natriuretic peptide receptor C/guanylat	Npr3	NM_012868	+1.42	0.0165

function		e cyclase C				
	Transcription factors	Activating transcription factor 3	Atf3	NM_012912	-1.67	0.0056
		Early growth response 1	Egr1	NM_012551	-1.80	0.0000
		Early growth response 2	Egr2	NM_053633	-1.54	0.0292
		Estrogen receptor 1	Esr1	NM_012689	+2.23	0.0424
		FBJ osteosarcoma oncogene	Fos	NM_022197	-2.04	0.0000
		Jun B proto-oncogene	Junb	NM_021836	-1.88	0.0026
		Nuclear	Nr4a1	NM_0243	-1.68	0.0055

		receptor subfamily 4, group A, member 1		88		
		Nuclear receptor subfamily 4, group A, member 3	Nr4a3	NM_017352	-2.32	0.0000
	Others	Aldehyde dehydrogenase 1 family, member A1	Aldh1a1	NM_022407	+2.07	0.0039
		Alpha-2-glycoprotein 1, zinc-binding	Azgp1	NM_012826	+1.51	0.0098
		Secreted frizzled-	Sfrp4	NM_053544	+1.36	0.0382

		related protein 4				
		Transthyretin	Ttr	NM_012681	+2.22	0.0000
Metabolism of folate, polyamine and methyl groups		Choline dehydrogenase	Chdh	NM_198731	+1.49	0.0412
		Folate receptor 1 (adult)	Folr1	NM_133527	+1.88	0.0147
		Glycine N-methyltransferase	Gnmt	NM_017084	-1.72	0.0168
		Methionine adenosyltransferase	Mat1a	NM_012860	+1.49	0.0092

		1, alpha				
Glucose and insulin homeostasis		Cytochrome P450, family 2, subfamily e, polypeptide 1	Cyp2e1	NM_031543	+1.71	0.0125
		Fructose-1,6-bisphosphatase 1	Fbp1	NM_012558	+2.50	0.0000
		Growth factor receptor bound protein 14	Grb14	NM_031623	+1.41	0.0316
		Glucagon receptor	Gcgr	NM_172091	-1.59	0.0312

Abbreviations: EWAT, epididymal white adipose tissue depot; FDR, false discovery rate; GSPE, grape seed procyanidin extract; HFD, high-fat diet. FC: Fold changes in HFD-GSPE vs HFD animals. The + sign indicates upregulation, and the - sign indicates downregulation in the HFD-GSPE animals. Genes

572 with FDR-derived P-values < 0.05 from the microarray data analysis are shown.

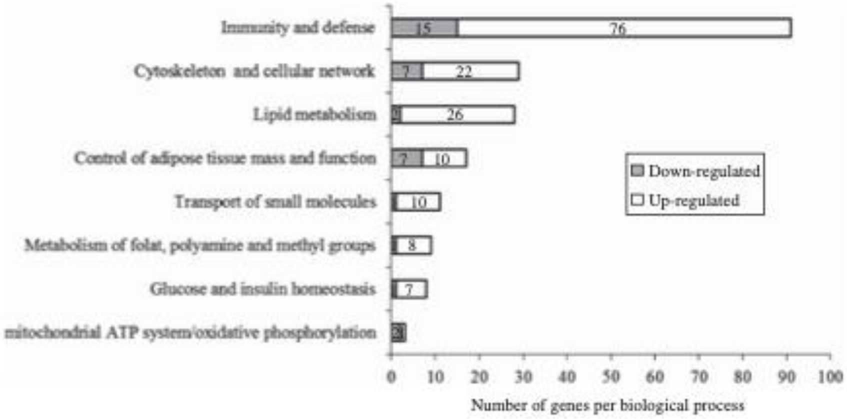
573 **Table 4. Quantitative PCR (Q-PCR) confirmation of array data**

