Grape seed procyanidins improve β-cell functionality under lipotoxic conditions due to their lipid-lowering effect

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Received 13 December 2011; received in revised form 4 April 2012; accepted 15 June 2012

Abstract

Procyanidins have positive effects on glucose metabolism in conditions involving slightly disrupted glucose homeostasis, but it is not clear how procyanidins interact with β-cells. In this work, we evaluate the effects of procyanidins on β-cell functionality under an insulin-resistance condition. After 13 weeks of cafeteria diet, female Wistar rats were treated with 25 mg of grape seed procyanidin extract (GSPE)/kg of body weight (BW) for 30 days. To determine the possible mechanisms of action of procyanidins, INS-1E cells were separately incubated in high-glucose, high-insulin and high-oleate media to reproduce the conditions the β-cells were subjected to during the cafeteria diet feeding. In vivo experiments showed that chronic GSPE treatment decreased insulin production, since C-peptide levels and insulin protein levels in plasma were lower than those of cafeteria-fed rats, as were insulin and Pdx1 mRNA levels in the pancreas. GSPE effects observed in vivo were reproduced in INS-1E cells cultured with high oleate for 3 days. GSPE treatment significantly reduces triglyceride content in β-cells treated with high oleate and in the pancreas of cafeteria-fed rats. Moreover, gene expression analysis of the pancreas of cafeteria-fed rats revealed that procyanidins up-regulated the expression of Cpt1a and down-regulated the expression of lipid synthesis-related genes such as Fasn and Srebf1. Procyanidin treatment counteracted the decrease of AMPK protein levels after cafeteria treatment. Procyanidins cause a lack of triglyceride accumulation in β-cells. This counteracts its negative effects on insulin production, allowing for healthy levels of insulin production under hyperlipidemic conditions.

Keywords: Procyanidins; High-fat diet; Oleate; Insulin secretion; β-Cell

1. Introduction

Procyanidins have positive effects on glucose metabolism in conditions of slightly disrupted glucose homeostasis [1], a property that makes these compounds very interesting as functional food ingredients. Part of this effect could be explained by the activity of procyanidins on adipose cells [2], but, in fact, in a rat cafeteria-diet model, grape seed procyanidins extract (GSPE)-treated animals had fewer instances of insulinemia and glycemia than did the cafeteria group. Literature analysis indicated that the mechanism of the interaction of procyanidins with β-cells is not completely understood [3]. On the other hand, we recently observed that, at some doses, procyanidins change β-cell functionality, modifying insulin synthesis and secretion under nonpathological conditions [4], through their effects on membrane potentials.

A cafeteria diet allows for development of insulin resistance with hyperglycemia and hypertriglyceridemia conditions, and it is thus a good model for most syndrome X human pathologies [5]. Peripheral tissues play a key role in these pathologies, working together with pancreatic β-cells. In conditions of insulin resistance, β-cells are in high-glucose and high-fatty acid conditions, and published studies have shown that prolonged exposure of pancreatic islets to elevated concentrations of fatty acids reduces insulin secretion in vitro [6,7]. This has also been implicated in the declining insulin secretory capacity of the β-cell, which accompanies the beginning of type 2 diabetes [8]. Like fatty acids, chronic hyperglycemia in β-cells causes defective insulin gene expression, diminished insulin content and defective insulin secretion [9]. While elevated levels of glucose or fatty acids can, by themselves, have detrimental effects on β-cell function in many experimental systems, the combination of both nutrients is synergistically harmful, and the term glucolipotoxicity has been coined to describe the phenomenon [10,11].

In the present study, our goal was to understand the relationship between procyanidins and insulin-producing cells under an insulin resistance condition. We first determine whether procyanidin extract could alleviate the deleterious effects of cafeteria diet on β-cell functionality in vivo. To analyze the biochemical mechanism of this postulated effect, we assess the actions of GSPE on β-cells cultured in high-glucose, high-insulin and high-fatty acid media.
2. Materials and methods

2.1. Chemicals

According to the manufacturer, GSPE (Les Dérivés Résiniques et Terpénniques, Dax, France) contained monomeric (16.6%), dimeric (18.8%), trimeric (16%), tetrameric (9.3%) and oligomeric procyandins (5–13 U: 35.7%) and phenolic acids (4.22%).

