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Modulatory effect of grape-seed procyanidins on local and systemic inflammation in diet-induced obesity rats

Ximena Terra, Víctor Pallarés, Anna Ardèvol, Cinta Bladé, Juan Fernández-Larrea, Gerard Pujadas, Josepa Salvadó, Lluís Arola, Mayte Blay*

Department of Biochemistry and Biotechnology, Nutrigenomic Research Group, Rovira i Virgili University, Tarragona, 43007, Spain

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Abstract

Chronic low-grade inflammation in obesity is characterized by macrophage accumulation in white adipose tissue (WAT) and abnormal cytokine production. We tested the hypothesis that grape-seed procyanidin extract (PE), with known anti-inflammatory and antioxidant effects, would improve local and systemic inflammation in diet-induced obesity rats. First, we analyzed the preventive effects of procyanidins (30 mg/kg per day) on rats fed a 60% kcal fat diet for 19 weeks. Second, we induced cafeteria diet obesity for 13 weeks to investigate the corrective effects of two PE doses (25 and 50 mg/kg per day) for 10 and 30 days.

In the preventive model, PE group had reduced not only body weight but also plasmatic systemic markers of inflammation tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP). The PE preventive treatment significantly showed an increased adiponectin expression and decreased TNF-α, interleukin-6 and CRP expression in mesenteric WAT and muscle TNF-α. A reduced NF-κB activity in liver is also observed which can be related to low expression rates of hepatic inflammatory markers found in PE group. Finally, PE dietary supplementation is linked to a reduced expression of Emr1 (specific marker of macrophage F4/80), which suggests a reduced macrophage infiltration of WAT.

In the corrective model, however, only the high dose of PE reduced CRP plasma levels in the short treatment without changes in plasmatic TNF-α.

In conclusion, orally ingested PE helps preventing imbalanced obesity cytokine pattern, but its corrective effects need to be further investigated. The dietary regular intake of food or drinks containing procyanidins might help prevent low-grade inflammatory-related diseases.
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Keywords: Procyanidins; IL-6; CRP; TNF-α; Adiponectin; Low-grade inflammation; Diet-induced obesity

1. Introduction

Metabolic syndrome comprises a cluster of risk factors for cardiovascular disease and type 2 diabetes mellitus [1,2]. Although the specific etiology for this increasingly important proinflammatory condition is not known, obesity and insulin resistance are generally present. Because of the escalating levels of obesity, diabetes, and cardiovascular disease in today's society, metabolic syndrome is receiving considerable attention. This high prevalence has led to the development of new strategies that can reverse its detrimental physiological alterations. Greater insight into the mechanisms behind the syndrome may improve our understanding of how to prevent and best manage this complex condition.

Recent studies have shown that adipocyte dysfunction plays an important role in the development of obesity and insulin resistance [3]. The adipocyte is the primary site of energy storage and accumulates triacylglycerol during nutritional excess. Adipocytes also synthesize and secrete biologically active molecules called adipokines. The altered production of proinflammatory molecules by adipose tissue has been implicated in the metabolic complications of obesity. Compared with the adipose tissue of lean individuals, the adipose tissue of obese individuals expresses increased amounts of such proinflammatory proteins as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), intracellular adhesion molecule-1 (ICAM-1), monocyte chemotactic protein-1 (MCP-1), and reduced adiponectin expression [4].

It has now been firmly established that obesity is associated with a low-grade proinflammatory state, characterized by macrophage infiltration of muscle and adipose tissue, adipocyte dysfunction and abnormal production of proinflammatory mediators [5,6]. 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 it has recently been recognized that adipocytes also have significant intrinsic inflammatory properties. Like macrophages, adipocytes are exquisitely sensitive to cytokine-mediated inflammatory signals. In turn, these stimuli induce the expression of

* Corresponding author. Department of Biochemistry and Biotechnology, C/ Marcel.lí Domingo s/n, 43007, Tarragona, Spain. Tel.: +34 977 558497; fax: +34 977558232.
E-mail address: mteresa.blay@urv.cat (M. Blay).

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inflammatory mediators such as IL-6, TNF-α and CRP. Although many of these activities are restricted to autocrine and paracrine effects, some of the cytokines that are secreted from adipocytes and adipose-resident macrophages make significant contributions to systemic inflammation. Interplay between macrophages and adipocytes by paracrine effects are presumably central in initiating and maintaining adipocyte dysfunction. Adipocytes increase in size as a consequence of hyperalimentation and large adipocytes release more (saturated) FFAs, which bind to macrophage toll-like receptor (TLR)-4. This results in NF-κB activation and, ultimately, to increased TNF-α production. In turn, macrophage-derived TNF-α activates human adipocytes, thereby further inducing lipolysis and enhancing the expression of ICAM-1, IL-6 and MCP-1. The diapedesis of monocytes from the blood to adipose tissue and differentiation into macrophages is further facilitated by MCP-1 and ICAM-1. This local paracrine loop involving adipocyte-derived FFAs and macrophage-derived TNF-α establishes a gradual vicious cycle that presumably leads to a proinflammatory state of both macrophages and adipocytes. It should be noted that large adipocytes produce less adiponectin. Since adiponectin normally inhibits TLR-activated NF-κB activity, it is assumed that low adiponectin levels reenforce the previously described loop [6].