Gene name	**Gene symbol**	**Sequence ID**	**FC array**	**FC Q-PCR**	**P-value array**	**P-value Q-PCR**	**Range of Ct values**
Aldehyde dehydrogenase 1 family, member A1	Aldh1a1	NM_022407	+2.07	+2.04	0.0039	0.0200	27.0–30.0
Chemokine (C-X-C motif) ligand 1	Cxcl1	NM_030845	-3.75	-4.33	0.0000	0.0105	26.0–32.0
Early growth response 1	Egr1	NM_012551	-1.80	-1.88	0.0000	0.0320	21.5–23.5
FBJ	Fos	NM_022	-2.04	-2.12	0.0000	0.0473	22.5–

osteosarcoma oncogene		197					26.0
Nuclear receptor subfamily 4, group A, member 3	Nr4a3	NM_017352	-2.32	-2.18	0.0000	0.0993	27.0–31.0
Monocyte chemoattractant protein-1	Mcp1	NM_031530	-1.37	-1.45	0.0871	0.1287	24.5–27.0

FC: Fold changes in HFD-GSPE vs HFD animals. The + sign indicates upregulation, and the - sign indicates downregulation in the HFD-GSPE animals. The P-values of the microarray data were all within false discovery rate (FDR)-derived P-values < 0.05. The P-value of the Q-PCR data is given (Student's t-test, P < 0.05). The ranges of the Ct values for the genes verified by Q-PCR are shown.

Figure 1



Immunity and defense
15
76
Cytoskeleton and cellular network
7
22
Lipid metabolism
2
26
Control of adipose tissue mass and function
7
10
Transport of small molecules
10
Metabolism of folat, polyamine and methyl groups
8
Glucose and insulin homeostasis
7
mitochondrial ATP system/oxidative phosphorylation
3
Down-regulated
Up-regulated
0
10
20
30
40
50
60
70
80
90
100
Number of genes per biological process

Supplementary table 1. Nucleotide sequences of primers used for Q-PCR amplification in the epidydimal white adipose tissue depot (EWAT).

Gene	**Forward primer (5' to 3')**	**Reverse primer (5' to 3')**	**Ref. or Acc. No.**
Aldh1a1	GCGATCTCCTCTCACATGGA	TCCTCAACGAAAATCCGGGA	NM_022407
Cxcl1	AGTGGCAGGGATTCACTTCA	CGCCATCGGTGCAATCTATC	NM_030845
Egr1	GAACAACCCTACGAGCACCT	TCACAAGGCCACTGACTAGG	NM_012551
Fos	CCCAAACCACGACCATGATG	AGTTGGCACTAGAGACGGAC	NM_022197
Hprt	TCCCAGCGTCGTGATTAGTGA	CCTTCATGACATCTCGAGCAAG	NM_012583
Mcp1	AGGCAGATGCAGTTAATGCCC	ACACCTGCTGCTGGTGATTCTC	NM_031530
Nr4a3	ATAGGAGCTCATCATCCGGC	TCCCGACACTGAGACACTTC	NM_031628
Ppia	CCAAACACAAATGGTTCCCAGT	ATTCCTGGACCCAAAACGCT	NM_017101
Rplp0	GGACCTCACCGAGATTAGGG	CCCACCTTGTCTCCAGTCTT	NM_022402

The table shows the nucleotide sequences of primers used for Q-PCR amplification in EWAT. *Aldh1a1*, aldehyde dehydrogenase 1 family, member A1; *Cxcl1*, chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha); *Egr1*, early growth response 1; *Fos*, FBJ osteosarcoma oncogene; *Hprt*, hypoxanthine phosphoribosyltransferase 1; *Mcp1*, monocyte chemoattractant protein-1; *Nr4a3*, nuclear receptor subfamily 4, group A, member 3; *Ppia*, peptidylprolyl isomerase A (cyclophilin A); *Rplp0*, ribosomal protein, large, P0. Primer pairs for PCR were designed using Primer3 software and sequence information obtained from Genbank.