2.2. Cell culture and treatment

INS-1E cells were kindly provided by Prof. Pierre Maechler, University of Geneva [12]. The cell line was cultured as previously described [13]. Cell culture reagents were obtained from BioWhittaker (Verviers, Belgium). Three different models were assayed: (1) High-glucose treatment: The cells were incubated with 25 mM glucose for 24 h with 5 or 25 mg/L of GSPE. (2) High-insulin treatment: After 24 h of deplletion, the cells were incubated for 24 h with 20 mM insulin (Novo Nordisk Pharma, Madrid, Spain) and with 1, 5 or 25 mg/L of GSPE. (3) High-oleate treatment: Cells were cultured for 72 h with 0.4 mM oleate (stock solution: 10 mM oleate; Sigma-Aldrich, St. Louis, MO, USA) dissolved in 12.5% fatty acid-free BSA (Sigma-Aldrich) [14] and during the last 24 h, cells were treated concomitantly with 25 mg GSPE/L.

2.3. Animal experimental procedures

For the cafeteria-fed animal study (six animals per group), the animals were treated as previously described [2]. Briefly, female rats were divided into two groups: a control group fed with a standard diet (Panlab A03) and a cafeteria-fed group fed with a cafeteria diet (bacon, sweets, biscuit with pâté, cheese, milk and sugar) and water plus the standard diet. After 11 weeks, obesity was induced in the animals and the cafeteria group was divided into two subgroups: (i) cafeteria group of rats treated with a vehicle (sweetened condensed milk) and (ii) cafeteria + 25 group of rats treated with 25 mg of GSPE/kg of body weight (BW) per day. After 10 days of GSPE treatment, six animals from each group were sacrificed (short treatment). After 30 days of GSPE treatment, the remaining six animals of each group were sacrificed (long treatment). For the high-fat animal study (six animals per group), the animals were treated as previously described [15]. Briefly, male rats were fed with a high-fat diet (control) or with a high-fat diet containing 1 mg of GSPE/L. After 19 weeks of treatment, the animals were sacrificed. Blood was collected from all the animals using heparin, 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.

2.4. Glucose-stimulated insulin secretion

The secretory responses to glucose were tested in INS-1E cells as previously described [13]. Glucose-stimulated insulin secretion (GSIS) was measured by Insulin ELISA (Merckodia).

2.5. Triglyceride content

INS-1E cells were cultured in 12-well plates and treated with 0.4 mM oleate for 3 days. During the last 24 h of oleate treatment, cells were incubated concomitantly with 25 mg/L of GSPE. Cells were collected in PBS containing 0.1% triton X-100 (Sigma-Aldrich), and the solution was sonicated. Triglycerides from the pancreas were extracted using the same buffer. Triglyceride content was determined using an enzymatic colorimetric kit (QCA). Protein content of each sample was measured using ImageJ 1.44p software; all proteins were quantified relative to the loading control.

2.6. Mitochondrial membrane potential (∆Ψm) and cell membrane potential measurements

Mitochondrial membrane potential (∆Ψm) and cell membrane potential were measured as described [12].

2.7. Western blot

Protein was extracted from the whole frozen pancreas using RIPA lysis buffer (15 mM Tris–HCl, 165 mM NaCl, 0.5% Na-deoxycholate, 1% Triton X-100 and 0.1% SDS), with a protease inhibitor cocktail (1:1000; Sigma-Aldrich) and 1 mM PMSF. Total protein levels of the lysate were determined using the Bradford method [16]. Proteins were loaded and run through a 12 % SDS-polyacrylamide gel. Samples were transferred to a PVDF membrane (Bio-Rad Laboratories, Hercules, CA, USA), blocked at room temperature using 5% (w/v) nonfat milk in TTBS buffer (Tris-buffered saline (TBS), 0.5% (v/v) Tween-20) and incubated with rabbit AMPK primary antibody (Cell Signaling Technology, Beverly, MA, USA) or anti-β-actin antibody (Sigma-Aldrich). After washing with TTBS, blots were incubated with peroxidase-conjugated anti-rabbit secondary antibody (GE Healthcare, Buckinghamshire, UK). Blots were washed thoroughly in TTBS, followed by TBS after immunoblotting. Immunoreactive protein was visualized with the ECL Plus Western blotting detection system (GE Healthcare) and with the Alpha Innotech system with software version 6.0.2v (Alpha Innotech, San Leonardo, CA, USA). Densitometric analysis of immunoblots was performed using ImageJ 1.44p software; all proteins were quantified relative to the loading control.