Procyanidins are phenolic compounds from the flavonoid group that are widely distributed in the human diet and which are particularly rich in such fruits as berries, blue corn and beans, and in many fruits and vegetables. This suggests that we ingest significant amounts of procyanidins from plant-based daily diets like the Mediterranean diet [7]. Of all the various classes of flavonoids, procyanidins are the ones that will potentially be most consumed in the diet. Not only does the concentration of procyanidins in food vary but also the specific procyanidin present [8]. Because the human diet contains a mixture of procyanidins and it is likely that the effects are due not to one molecule but to the synergic effects of the flavonoids [9,10], we assessed the potential health benefits of a grape-seed procyanidin extract (PE) on the obesity-derived proinflammatory state.

Flavonoids function as powerful antioxidants and have anti-inflammatory activities in vitro [11] and in vivo [12]. They have proved to have antioxidant/free radical scavenging abilities; they can chelate minerals, modulate mammalian enzyme activity, enhance intracellular signaling, strengthen membranes, and bind to receptor sites, among other things. Apart from its antioxidant capacity, anti-inflammatory procyanidins are widely sought to provide wide-ranging health protection and bolster the human immune system because inflammation is such a prominent early symptom of so many chronic human illnesses and injuries [13].

In our preliminary study [14], we showed that procyanidins prevent low-grade inflammation in vivo by adjusting adipose tissue cytokine imbalance, enhancing anti-inflammatory molecules and diminishing proinflammatory ones. In the present study, we evaluated the effectiveness of procyanidins in two experimental models of obesity, and when they were administered as a corrective or preventive treatment. We focused on the putative modulatory effects of procyanidins on cytokine expression in white adipose tissue, muscle and liver to gain insight into the mechanisms that underlie the anti-inflammatory effects ascribed to procyanidins.

2. Materials and methods

2.1. Chemicals

Grape-seed 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 %) according to HPLC analysis.

2.2. Animal experimental procedures

Wistar female rats, weighing between 160 and 175 g, were purchased from Charles River Laboratories (Barcelona, Spain), housed in cages and subjected to a standard 12 h/12 h light–dark cycle. After 1 week in quarantine the animals were divided into the different experimental groups. The schematic diagram of procyanidin treatments are presented in Fig. 1.

2.2.1. Preventive treatment

Semipurified diets were obtained from Research Diets (USA). Briefly, three diets were used (Table 1): a low-fat diet (LF), a high-fat diet (HF) and a high-fat diet supplemented with PE (HFPE). The standard control diet was LF. The HFPE differs from HF in the PE content which was 1 mg of PE/g of feed (approximately 11 mg/animal per day, 30 mg/kg per day). At ~15 weeks of age, the rats were randomly assigned to be fed ad libitum LF (n=6), HF (n=6) or HFPE (n=6) diets. The experimental period lasted 19 weeks. Rats were weighed and sacrificed by beheading after a 3-h fast.

