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Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet

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Abstract

Objective: The main objective of this study was to evaluate the effect of procyanidin intake on the level of inflammatory mediators in rats fed a hyperlipidic diet, which are a model of low-grade inflammation as they show an altered cytokine production.
Design: Male Zucker *Fa/fa* rats were randomly grouped to receive a low-fat (LF) diet, a high-fat (HF) diet or a high-fat diet supplemented with procyanidins from grape seed (HFPE) (345 mg/kg feed) for 19 weeks and were then euthanized. We determined biochemical parameters, C-reactive protein (CRP) and IL-6 levels in plasma. Adipose tissue depots and body weight were also determined. We assessed CRP, IL-6, TNF-α and adiponectin gene expression in liver and white adipose tissue (WAT).
Results: As expected, rats fed the HF diet show an enhanced production of CRP. Our results demonstrate that the HFPE diet decreases rat plasma CRP levels but not IL-6 levels. The decrease in plasma CRP in HFPE rats is related to a down-regulation of CRP mRNA expression in the liver and mesenteric WAT. We have also shown a decrease in the expression of the proinflammatory cytokines TNF-α and IL-6 in the mesenteric WAT. In contrast, adiponectin mRNA is increased in this tissue due to the procyanidin treatment.

As previously reported, CRP plasma levels correlate positively with its expression in the mesenteric WAT, suggesting that procyanidin extract (PE) modulates CRP at the synthesis level. CRP plasma levels also correlate positively with body weight. As expected, body weight is associated with the adiposity index. Also, TNF-α expression and IL-6 expression have a strong positive correlation. In contrast, the expression of the anti-inflammatory cytokine adiponectin correlates negatively with the expression of TNF-α and IL-6 in the mesenteric WAT.
Conclusion: These results suggest a beneficial effect of PE on low-grade inflammatory diseases, which may be associated with the inhibition of the proinflammatory molecules CRP, IL-6 and TNF-α and the enhanced production of the anti-inflammatory cytokine adiponectin. These findings provide a strong impetus to explore the effects of dietary polyphenols in reducing obesity-related adipokine dysregulation to manage cardiovascular and metabolic risk factors.

Keywords: Procyanidins; IL-6; CRP; TNF-α; Adiponectin; Low-grade inflammation

1. Introduction

Procyanidins are phenolic compounds from the flavonoids group that are widely found in cereals, vegetables and fruits like grapes, berries, cocoa and apples. They have a broad range of biological activities [1]. They function as powerful antioxidants and exert anti-inflammatory activities in vitro. Recent studies have shown potent anti-inflammatory properties of procyanidins on experimental inflammation in rats and mice [2,3]. Its mechanisms of anti-inflammatory action remain poorly understood and are relevant to oxygen free radical scavenging, antilipid peroxidation, inhibition of the formation of inflammatory cytokines, alterations in cell membranes receptors, intracellular signaling pathway proteins and modulation of gene expression [4].

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Obesity is associated with a state of chronic inflammation characterized by macrophage infiltration of muscle and adipose tissue and abnormal production of proinflammatory mediators. In addition to adipocytes, adipose tissue contains fibroblasts, preadipocytes, tissue-resident macrophages and vascular constituents. Macrophages are known to be crucial contributors to inflammation, but more recently, it has been recognized that adipocytes demonstrate significant intrinsic inflammatory properties as well. Like macrophages, the adipocyte is exquisitely sensitive to infectious disease agents and cytokine-mediated inflammatory signals. In turn, these stimuli induce the expression of inflammatory mediators such as IL-6, TNF-α and SAA. Although many of these activities are restricted to autocrine and paracrine effects, some of these cytokines that are secreted from adipocytes and adipose-resident macrophages make significant contributions to systemic inflammation [5].

Adipose tissue is not usually thought of as an immune or inflammatory organ. However, the discovery of elevated secretion of these factors from obese adipose tissue provided the first evidence of a direct connection between obesity and systemic inflammation [5].

The altered production of proinflammatory molecules (so-called "adipokines") by adipose tissue has been implicated in the metabolic complications of obesity [6]. Compared with adipose tissue of lean individuals, adipose tissue of obese individuals expresses increased amounts of proinflammatory proteins such as TNF-α, IL-6, inducible nitric oxide synthase, C-reactive protein (CRP), soluble ICAM and monocyte chemotactic protein-1, as well as reduced adiponectin expression [7].