2.8. Quantitative RT-PCR

Total RNA from pancreas was extracted using Trizol reagent following the manufacturer’s instructions. Total RNA from INS-1E cells was isolated using a miRNeasy Mini Kit (Qiagen, Barcelona, Spain). CDNA from all the experiments was generated using the TaqMan Biocycler’s kit and it was subjected to quantitative RT-PCR amplification using Taqman Master Mix (Applied Biosystems, Foster City, CA, USA). Specific Taqman probes (Applied Biosystems) were used for different genes: Rn01774648_g1 for insulin, Rn00565839_m1 for insulin degrading enzyme (IDE), Rn00755951_m1 for pancreatic duodenal homeobox 1 (Pdx1), Rn00561265_m1 for glucokinase, Rn01754856_m1 for mitochondrial uncoupling protein 2 (Ucp2), Rn00569117_m1 for fatty acid synthase (Fasn), Rn01495769_m1 for sterol regulatory element-binding protein 1c (Srebf1), Rn00580702_m1 for carnitine palmitoltransferase-1a (Cpt1a) and Rn04049945_m1 for peroxisome proliferator-activated receptor γ (PPAR-γ). β-Actin was used as the reference gene (Rn00667869_m1). Reactions were run on a quantitative RT-PCR 7300 System (Applied Biosystems) according to the manufacturer’s instructions.

2.9. Calculations and statistical analysis

Results are expressed as the mean±S.E.M. Effects were assessed by Student’s t test. All calculations were performed with SPSS software.

3. Results

3.1. GSPE decreases insulin production

For animals in which we previously induced damage by cafeteria-diet treatment for 13 weeks, 30 days of daily treatment with 25 mg GSPE/kg BW improved glyceremia and lowered insulinemia [2]. Peripheral effects were seen in the adipose tissue of these animals [2], and now we show that β-cell insulin production is lower, with an even stronger effect on mRNA levels (Table 1). The amount of insulin protein levels in the pancreas and that of C-peptide levels in the plasma were also lower (Fig. 1A and B, respectively). In fact, GSPE-treated rats had insulin gene expression and C-peptide levels similar to those of the control group. The strong effect on insulin synthesis agrees with the decrease in levels of the upstream insulin effector Pdx1 (Table 1), despite no statistically significant differences being observed. It must be highlighted that the Pdx1 mRNA levels from the cafeteria group were not different compared with the levels in the control group [17]. However, we did observe a decrease in Ucp2 gene expression (Table 1).

Insulin plasma levels depend on insulin production but also on insulin clearance. In normal healthy animals, we have shown IDE to be a target for GSPE [4]. However, although the cafeteria diet modifies the activity and expression of IDE in liver and white adipose tissue

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cafeteria</th>
<th>Cafeteria+ GSPE</th>
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</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>5.11±1.4</td>
<td>1.48±0.0*</td>
</tr>
<tr>
<td>Pdx1</td>
<td>4.07±1.0</td>
<td>2.32±0.3</td>
</tr>
<tr>
<td>Ucp2</td>
<td>1.64±0.3</td>
<td>0.65±0.1*</td>
</tr>
<tr>
<td>Cpt1a</td>
<td>1.63±0.2</td>
<td>2.20±0.3*</td>
</tr>
<tr>
<td>Fasn</td>
<td>0.75±0.1</td>
<td>0.31±0.0*</td>
</tr>
<tr>
<td>Srebf1</td>
<td>1.28±0.1</td>
<td>1.03±0.1</td>
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</tbody>
</table>

Rats were fed with a cafeteria diet for 13 weeks and then were orally treated with 25 mg GSPE/kg BW for 30 days. Data are the mean±S.E.M. of six animals. *Indicates statistically significant differences between treatments (P<.05). Control values are published in Ref. [17].
procyanidins act in limiting insulin production. High-glucose medium for 24 h provoked a very high decrease in insulin mRNA levels that was not counteracted by GSPE treatment (control: 11 mM glucose: 2.13 ± 0.0; 25 mM glucose+5 mg GSPE/L: 1.01 ± 0.1; 25 mM glucose+25 mg GSPE/L: 0.96 ± 0.1; vs. control 25 mM glucose: 1.00 ± 0.0). Glucokinase mRNA was also decreased by hyperglycemia, an effect that was statistically reinforced by concomitant treatment with 25 mg GSPE/L (control low glucose (11 mM): 1.38 ± 0.0; 25 mM glucose+5 mg GSPE/L: 0.99 ± 0.0; 25 mM glucose+25 mg GSPE/L: 0.87 ± 0.0; vs. control 25 mM glucose: 1.00 ± 0.0). High insulin treatment for 24 h did not modify insulin mRNA levels, but concomitant GSPE treatment induced a tendency to increase insulin gene expression, being only statistically significant at 5 mg GSPE/L (20 nM insulin: 0.96 ± 0.1; 20 nM insulin+1 mg GSPE/L: 1.06 ± 0.1; 20 nM insulin+5 mg GSPE/L: 1.13 ± 0.1; 20 nM insulin+25 mg GSPE/L: 1.06 ± 0.1; vs. control: 1.00 ± 0.0).