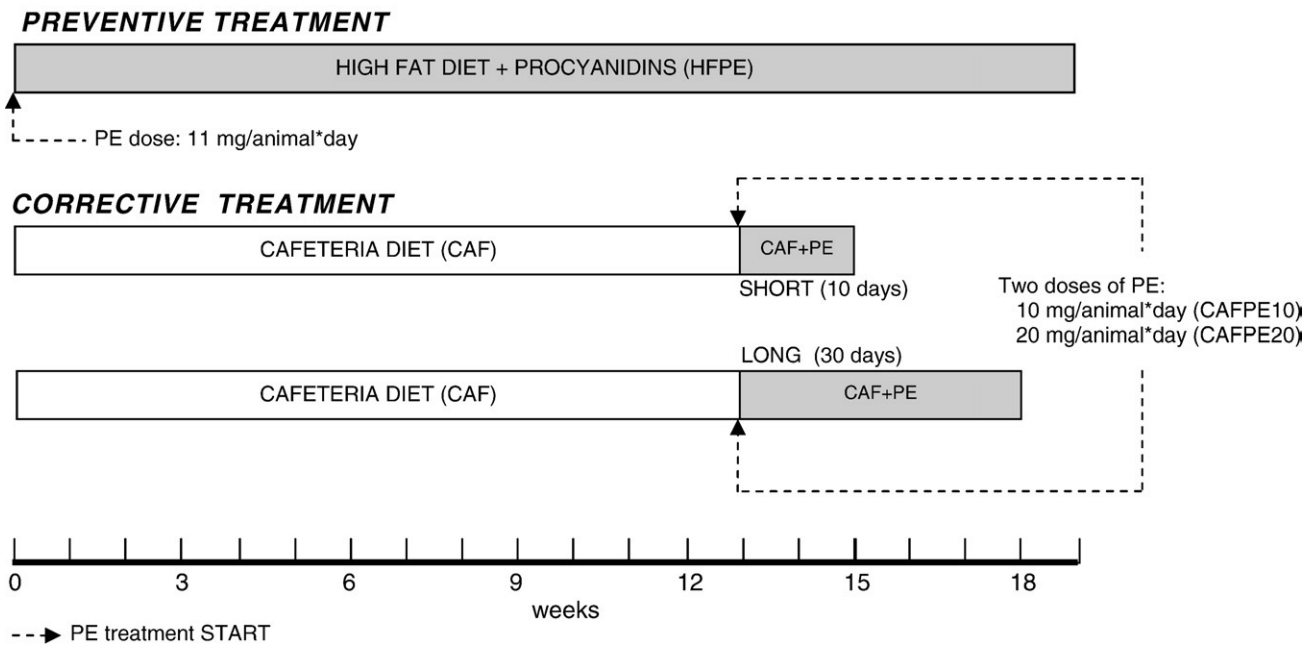

Fig. 1. Schematic diagram of procyanidin treatments.

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			
Corn starch	545.0	0.0	0.0
Maltodextrine	118.5	121.1	121.2
Sucrose	0.0	129.1	129.2
Lard	19.0	316.3	316.6
Soy bean oil	23.7	32.3	32.3
PE	0.0	0.0	1.0
Energy (kcal/g)	3.85	5.24	5.24
Protein (% energy)	20.0	20.0	20.0
Carbohydrate (% energy)	70.0	20.0	20.0
Fat (% energy)	10.00	59.90	59.90

LF, low-fat diet; HF, high-fat diet; HFPE, high-fat diet supplemented with procyanidins from grape seed. Vitamin and mineral mixture was also added to all the diets.

2.2.2. Corrective treatment

After the quarantine, 46 rats were divided into two groups: a control group (12 animals) fed with a standard diet (Panlab A-03, Barcelona, Spain) and another group (36 animals) fed with a cafeteria diet consisting of bacon, sweets, biscuits with pâté, cheese, muffins, carrots, milk with sugar and water plus the standard diet [15]. Animals were fed ad libitum and the food was renewed daily. The animals were fed the cafeteria diet for 13 weeks. They were divided into three subgroups (12 animals/group): rats treated with vehicle (CAF), rats treated with 10 mg of PE/animal per day (CAFPE10) or rats treated with 20 mg of PE per animal per day (CAFPE20) in sweetened condensed milk (25 and 50 mg/kg per day, respectively). After 10 days of GSPE treatment, six animals of each group were sacrificed (short treatment) and the other animals were fed for another 20 days (long treatment). At 9 a.m. on the last experimental day, rats were treated with PE or vehicle and the food was renewed. At 11 a.m. food was withdrawn and after a 3-h fast the animals were beheaded.

In both treatments, blood was collected using heparin as anticoagulant. Plasma was obtained by centrifugation and animal tissues were excised, frozen immediately in liquid nitrogen and stored at −80°C until analysis. All the procedures were approved by the Experimental Animals Ethics Committee of the Rovira i Virgili University.

2.3. Measurement of food intake and body weight gain

Body weight changes and caloric ingestion were monitored weekly throughout the experiment. Liver and adipose tissue fat pads (mesenteric, retroperitoneal and periovaric) were excised separately and weighed. The adiposity index was calculated as the total adipose tissue weight vs. total body weight.

2.4. Measurement of CRP and TNF-α levels

CRP and TNF-α levels were measured in plasma, liver and mesenteric adipose tissue. Tissues were homogenized on ice in a Triton X-100 cell lysis buffer [16]. After centrifugation for 20 min at 4°C, the supernatant was used for the assay.

CRP levels were quantified using a specific enzyme immunoassay according to the manufacturer's instructions (Immunology Consultants Laboratory, Inc., USA). The assay is a double polyclonal antibody sandwich enzyme immunoassay. TNF-α levels were quantified using a quantitative enzyme-linked immunosorbent assay according to the manufacturer's instructions (Invitrogen, S.A. Barcelona).

Table 2
Tissue weight of rats fed the different diets

Preventive treatment			
	LF	HF	HFPE
Total energy intake (kcal)	1075.4±54.4[a]	1410.3±21.7[b]	1306.0±46.3[b]
Tissue weight (g)			
Liver	10.1±0.4[ab]	11.1±0.4[b]	9.6±0.5[a]
Periovaric+ retroperitoneal fat	19.9±1.5[a]	24.4±1.3[b]	27.5±2.6[b]
Mesenteric fat	5.7±1.1[a]	8.2±0.5[b]	8.7±2.0[b]
Adiposity index	7.8±0.9[a]	9.1±0.4[a]	10.4±1.5[a]

Corrective treatment Short (10 days)	LF	CAF	CAFPE10	CAFPE20
Tissue weight (g)				
Periovaric+ retroperitoneal fat	15.4±2.5[a]	46.3±4.8[b]	37.7±5.8[b]	37.9±3.7[b]
Mesenteric fat	4.7±0.5[a]	15.7±3.1[b]	18.3±0.6[b]	13.7±2.1[b]
Adiposity index (%)	7.0±0.9[a]	14.5±0.9[b]	13.9±1.0[b]	14.2±1.0[b]
Long (30 days)				
Tissue weight (g)				
Periovaric+ retroperitoneal fat	12.7±0.7[a]	47.7±2.9[b]	46.5±3.1[b]	48.9±3.6[b]
Mesenteric fat	3.9±0.4[a]	20.2±2.6[b]	20.3±2.3[b]	13.0±1.9[c]
Adiposity index (%)	6.4±0.4[a]	15.7±0.7[bc]	13.6±0.3[c]	15.9±0.7[b]