CRP is an acute-phase protein that binds specifically to phosphorylcholine as a component of microbial capsular polysaccharide and participates in the innate immune response against microorganisms. CRP is the most extensively studied marker of systemic inflammation in humans. A large number of studies have further strengthened the association of elevated CRP levels with nearly all the important cardiovascular risk factors, including insulin resistance, diabetes, metabolic syndrome, hypertension, smoking and dyslipidemia. The regulation of this protein in the liver is believed to be driven by IL-6, IL-1 and TNF-α [8] from visceral adipose tissue draining directly into the portal system that causes the obesity-associated rise of CRP production. Furthermore, in addition to liver-derived CRP, newer data show that adipose tissue itself may contribute to obesity-associated increased CRP levels [9,10].

Adiponectin, the most abundantly secreted adipocytokine from differentiated adipocytes, has potent vasculoprotective, angiogenic, anti-inflammatory and antiatherogenic properties. High adiponectin levels are associated with a reduced risk of myocardial infraction in men, while low serum adiponectin levels are reported in obese individuals and in those with hypertension, coronary artery disease and type 2 diabetes [11]. Adiponectin has inflammatory-modulating activities demonstrated in clinical studies showing inverse associations between adiponectin levels and serum markers of inflammation [12]. Although it is not clear how or whether adiponectin itself has anti-inflammatory properties, it is clear that adiponectin production by adipose can be inhibited by systemic inflammation and confers protection against the metabolic syndrome and diabetes [13,14].

TNF-α, a proinflammatory cytokine originally defined by its antitumor activity, has a strong link with obesity. Some authors have reported that adipocytes directly express TNF-α in rodents and led to the concept of a role for inflammation in obesity. These observations were paralleled by human studies showing increased TNF-α expression in the adipose tissue of individuals who were obese and decreased TNF-α expression after weight loss. Evidence supporting a key role for TNF-α in obesity-related insulin resistance came from studies showing that *ob/ob* mice (leptin-deficient mice with evidence of insulin resistance) that were also deficient for TNF-α or TNF receptors (TNFRs) had improved insulin sensitivity in diet-induced obesity compared with TNF-α- and TNFR-sufficient *ob/ob* mice [15].

IL-6, a stress-induced inflammatory cytokine, is directly implicated in atherogenesis. High levels of IL-6 are thought to be responsible for the increase in acute-phase proteins seen in obese patients, in particular, CRP [11]. Obesity-associated induction of adipose IL-6 production induces CRP secretion, and there are data that suggest that IL-6 decreases lipoprotein lipase activity, which results in increased macrophage uptake of lipids [16]. In addition, IL-6 was significantly associated with body mass index, waist circumference and visceral adiposity in obese subjects. Adipocytes and macrophages both contribute to white adipose tissue (WAT)-derived IL-6, although the ultimate stimulus for IL-6 production in the presence of high adiposity is currently unknown.

Understanding the mechanisms that lead from obesity to inflammation will have important implications for the design of the new therapies to reduce the morbidity and mortality of obesity. The main objective of the present study was to examine the putative modulatory effects of procyanidin extract (PE) on cytokine expression and CRP and IL-6 release in rats fed the high-fat (HF) diet to gain insight on the mechanisms that underlie the anti-inflammatory effects ascribed to procyanidins.

2. Materials and methods

2.1. Chemicals

Grape seed PE was provided by Les Dérives Résiniques et Terpéniques (Dax, France). According to the manufacturer, the PE contained essentially monomeric (21.3%), dimeric (17.4%), trimeric (16.3%), tetrameric (13.3%) and oligomeric (5–13 units) (31.7%) procyanidins and phenolic acids (4.7%).