Supplementary Table 2: MCP1 protein levels in 30-day-old male offspring of rats that were fed a high-fat diet (HFD) and that were supplemented with GSPE or vehicle during the gestation and lactation periods.

	HFD	**HFD-GSPE**
MCP1 (ng/g protein)		
RWAT	59.5 ± 3.5	70.6 ± 4.5
MWAT	86.8 ± 9.3	69.5 ± 5.5
EWAT	89.2 ± 8.2	86.3 ± 7.0
IWAT	42.8 ± 9.7	66.0 ± 14.8

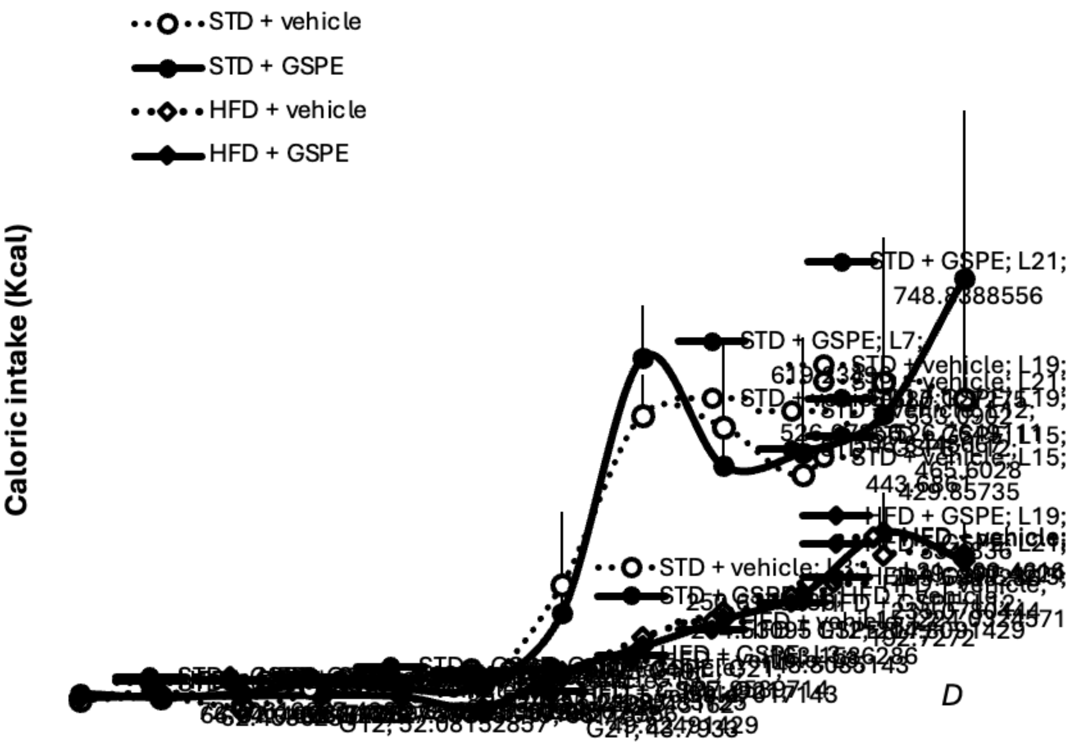
MCP1 protein levels in the retroperitoneal, mesenteric, epididymal and inguinal white adipose tissue depots (RWAT, MWAT, EWAT and IWAT, respectively). The levels of MCP-1 in the homogenate were measured using the rat MCP-1 ELISA Kit (Thermo Scientific, Rockford, USA). The amount of MCP-1 was normalized for protein content, which was determined using the Bradford protein assay, and is expressed as ng per gram of total protein. The data are expressed as the mean ± SEM (n = 8). No significant changes were found in the MCP-1 content between the HFD-GSPE and the HFD groups in any of the adipose depots that were analyzed.

Supplementary Table 3. Top ten genes differentially expressed in the EWAT of HFD-GSPE rats.