LF, low-fat diet; HF, high-fat diet; HFPE, high-fat diet supplemented with procyanidins from grape seed; CAF, cafeteria diet; CAFPE10, cafeteria diet supplemented with procyanidins from grape seed 10 mg/day per animal; CAFPE20, cafeteria diet supplemented with procyanidins from grape seed 20 mg/day per animal. Values are mean±S.E.M. (n=6 per group). The significance of difference among the groups was analyzed by ANOVA ($P < .05$). Different superscript letters[a,b,c] indicate significative differences between groups. Two letters indicate that the value is an intermediate value between a and b, as assessed by Tukey test for pairwise comparisons.

2.5. NF-κB activity assay

Nuclear protein extracts were prepared from the liver tissue of HF and HFPE rats. Tissue was homogenized in 0.25 M sucrose buffer. After centrifugation at 800×g for 10 min, the resulting pellet was centrifuged at 800×g for 5 min in hypotonic buffer to provide nuclei. Nuclear extract was obtained by incubation with lysis buffer following the manufacturer's instructions (TransAM NF-κB p65 kit; Active Motif, UK). Protein concentrations were then quantified using a Bradford assay, and equal amounts of protein were used in a colorimetric NF-κB assay specific for the activated form of the p65 subunit of NF-κB (TransAM NF-κB p65 kit; Active Motif, UK).

2.6. Quantitative real-time PCR

Tissue RNA extraction and real-time PCR analysis were performed for CRP, IL-6, adiponectin, TNF-α, Erm1 and GAPDH, as previously described, with a SYBR green dye [14].

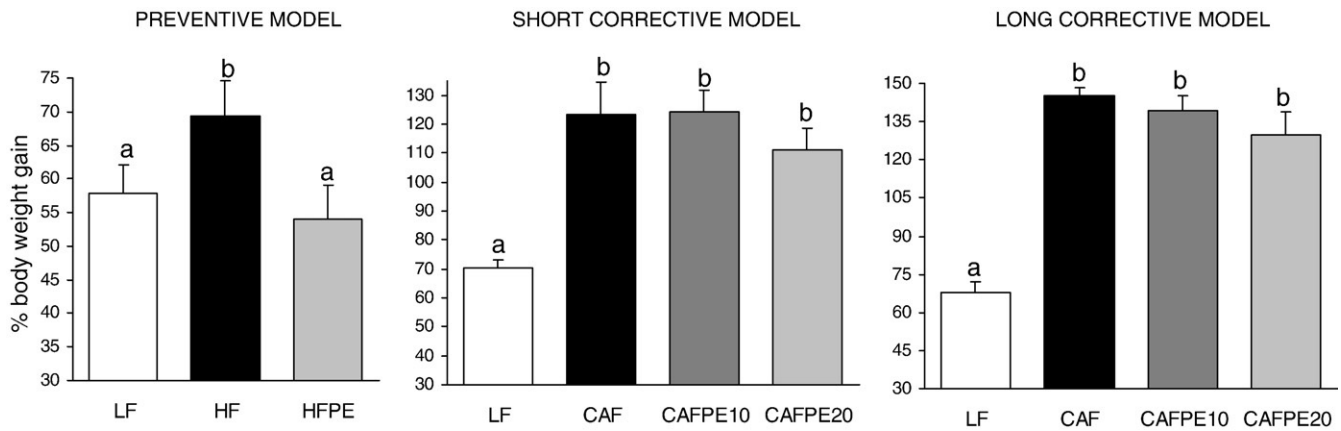

Fig. 2. Effect of procyanidins on the body weight gain of the different obesity models. LF, low-fat diet; HF, high-fat diet; HFPE, high-fat diet supplemented with procyanidins from grape seed; CAF, cafeteria diet; CAFPE10, cafeteria diet supplemented with procyanidins from grape seed 10 mg/day per animal; CAFPE20, cafeteria diet supplemented with procyanidins from grape seed 20 mg/day per animal. Values are mean±S.E.M. (n=6). The significance of difference among the groups was analyzed by ANOVA ($P < .05$).

2.7. Calculations and statistical analysis

Results are expressed as mean values±S.E.M. Effects were assessed using ANOVA or Student's *t* test. We used Tukey's test of honestly significant differences to make pairwise comparisons. All calculations were performed using SPSS 15.0 software.

3. Results

3.1. The effect of procyanidins on body weight

Wistar female rats fed both hyperlipidic diets showed higher body weights than control rats (Fig. 2), which indicated that both experimental models induced a highly significant obesity.

3.1.1. Preventive treatment

The PE preventive treatment produced significant body weight loss and also diminished body weight gain (Fig. 2). This treatment with PE also reduced liver weight, but no changes were found in adiposity or the weight of fat depots (Table 2).