2.2. Diets

Semipurified diets were obtained from Research Diets (USA). Briefly, three diets were used (Table 1). The low-fat

Table 1
Composition of the LF, HF and HFPE test diets

	Test diets		
	LF (g/kg diet)	HF (g/kg diet)	HFPE (g/kg diet)
Ingredients			
Casein	190	190	190
DL-Methionine	3	3	3
Cornstarch	498.5	215	215
Maltodextrin	35	75	75
Sucrose	290	290	290
Cellulose	30	30	30
Butter fat	14.7	44.2	44.2
Corn oil	39.3	118	118
Mineral mixture	40	40	40
Vitamin mixture	11	11	11
PE	0	0	0.32
Energy (kcal/g)	3.9	4.41	4.41
Protein (% energy)	16.8	16.8	16.8
Carbohydrate (% energy)	72.6	51.4	51.4
Fat (% energy)	10.6	31.8	31.8

(LF) diet, the hyperlipidic (HF) diet and the hyperlipidic with PE (HFPE) diet had equal protein percentage. The standard control diet was the LF diet. The HFPE diet differs from the HF diet in PE content, which was 0.32 mg of PE per gram of feed. The procyanidin dose used corresponds to the estimated amount of procyanidins that humans consume daily.

2.3. Experimental design and euthanasia

Male Zucker *Fa/fa* rats (Charles River Laboratories, Spain) were used in all studies (n=30). Rats were left 1 week in quarantine. At ~15 weeks of age, rats were randomly assigned to receive the LF (n=10), HF (n=10) or HFPE (n=10) diet ad libitum. Rats were housed in cages by pairs and subjected to a standard 12-h light:12-h dark cycle. The experimental period lasted 19 weeks. After rats were weighed, they were anesthetized by sodium pentobarbital (100 mg/kg ip) and euthanized by exsanguination after 6 h of fasting. Blood was obtained from abdominal aorta. The entire liver and adipose tissues were dissected out, weighed and snap frozen in liquid N_2 and stored at −80°C. All the procedures were performed with the approval of the ethics committee of our center and followed the laws concerning animal experimentation of the Government of Catalonia.

2.4. Measurement of adiposity, food intake and body weight gain

Body weight changes and caloric ingestion were monitored weekly during the whole experiment. Adipose tissue fat pads (mesenteric, retroperitoneal and epididymal) were excised separately and weighed. Adiposity index was calculated as total adipose tissue weight versus total body weight.

2.5. Measurement of biochemical parameters

After sacrifice, blood was collected and heparinized plasma was obtained by centrifugation. Total cholesterol levels and total plasma glucose levels were measured by enzymatic colorimetric methods (QCA S.L.). Determination of the GSH/GSSG ratio was assessed by colorimetric assay from Oxford Biomedical Research according to the manufacturer's instructions.

2.6. Measurement of CRP levels

Plasma CRP levels were quantified using a specific enzyme immunoassay (EIA) according to the manufacturer's instructions (Helica Biosystems). The assay is a double polyclonal antibody sandwich EIA.

2.7. Measurement of IL-6 and adiponectin plasma levels

Plasma IL-6 and adiponectin levels were quantified using specific EIAs according to the manufacturer's instructions (Biosource International, Inc.). The assays are based on a sandwich EIA.

2.8. mRNA analysis of CRP, IL-6, TNF-α and adiponectin genes by real-time RT-PCR

RNA from liver tissue was isolated with High Pure RNA Isolation Kit from Roche. RNA from adipose tissue was isolated using Trizol reagent (Invitrogen) following the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using oligo-dT and Superscript II Reverse Transcriptase (Life Technologies). cDNA (20 ng) was subjected to quantitative RT-PCR amplification using SYBR Green Master Mix (Applied Biosystems). The forward and reverse primers for rat genes are shown in Table 2. Reactions were run on a quantitative Real-Time PCR System (Applied Biosystems); the thermal profile settings were 50°C for 2 min and 95°C for 2 min and then 40 cycles at 95°C for 15 s and 60°C for 2 min. Relative expression levels of the mRNA of the target genes were normalized to GAPDH mRNA levels.

2.9. Calculations and statistical analysis

Results are expressed as mean±S.E.M. Effects were assessed using ANOVA or Student's t test. We used Tukey's test for honestly significant differences to make pairwise comparisons. Spearman's rank correlation test between the

Table 2
Rat-specific primer sequences

Gene	Primer sequence
CRP	F: 5′ TTTGTGCTATCTCCAGAACAGATCA 3′
(NM 017096)	R: 5′ GCCCGCCAGTTCAAAACAT 3′
IL-6	F: 5′ CCCAACTTCCAATGCTCTCCTAATG 3′
(NM 012589)	R: 5′ GCACACTGAGTTTGCCGAATAGACC 3′
Adiponectin	F: 5′ GGCCGTTCTCTTCACCTACG 3′
(NM 144744)	R: 5′ GGCTCCATGCTCCTCCATCT 3′
TNF-α	F: 5′ CGTCAGCCGATTTGCCATTTC 3′
(NM 12675)	R: 5′ TGGGCTCATACCAGGGCTTGAG 3′
GAPDH	F: 5′ CAT GGC CTT CCG TGT TCC T 3′
(NM 023964)	R: 5′ CCT GCT TCA CCA CCT TCT TGA 3′