	Gene name	Gene symbol	Sequence ID	FC	P-value	Biological process	Specific pathway
Down-regulated	*Chemokine (C-X-C motif) ligand 1*	*Cxcl1*	NM_030845	- 3.75	0.0000	Immune and defense	Cytokine/chemokine mediated immunity
	Actin, beta	*Actb*	NM_031144	- 3.26	0.0375	Cytoskeleton and cellular network	Cytoskeletal filaments
	Acyl-CoA thioesterase 5	*Acot5*	NM_001079709	- 3.12	0.0000	Lipid metabolism	Fatty acid metabolism
	Heat shock 70kD protein 1B	*Hspa1b*	NM_212504	- 2.89	0.0000	Immune and defense	Response to stress
	Nuclear receptor subfamily 4, group A, member 3	*Nr4a3*	NM_017352	- 2.32	0.0000	Control of adipose tissue mass and function	Transcription factors
	Chemokine (C-X-C motif) ligand 2	*Cxcl2*	NM_053647	- 2.31	0.0000	Immune and defense	Cytokine/chemokine mediated immunity
	FBJ osteosarcoma oncogene	*Fos*	NM_022197	- 2.04	0.0000	Control of adipose tissue mass and function	Transcription factors
	Claudin 23	*Cldn23*	NM_001033062	- 2.00	0.0001	Cytoskeleton and cellular network	Intercellular junctions
	Similar to F43G9.2	*LOC681647*	XM_001057182	- 1.99	0.0159	Unknown	Unknown
	Unknown	*Unknown*	ENSRNOT00000026303	- 1.94	0.0007	Unknown	Unknown
Up-regulated	*Cytochrome P450, family 2, subfamily d, polypeptide 5*	*Cyp2d5*	NM_173304	+ 3.16	0.0000	Immune and defense	Response to stress
	RT1 class II, locus Bb	*RT1-Bb*	NM_001004084	+ 3.11	0.0018	Immune and defense	Others
	Alpha-fetoprotein	*Afp*	NM_012493	+ 3.02	0.0000	Immune and defense	Acute phase response
	Cytochrome P450, family 2, subfamily d, polypeptide 1	*Cyp2d1*	NM_153313	+ 2.96	0.0000	Immune and defense	Response to stress
	Similar to RIKEN cDNA 4930555I21	*MGC114440*	NM_001025772	+ 2.94	0.0029	Unknown	Unknown
	Prostaglandin D2 synthase	*Ptgds*	NM_013015	+ 2.71	0.0000	Immune and defense	Eicosanoid pathway mediated inflammation
	Cortexin 3	*Ctxn3*	NM_001134696	+ 2.65	0.0000	Others	Others
	Cadherin 16	*Cdh16*	NM_001012055	+ 2.59	0.0000	Cytoskeleton and cellular network	Cell adhesion
	Similar to 4930506M07Rik protein	*RGD1311558*	NM_001079705	+ 2.53	0.0073	Unknown	Unknown
	Similar to chromosome 9 open reading frame 7	*RGD1311501*	NM_001106561	+ 2.53	0.0000	Unknown	Unknown

Top ten down-regulated and up-regulated genes differentially expressed in the HFD-GSPE *vs* the HFD group. FC: Fold changes in HFD-GSPE vs. HFD animals. The + sign indicates up-regulation, and the – sign indicates down-regulation in the HFD-GSPE animals. Genes with FDR-derived p-values <0.05 from the microarray data analysis are shown.

Supplementary Figure 1



Energy intake of dams fed with the standard diet (STD) or the high fat diet (HFD) and treated with either vehicle (vehicle) or GSPE (GSPE) during gestation and lactation. G refers to gestation and L refers to lactation. To calculate the energy intake, we assumed the energy content of the STD to be 299 Kcal/100 g and that of the HFD to be 524 Kcal/100 g, as provided by the manufacturer. Energy intake is shown at days 3, 7, 12, 15, 19 and 21for both periods. The data are provided as the mean ± SEM (n = 8). *D*: the effect of the type of diet (repeated measures ANOVA, p<0.05).