3.1.2. Corrective treatment

The most significant differences in body weight were found in the corrective model, where the animals that were fed the cafeteria diet increased their body weight by 40%. Also, the fat pad weights of rats fed both diets were higher than those of the LF diet (Table 2). The corrective treatment with PE did not produce any significant reduction in body weight, although rats treated with procyanidins tended to weigh less. Some doses of PE showed tissue weight reductions compared with its obese controls, as shown with the highest dose of PE that was able to reduce the mesenteric fat in the long corrective model.

3.2. PE treatment modulates adipose, hepatic, muscular and systemic inflammation

Given our previous results [14], we went on to investigate the modulatory effect of procyanidins on both local and systemic manifestations of inflammation.

3.3. Visceral adipose tissue

We analyzed the effect of preventive procyanidin treatment on the expression of genes known to be closely related to the inflammatory process in the visceral adipose tissue of rats fed a standard LF diet, HF diet or HFPE (Fig. 3). We found that HF rats had higher CRP, TNF-α and IL-6 mRNA levels than control LF rats and HFPE rats. Furthermore, CRP and TNF-α protein levels were reduced in HFPE (Table 3), which is consistent with its gene expression. Thus, the cytokine levels increased by the HF diet were diminished by procyanidin treatment in this tissue. PE also stimulated the expression of the anti-inflammatory cytokine adiponectin. Emr1, which encodes the expression of F4/80, a surface marker of mature macrophages, increased in the HF group but decreased in the HFPE group.

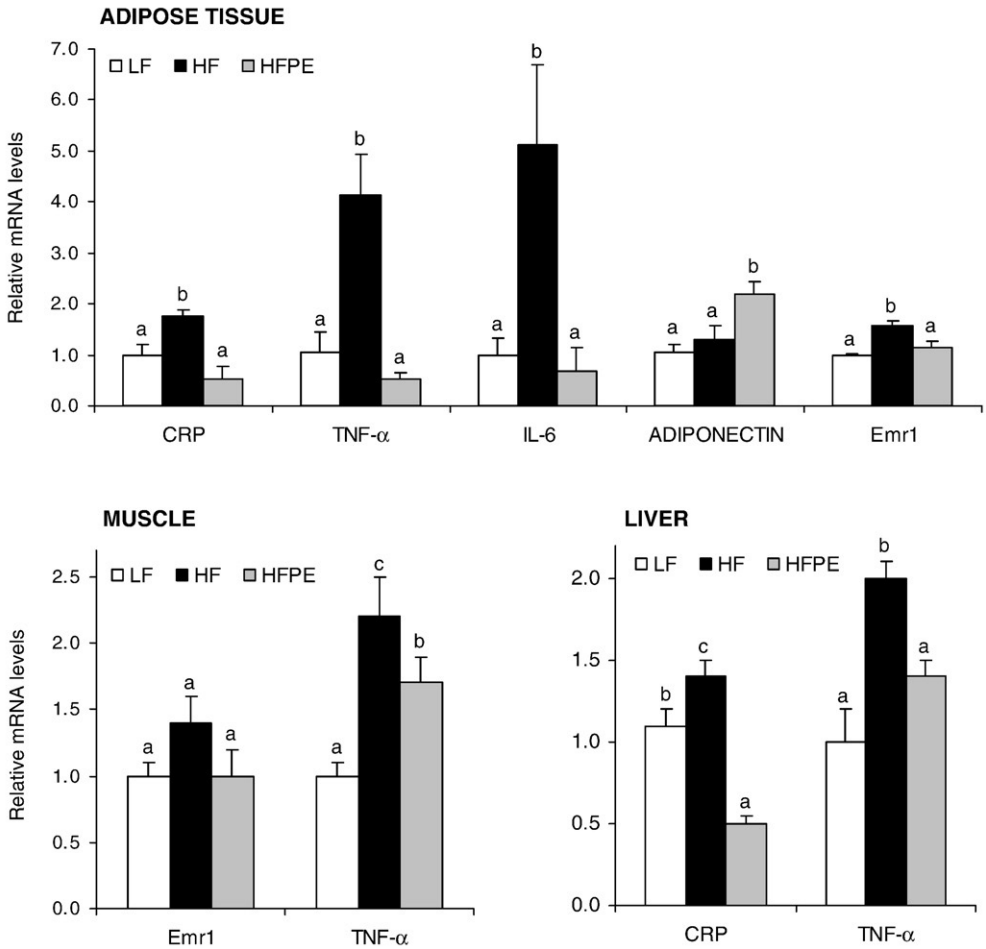

Fig. 3. Procyanidin effect on gene expression in mesenteric adipose tissue, muscle and liver from the preventive treatment rats. mRNA was extracted, corresponding cDNA was synthesized, and CRP, IL-6, adiponectin, TNF-α and Emr1 gene expression was measured by quantitative real-time RT-PCR. LF, low-fat diet; HF, high-fat diet; HFPE, high-fat diet supplemented with procyanidins from grape seed. ANOVA test was used to evaluate significance between groups ($P < .05$).