Table 3
Body weight gain, food and fat intake and adipose weight of Zucker rats fed LF, HF and HFPE diets for 19 weeks

	Test diets		
	LF (n=10)	HF (n=10)	HFPE (n=10)
Body weight gain (%)	177.55±3.78[a]	188.66±2.27[b]	181.58±2.44[a,b] (n=9)
Fat intake (kcal)	211.81±4.07[a]	662.07±19.59[b]	664.18±18.08[b]
Total energy intake (kcal)	1998.19±38.37	2082.64±61.61	2088.64±56.85
Tissue weight (g)			
Epididymal fat	8.27±0.16[a]	8.45±0.52[a,b]	9.04±0.46[b]
Mesenteric fat	6.16±0.70	7.00±0.66	6.80±0.27
Retroperitoneal fat	9.04±0.56[a]	11.13±0.71[b]	10.6±0.55[a,b]
Adiposity index	4.43±0.20	5.06±0.14	5.07±0.19

Values are expressed as mean±S.E.M. The significance of difference among the three groups was analyzed by ANOVA. Values not showing a superscript letter among the three diet groups are not significantly different (P<05).

three experimental groups was assessed. All calculations were performed using SPSS 14.0 software.

3. Results

3.1. Food intake, body weight and adipose tissue weight

Zucker *Fa/fa* rats fed the HF diet had significantly higher body weights than control rats fed the LF diet (P<.05; Table 3). In spite of fat intake being significantly increased, the total energy intakes of the three groups of rats were comparable (P>.05), indicating that higher body weight gains in the HF group may be related to higher fat intake but not to higher energy intake.

The epididymal and retroperitoneal fat pad weights of rats fed the HF diet were higher than the weights of those fed the LF diet (P<05; Table 3), although mesenteric weight remained unchanged. Adiposity index in the three groups (LF, HF and HFPE) was unchanged.

A positive correlation was found between adiposity index and body weight (ρ=.408, P<05) (Table 6).

3.2. Diet effect on metabolic variables and GSH/GSSG ratio

The total plasma cholesterol levels of Zucker rats fed the HFPE, HF or LF diet did not change significantly, neither by diet nor by procyanidin ingestion. Glucose plasma levels were not significantly increased by the HF diet compared to the LF diet, but the HFPE diet reduced glucose levels significantly (Table 4).

In plasma analysis, rats fed the HF and HFPE diets showed a reduced GSH/GSSG ratio compared to those fed the LF diet, whereas no significant difference was found by PE treatment (Table 4).

3.3. PE modulates CRP and adiponectin plasma levels in rats fed the hyperlipidic diet without modifying IL-6 levels

CRP plasma levels were increased in HF rats, thus indicating a low-grade inflammation similar to that found in overweight/obese individuals. Moreover, HFPE administration to rats, that is, a daily ingestion per animal of nearly 7 mg of PE during 19 weeks of treatment, resulted in an important decrease in CRP that is in the range found in rats fed a standard diet (LF). In contrast to most adipocyte hormones, the anti-inflammatory cytokine adiponectin is decreased in obesity and increased in response to weight reduction. In this work, we found a decrease in adiponectin plasma levels in HF rats. Furthermore, adiponectin plasma levels were increased significantly in rats fed the HFPE diet (Table 4). We then measured IL-6 levels in the plasma of animals and found no difference in IL-6 levels between the

Table 4
Plasma analysis of markers of oxidative stress, inflammation and metabolic variables

	Group		
	LF	HF	HFPE
Oxidative stress			
GSH/GSSG	133.63±31.81 (n=6)	6.67±2.39 * (n=8)	11.96±1.84 * (n=8)
Inflammation			
CPR (μg/ml)	203.08±73.63 (n=7)	472.19±149.47 * (n=8)	109.48±32.32 ** (n=8)
IL-6 (pg/ml)	85.07±3.11 (n=8)	93.75±1.35 * (n=9)	96.27±3.13 * (n=7)
Adiponectin (μg/ml)	3.21±0.24 (n=9)	2.63±0.12 * (n=8)	3.37±0.42 ** (n=10)
Metabolic variables			
Total cholesterol (mg/ml)	1.29±0.05 (n=10)	1.22±0.08 (n=9)	1.12±0.05 (n=9)
Glucose (mg/dl)	186.35±11.36 (n=9)	207.09±10.46 (n=9)	177.46±7.96 ** (n=9)

Student's t test was used. Values are expressed as mean±S.E.M.
* P<05 compared to LF.
** P<05 compared to HF.

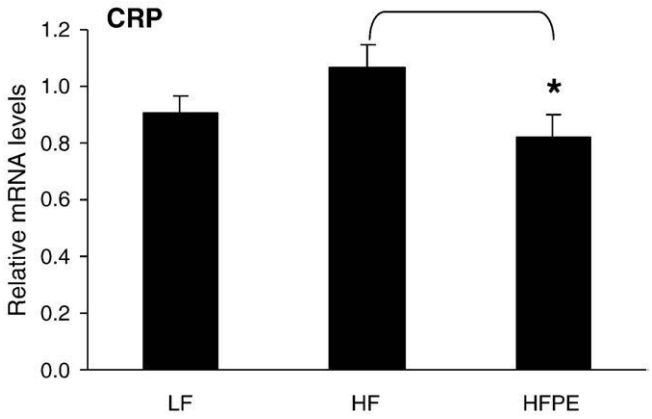

Fig. 1. Diet effect on CRP expression in liver. Liver mRNA was extracted, corresponding cDNA was synthesized and CRP gene expression was measured by quantitative real-time RT-PCR. Student's *t* test was used to evaluate significance between groups ($P<.05$).

HF group and the HFPE group, but both groups had higher IL-6 levels than rats receiving the standard LF diet.

3.4. PE acts by down-regulating mRNA CRP levels in the liver and mesenteric adipose tissue

Real-time PCR analysis of CRP in the liver showed differences between HF and HFPE rats. As expected, procyanidins caused a decrease in the synthesis of CRP mRNA in the liver with respect to rats receiving the same diet without procyanidins (Fig. 1).

Because CRP from adipose tissue is also an important source of this pentraxin in obese rats, we determined the CRP levels in adipose tissue of different origins: mesenteric, epididymal and retroperitoneal.

In mesenteric adipose tissue, we found that HF rats had higher CRP mRNA levels than control LF rats and HFPE rats. Thus, the HF diet increased CRP levels that were diminished by procyanidin treatment. In retroperitoneal and epididymal adipose tissue, nonsignificant differences were found between procyanidin treatment and diet (Table 5).

3.5. PE modulates gene expression in the mesenteric adipose tissue

Quantitative RT-PCR analysis of the mRNAs for CRP, IL-6, TNF-α and adiponectin genes in the mesenteric adipose tissue of rats fed the standard LF diet, HF diet or HFPE diet was performed. As we have previously described, procyanidin treatment modified not only CRP levels in mesenteric adipose tissue (Fig. 2A) but also IL-6, TNF-α and adiponectin gene expression. Our results show that IL-6 gene expression level was up-regulated by the HF diet and reduced significantly by procyanidin treatment (Fig. 2B). TNF-α gene expression level was also reduced significantly by procyanidins (Fig. 2C). On the contrary, the anti-inflammatory cytokine adiponectin was increased by PE treatment (Fig. 2D).

3.6. Correlations of CRP, IL-6, adiponectin and TNF-α expression in mesenteric adipose tissue and CRP and IL-6 plasma levels

To test possible associations between mRNA levels of CRP, IL-6, adiponectin and TNF-α in mesenteric adipose tissue; CRP and IL-6 plasma levels; body weight; and adiposity index, we performed Spearman's rank correlation test, analyzing the data from the three experimental groups. As shown in Table 6, a significant positive correlation between CRP expression and CRP plasma levels ($\rho=.623$, $P<001$) was found as expected. Furthermore, TNF-α expression and IL-6 expression correlated positively ($\rho=.745$, $P<.001$).

There were significant negative correlations between IL-6 and adiponectin expression ($\rho=-.436$, $P<.05$) and between TNF-α and adiponectin expression ($\rho=-.457$ $P<.05$). Finally, body weight correlated positively with CRP plasma levels ($\rho=.475$, $P<.05$).