Table 3
Cytokine levels in plasma and various tissues

Preventive treatment	LF	HF	HFPE
CRP			
Plasma (μg/ml)	387.4±28.2[ab]	658.1±147.5[b]	313.7±21.7[a]
Adipose tissue (ng/100 μg protein)	33.6±1.3[a]	74.22±2.9[b]	34.6±3.4[a]
Liver (ng/100 μg protein)	71.5±4.2[a]	139.1±2.3[b]	139.4±3.2[b]
TNF-α			
Plasma (pg/ml)	NA (<4)	41.7±9.3[a]	15.2±3.7[b]
Adipose tissue (pg/100 μg protein)	NA (<4)	76.9±5.6[a]	60.7±4.3[b]

Corrective treatment	CAF	CAFPE10	CAFPE20
CRP			
Plasma short treatment (μg/ml)	798.4±27.7[a]	871.1±110.6[a]	430.4±44.1[b]
Plasma long treatment (μg/ml)	1050.1±230.0[a]	1230.7±267.3[a]	1029.1±276.2[a]
TNF-α			
Plasma short treatment (pg/ml)	21.3±3.8[a]	4.0±0.6[b]	5.4±0.1[b]
Plasma long treatment (pg/ml)	15.8±1.3[a]	11.2±1.8[a]	11.6±3.7[a]

LF, low-fat diet; HF, high-fat diet; HFPE, high-fat diet supplemented with procyanidins from grape seed; CAF, cafeteria diet; CAFPE10, cafeteria diet supplemented with procyanidins from grape seed 10 mg/day*animal; CAFPE20, cafeteria diet supplemented with procyanidins from grape seed 20 mg/day*animal. Values are mean±S.E.M (n=6 per group). The significance of difference among the groups was analyzed by ANOVA ($P < .05$). Different superscript letters[a,b,c] indicate significative differences between groups. Two letters indicate that the value is an intermediate value between a and b, as assessed by Tukey test for pairwise comparisons. NA, not available.

3.4. Muscle

We determined the effect of PE on local inflammation by real-time PCR analysis of gene expression in muscle from the preventive model rats. As shown in Fig. 4, the analysis of TNF-α expression in muscle revealed a marked increase in HF rats and a significant reduction in TNF-α expression in rats fed the HFPE diet. We also assessed the expression of Emr1. Although its expression was higher in HF rats than in HFPE rats, this difference was not significant in muscle.

3.5. Liver

We also analyzed CRP and TNF-α hepatic gene expression in this model. Both parameters were increased by the HF diet, and treatment with PE significantly decreased CRP and TNF-α expression (Fig. 3). The hepatic analysis of CRP protein expression revealed that the HF diet significantly increased this parameter, but procyanidin treatment was not able to reduce it. Moreover, using a specific assay for NF-κB p65 subunit activity, we determined that there was significantly less p65 activity in liver nuclear extracts derived from the HFPE group than in HF rat livers (Fig. 4).

3.6. Systemic inflammation

To further investigate the effect of procyanidins on inflammation, we analyzed CRP and TNF-α plasma levels in both models of obesity.

3.6.1. Preventive treatment

CRP plasma levels were increased in HF rats, thus indicating low-grade inflammation induced by a high-fat diet, similar to that found in humans with overweight/obesity (Table 3). Moreover, the administration of HFPE to rats — that is to say, a daily ingestion per animal of nearly 11 mg of PE for 19 weeks — resulted in such a decrease in CRP that it fell to the range of levels found in rats fed the control diet (LF).

We also investigated the possibility that procyanidins could reduce systemic inflammation by modulating TNF-α. Interestingly, in the preventive model, TNF-α plasma levels increased in HF rats and decreased in PE rats.

3.6.2. Corrective treatment

The cafeteria diet increased CRP plasma levels in the short and long treatment in comparison to rats fed the standard diet. In this model, however, only the high dose of PE (20 mg/day per animal) reduced CRP plasma levels in the short treatment (Table 3).

Rats fed the cafeteria diet (CAF) had higher levels of TNF-α than CAFPE10 and CAFPE20 groups in the short treatment, although no changes in TNF-α plasma levels were detected between groups from the long corrective treatment (Table 3).

4. Discussion

Obesity is the key player in metabolic syndrome. Improving adipocyte dysfunction is one of the crucial ways of preventing this syndrome. In this study we have tried to increase our understanding of how procyanidins regulate the pathways responsible for preventing obesity and modulating the associated low-grade inflammation. In our previous investigations, we demonstrated that procyanidins were able to modulate adipokine expression in rats fed a 30% fat diet [14]. Now we wanted to evaluate the effect of procyanidins at different times and doses, and in different models of obesity. To prove how effective they are at preventing or treating diseases, our experimental design included several treatment conditions. We analyzed two different diets as models of a disturbed metabolic state. In the first, we analyzed the preventive effects of procyanidins on rats fed a 60% fat diet. In the second, we induced obesity by feeding the rats with a cafeteria diet, which is a rapid inducer of obesity. We analyzed the corrective effects of two PE doses (10 and 20 mg/day) over two periods of time (short and long treatments). These two models gave us a greater scope for analyzing the duration of the treatment, and the preventive and curative effects of procyanidins.