There was no significant correlation of IL-6 plasma levels with any of the parameters measured. Liver CRP expression level was also examined, but no significant correlation between any of the parameters measured was found (data not shown).

4. Discussion

The primary function of adipose tissue is to store energy in the form of triglycerides during periods of energy excess and to release energy during fasting or starvation as free fatty acids and glycerol. Adipose tissue secretes a variety of peptides called adipokines including leptin, adiponectin, TNF-α, IL-6 and resistin, which have endocrine, autocrine and paracrine effects on the brain, liver and skeletal muscles [17]. Dysfunction of adipose tissue can result in insulin resistance and obesity-linked metabolic and vascular diseases. Obesity is associated with a chronic inflammatory response, which is characterized by abnormal cytokine production, increased synthesis of acute-phase reactants, such as CRP, and the activation of proinflammatory signaling

Table 5
Effect of the HFPE diet on CRP relative expression in adipose tissue

Group	Adipose tissue		
	Mesenteric	Retroperitoneal	Epididymal
LF	1.048±0.152[a]	1.049±1.152	1.022±0.274
HF	1.835±0.191[b]	1.166±0.124	1.345±0.270
HFPE	0.963±0.117[a]	1.173±0.100	1.398±0.145

Adipose tissue mRNA was extracted, corresponding cDNA was synthesized and CRP gene expression was measured by quantitative real-time RT-PCR. Results are expressed as relative expression levels normalized to the expression of the control group (LF). Values are expressed as mean±S.E.M. The significance of difference among the three groups was analyzed by ANOVA. Values not showing a superscript letter among the three groups are not significantly different ($P>.05$).

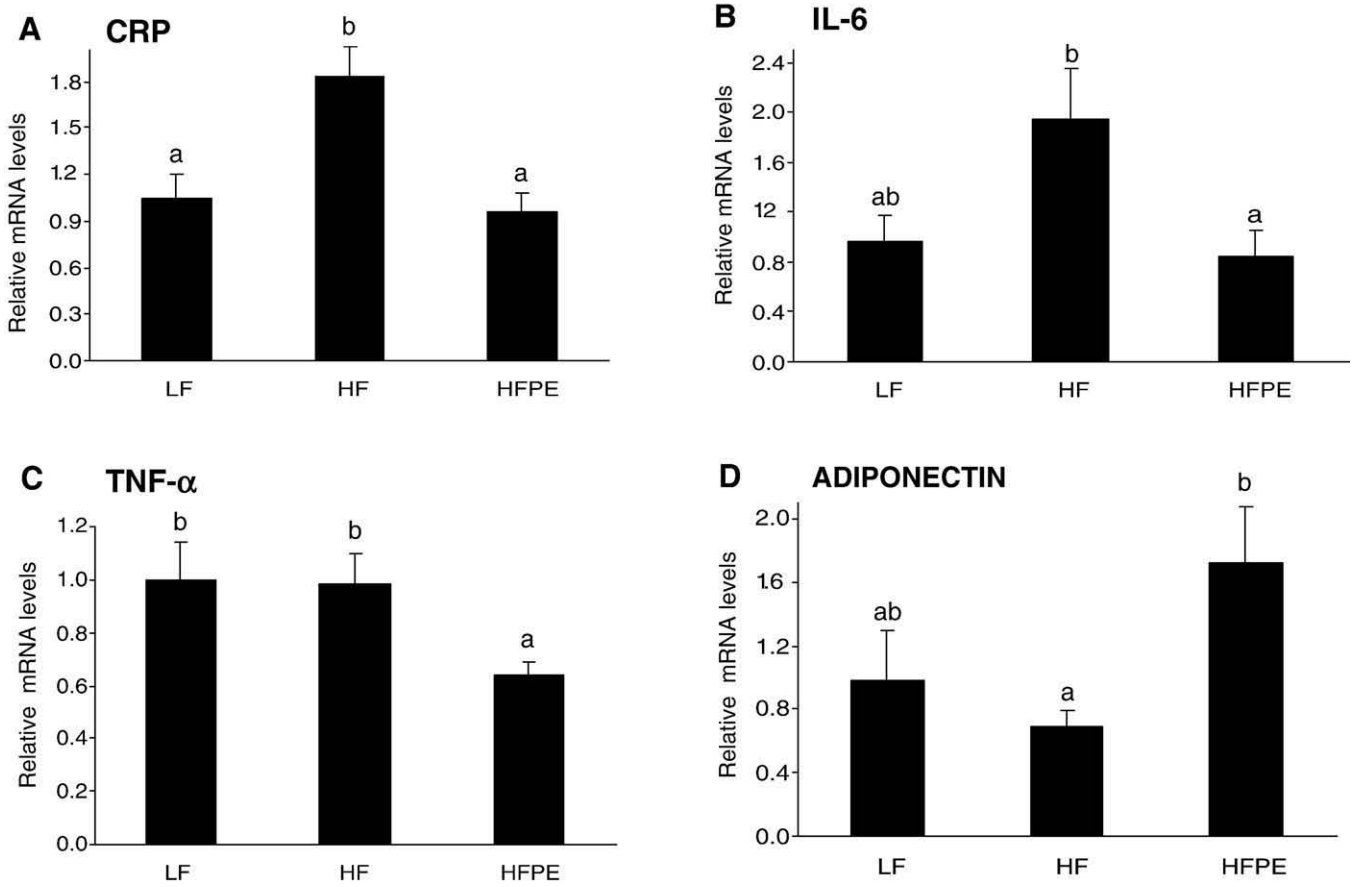

Fig. 2. Diet effect on gene expression in mesenteric adipose tissue. mRNA was extracted, corresponding cDNA was synthesized and CRP, IL-6, adiponectin and TNF-α gene expression was measured by quantitative real-time RT-PCR. ANOVA test was used to evaluate significance between groups ($P<.05$).

pathways [15]. It remains highly likely that adipokines contribute to obesity-associated systemic inflammation and remain potentially important targets for prevention of inflammation-induced insulin resistance or vasculopathy.

Procyanidins have been postulated to possess anti-inflammatory and immunomodulatory activities in vitro and in vivo [18]. In this work, we show that PE acts as an anti-inflammatory substance in vivo. To assess the effect of procyanidins, we compared the ability of PE to modify inflammatory parameters in Zucker *Fa/fa* rats after 19 weeks on a non-hyperlipidic diet, a hyperlipidic diet or a hyperlipidic diet with PE.

We found that feeding rats with the hyperlipidic diet resulted in a moderate increase in body weight as expected, as well as a less pronounced increase in rats receiving procyanidins, as we demonstrated before [19]. Body weight was positively related with adiposity index, comparing all the experimental groups. Moreover, biochemical parameters measured in plasma indicate that the HF diet produced a marked increase in oxidative stress, although it was not attenuated by procyanidin treatment. In contrast, total cholesterol was unchanged and glucose levels were reduced in HFPE-fed rats.

Previous studies indicate that a chronic low-grade inflammation is involved in the pathogenesis of atherosclerosis, and an elevated, highly sensitive CRP level is a risk factor for coronary artery disease. CRP is also a well-known systemic marker for inflammation in human and rats [20]. Plasma CRP levels were also strongly associated with obesity and obesity-related diseases, including insulin resistance, diabetes mellitus and hyperlipidemia. In this work, we found that CRP plasma levels were increased because of the HF diet up to 472 μg/ml, which is at the upper range in normal laboratory healthy rats [21]. We demonstrate that ingestion of procyanidins diminishes CRP levels, thus reducing the diet-induced low-grade inflammation. In addition, plasma CRP levels were positively associated with total body fat mass and CRP expression levels in mesenteric adipose tissue. We examined CRP expression in liver where procyanidins reduced its mRNA. Some authors have recently reported the same properties of red wine phenolics that reduced CRP expression in the human hepatic cell line Hep3B [22].