Firstly, we analyzed the effects of PE on body weight. In the preventive model, we found that feeding rats with the hyperlipidic diet resulted in an increase of body weight as expected [17], but also a less pronounced increase in rats receiving procyanidins [14]. Moreover, rats fed the cafeteria diet increased in weight considerably more than the control group, but procyanidin treatment had little

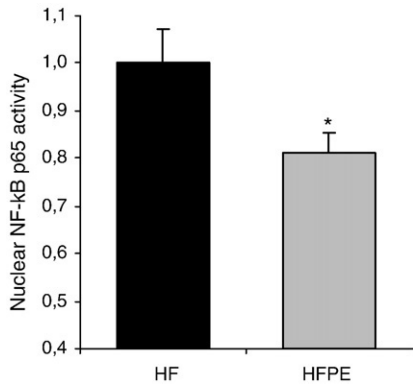

Fig. 4. Procyanidin effect on NF-κB activity in liver from the preventive treatment rats. PE significantly decreases hepatic NF-κB activity after 19 weeks (n=6 per group). Student's t test was used to evaluate significance between groups ($P < .05$).

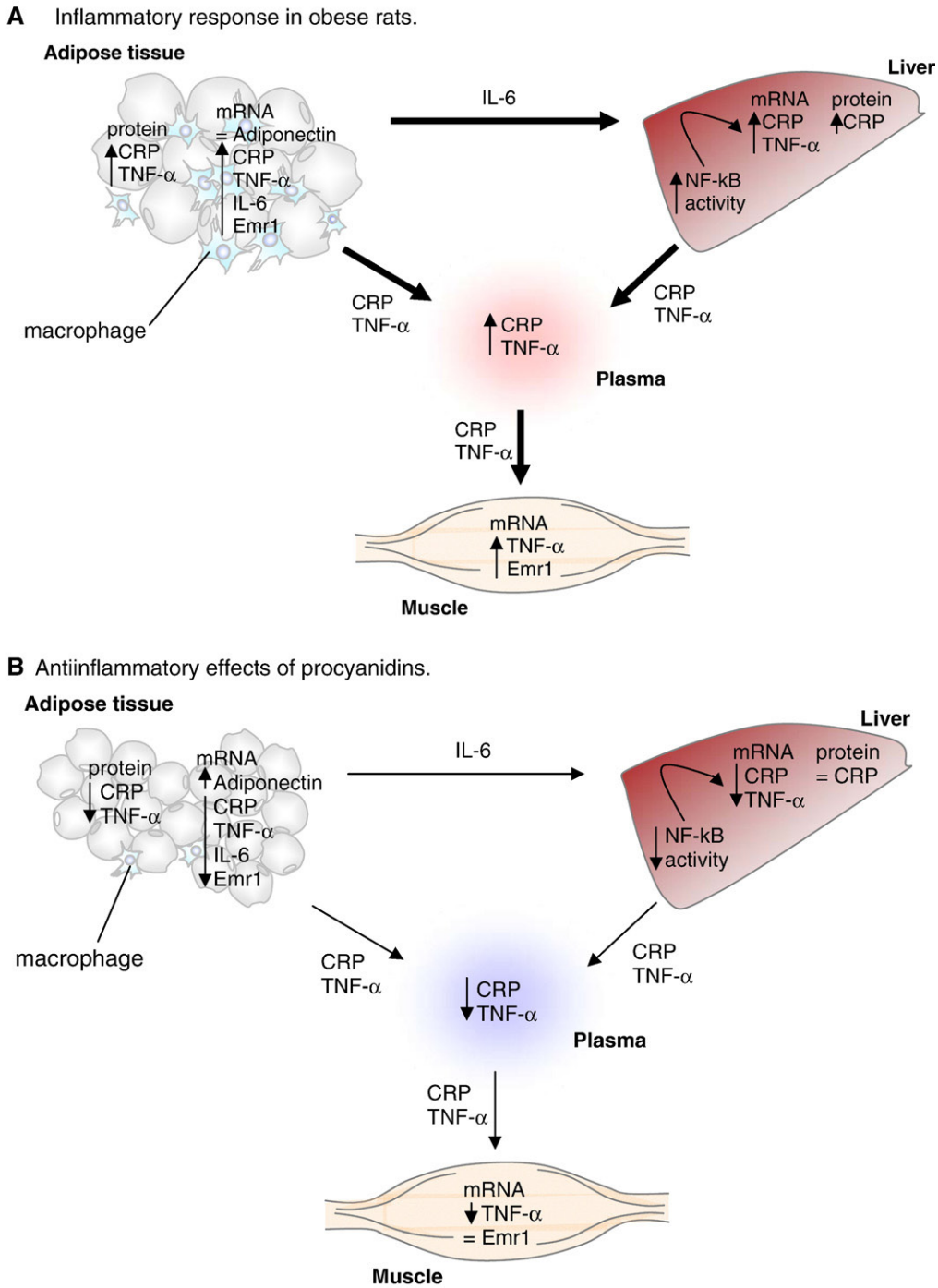

Fig. 5. Schematic diagram of procyanidin effects in the preventive model rats.

effect on this parameter. The reason for these differences between models is unclear but they might be explained by the different degree of obesity in each model. In our previous studies we also fed rats diets with a lower percentage of fat. This suggested that procyanidins administered after obesity has been established cannot compensate for the high body weight caused by the cafeteria diet.

To further investigate the effects of procyanidins on systemic manifestations of inflammation, we analyzed CRP and TNF-α plasma levels, which are markers of systemic inflammation, in both models of obesity [18,19]. In the preventive model, procyanidins diminished the diet-induced systemic inflammation by reducing the TNF-α and CRP plasma levels. On the other hand, the short corrective treatment with a high dose of procyanidins lowered CRP plasma levels. These results indicate that when the overproduction of CRP in obese rats is not extremely high a short curative dose of procyanidins might effectively diminish CRP plasma levels. TNF-α levels were also reduced by both doses of procyanidins in the short treatment. However, in the long corrective treatment, no changes in CRP or TNF-α plasma levels were detected.

We have focused on the cytokine expression profile in mesenteric adipose tissue as a representative of visceral adipose tissue, not only because it appears to be an important point in the development of low-grade inflammation [20] but also because we have previously shown that adipose tissue can be an important target for procyanidins

[21]. In this study, we have shown that IL-6, CRP, and TNF-α high-fat diet-induced gene expression was reduced by the adding PE to the diet, as we have previously reported [14]. Also, the gene expression of the anti-inflammatory molecule adiponectin was enhanced. Furthermore, procyanidin treatment reduced TNF-α and CRP protein levels in mesenteric adipose tissue, indicating that local inflammation in this tissue was prevented.

Only relatively recently have obese individuals been described to have increased macrophage infiltration of adipose tissue, but it now appears that the infiltration rate of monocytes into visceral adipose tissue is higher than into subcutaneous adipose tissue [6]. Macrophages have been identified as the primary source of many of the circulating inflammatory molecules that are detected in the obese state [22]. Weisberg et al. [5] demonstrated that adipose tissue macrophages are responsible for almost all adipose tissue TNF-α expression and significant amounts of IL-6 expression in mice. Furthermore, the preponderance of evidence supports the hypothesis that the amounts of TNF-α mRNA and TNF-α released by adipose tissue are enhanced in human obesity and that the vast majority of TNF-α releases by adipose tissue come from the nonfat cells in this tissue [23]. Because obesity is associated with macrophage accumulation in adipose tissue [24,25], we wished to examine the effects of procyanidins on macrophage infiltration by measuring the expression levels of Emr1, the gene encoding a specific marker for mature macrophages (F4/80) in the mesenteric adipose tissue. We found that macrophage presence increased significantly more than in control rats and, interestingly, procyanidins reduced this level. So, the inhibition of the cytokine expression in this tissue might be due to a decrease in the number of macrophages, but procyanidins might also directly affect the proinflammatory pathways in the adipocyte and the macrophage.

The stimulus for macrophage influx into fat is largely unknown and probably involves a complex pathophysiology that includes physical and chemical adipocyte injury and cytokine cross-talk with increased local expression of TNF-α, MCP-1 and I-CAMs [26]. We found that procyanidin treatment decreased NF-κB activity in liver, which is directly associated with the decreased hepatic expression of such inflammatory molecules as TNF-α and CRP, as we found in our study. We hypothesize that procyanidins also reduce MCP-1 secretion in adipocytes partially due to the diminished TNF-α levels that we have detected in adipose tissue, muscle and liver, which might be a consequence of the inhibitory effects of procyanidins on NF-κB activation [13,27]. As we have previously proposed, the local enhancement in adiponectin expression produced by PE might also be partly responsible for the reduced cytokine expression levels. On the other hand, TNF-α has been shown to induce the expression of adhesion molecules (I-CAM) and chemokines in human endothelial cells. So, another mechanism for procyanidin effects might be the result of diminishing I-CAM expression, which activates macrophages [6]. Some authors have recently reported similar results in murine obesity models and in vitro experiments with such other flavonoids as curcumin, resveratrol and a GSPE [25,28,29].

We found reduced cytokine expression in liver, muscle and mesenteric adipose tissue, which lowers local inflammation and possibly also systemic inflammation [30,31]. In any case, this is an interesting finding that demonstrates the potential effects of procyanidins on such low-grade inflammation-related diseases as obesity (Fig. 5).

In summary, we have shown that procyanidins modulate tissue and plasma cytokine levels partially by reducing macrophage infiltration in adipose tissue and NF-κB activity in liver. However, although procyanidins are effective at preventing the onset of inflammation, it is unclear whether procyanidins exert anti-inflammatory effects once inflammation is established and whether they can help to resolve inflammation.

In conclusion, our studies revealed that daily consumption of procyanidins prevent both systemic and local low-grade inflammation in adipose tissue, muscle and liver, which might improve obesity-induced insulin resistance in these tissues. Given these interesting findings, further investigation of the effects of procyanidins in human patients is required.

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