In the mesenteric adipose tissue, CRP was also down-regulated by the HFPE diet, while in retroperitoneal and epididymal adipose tissue, it was unchanged by either the HF

Table 6
Spearman's correlation coefficients (ρ) of CRP, IL-6, adiponectin and TNF-α expression in mesenteric adipose tissue, plasma levels of CRP and IL-6 and mass parameters

	mRNA levels in mesenteric adipose tissue			Plasma levels		Mass parameters	
	IL-6	Adiponectin	TNF-α	CRP	IL-6	Body weight	Adiposity
CRP mRNA							
ρ	.233	.216	−.096	.623**	.0409	.357	.178
P	.336	.348	.686	.008	.092	.102	.428
n	19	21	20	17	18	22	22
IL-6 mRNA							
ρ		−.436*	.745**	.256	.360	.209	.065
P		.033	.000	.277	.131	.337	.780
n		24	23	20	19	23	21
Adiponectin mRNA							
ρ			−.457*	−.118	.225	−.065	.148
P			.022	.610	.327	.756	.500
n			25	21	21	25	23
TNF-α mRNA							
ρ				−.054	.105	−.071	.014
P				.821	.660	.737	.950
n				20	20	25	23
CRP plasma							
ρ					.014	.475*	.081
P					.954	.029	.743
n					19	21	19
IL-6 plasma							
ρ						.092	.297
P						.683	.191
n						22	21
Body weight							
ρ							.408*
P							.039
N							18

* $P<05$.
** $P<01$.

diet or procyanidins. These variations found in CRP expression between the adipose tissues examined might be due to the different degree of macrophage infiltration in the WATs and the resultant different pattern of cytokine release [7,23], although the reason for this difference is unclear.

Taken together, the increased CRP expression in mesenteric adipose tissue may partially account for the elevation of plasma CRP and the effect of procyanidins on the adipose tissue may be responsible for the reduction of CRP protein and expression levels shown with the HFPE diet.

On the contrary, we have not detected changes in IL-6 plasma levels due to procyanidin treatment, although IL-6 levels were increased by the HF diet. There were no positive correlations between IL-6/CRP levels as could be expected [24].

It has been recently reported that adiponectin-deficient mice exhibit severe diet-induced insulin resistance and enhanced neointimal thickening after vascular injury [10]. These findings suggest that adiponectin has anti-inflammatory properties and acts as an endogenous modulator of obesity-related diseases. Furthermore, administration of adiponectin to obese or diabetic mice causes weight loss and also enhances insulin sensitivity and reduces the plasma glucose level by suppressing hepatic glucose production [25]. Previous studies in our group [26] demonstrate that PE acts as an enhancer of the glucose uptake in 3T3-L1 adipocytes and show that an acute gavage of PE (250 mg PE/kg body weight) significantly reduced blood glucose levels in streptozotocin-induced diabetic rats. In this work, adiponectin plasma levels and adiponectin expression in mesenteric adipose tissue of HFPE rats were highly increased compared with HF rats. Then, the reduction of glucose plasma levels may be driven as a consequence of the enhanced adiponectin expression that PE produces. These findings suggest that procyanidins act as anti-inflammatory molecules in vivo by increasing adiponectin expression. In agreement with our results, the monomeric procyanidin, catechin, has been recently described as an inducer of adiponectin expression in the adipocyte cell line 3T3-L1 [27].

In the current study, procyanidin treatment decreased IL-6 mRNA levels in the mesenteric WAT. In addition, IL-6 expression was negatively correlated with adiponectin expression, suggesting that the expression of IL-6 was negatively regulated by adiponectin in adipose tissue, as shown by other investigators [10].

Our results show that TNF-α expression in adipose tissue was also reduced by the HFPE diet and that this mRNA expression had a strong negative association with adiponectin expression. TNF-α suppresses the transcription of adiponectin in an adipocyte cell line, which might explain the lower levels of serum adiponectin in individuals who are obese [28].

The mechanisms regulating CRP synthesis at extrahepatic sites are unknown. CRP induction in hepatocytes is principally regulated at the transcriptional level by the cytokine IL-6. This cytokine controls expression of many acute-phase protein genes through activation of the transcription factors STAT3, C/EBP family members and Rel proteins (NF-kB) [29]. In searching for the mechanisms involved in inflammation-associated diseases, we have previously demonstrated that PE inhibits NF-kB activation in vitro [18].

We propose that the inhibition of the NF-kB pathway produced by procyanidins down-regulates TNF-α and IL-6 expression, which may explain the increase of adiponectin expression and the indirect reduction of CRP plasma and mRNA levels.

In summary, we have shown for the first time that procyanidins prevent low-grade inflammation in vivo, by adjusting adipose tissue cytokine imbalance, enhancing anti-inflammatory molecules and diminishing proinflammatory ones. Further studies are needed to elucidate the mechanism by which procyanidins may act as anti-inflammatory agents in obese humans.

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