Table 2 shows the effects of GSPE on high-oleate culture medium. In this condition, there was an increase in insulin mRNA and GSPE limited this gene expression increase, similar to what we have observed in the in vivo studies. Glucokinase mRNA showed a similar pattern: levels were increased by oleate and GSPE limited the oleate effect. Pdx1 gene expression was unmodified by oleate treatment (oleate: 0.97 ± 0.03; oleate+GSPE: 0.96 ± 0.06; vs. control: 1.00 ± 0.03). On the other hand, Ucp2 mRNA levels were up-regulated by oleate (Table 2). However, in this case, GSPE did not mitigate the effects of oleate on Ucp2 mRNA levels.

Thus, of all the conditions assayed for culture of β-cells, only hyperlipidemia reproduced the effects we had obtained in vivo, i.e., the cafeteria diet induced high insulin expression levels that could be counteracted by the addition of procyanidins. Moreover, high-oleate treatment altered not only insulin secretion, mainly basal secretion, but also the GSIS (Fig. 2A). GSPE treatment slightly improved the oleate effect on insulin secretion (Fig. 2A). Therefore, GSPE seems to act on β-cell lipid metabolism to exert its bioactivity on insulin production.

3.3. Mechanism of action of GSPE on β-cells under hyperlipidemic stress

Data regarding how procyanidins affect β-cells are limited [3]. Working on an undamaged cell line (INS-1E), we have found that GSPE alters insulin secretion through its uncoupling action on cell membranes [4]. Olate uncouples mitochondrial plasma membrane potential [14,18]. To identify the target sites of GSPE on β-cells, we measured the mitochondrial membrane potential under oleate treatment altered not only insulin secretion, mainly basal secretion, but also the GSIS (Fig. 2A). GSPE treatment slightly improved the oleate effect on insulin secretion (Fig. 2A). Therefore, GSPE must use other mechanisms to improve the function of damaged oleate cells.

One of the most obvious effects of GSPE is its ability to improve lipid metabolism [19]. Under oleate treatment, β-cells have higher levels of triglyceride stores [20], so we measured the triglycerides that accumulated in β-cells. Oleate treatment doubles the amount of triglycerides (2.05 ± 0.1) vs. control cells (1.00 ± 0.0), while GSPE

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| Gene expression in INS-1E cells treated with oleate and GSPE |
|-----------------|-----------------|-----------------|
| Control         | Oleate          | Oleate+GSPE     |
| Insulin         | 1.00±0.0        | 1.64±0.1         | 1.24±0.1* |
| Glucokinase     | 1.00±0.0        | 1.20±0.1*        | 1.08±0.1 |
| Ucp2            | 1.00±0.0        | 1.80±0.2*        | 1.71±0.1* |

* Indicates a significant difference (P<0.05) vs. control group.
† Indicates a significant difference (P<0.05) vs. oleate group.

The effects of GSPE on glucose homeostasis are very dependent on the degree of damage. Thus, to gain further evidence of the effects of GSPE on the pancreas as described above, we analyzed relevant data from other animal models. A similar study that used a shorter GSPE treatment of 10 days did not show a statistically significant effect on insulin mRNA, but there was a tendency towards decreased gene expression (cafeteria: 1.07±0.2; cafeteria+25: 0.76±0.1; vs. control: 1.16±0.3). When we compared the insulin plasma levels to mRNA expression levels, there was a statistically significant increase due to GSPE treatment, suggesting a limited production vs. the amount of circulating insulin (cafeteria: 1.05±0.3; cafeteria+25: 1.83±0.2; vs. control: 0.63±0.2). This parameter was also clearly increased after 30 days of GSPE treatment (cafeteria: 1.50±0.4; cafeteria+25: 2.70±0.7; vs. control: 1.22±0.3).

By contrast, an equivalent dose of GSPE simultaneously administered by feed pellets in a high-fat diet (HF) to another group of rats did not cause any statistically significant change in insulin measurements. Despite a tendency towards lower mRNA levels (HF+30: 0.94±0.2; vs. HF: 1.32±0.3), plasma insulin levels were unchanged (HF+30: 5.20±0.5; vs. HF: 4.77±0.8), as was the ratio of plasma insulin to mRNA insulin (HF+30: 5.95±1.2; vs. HF: 5.46±1.5). It must be highlighted that this third model showed only moderate signs of glucose homeostasis disruption [15]. By contrast, cafeteria animal models showed almost all the metabolic syndrome alterations: hyperglycemia, hyperinsulinemia and increased plasma free fatty acids.

3.2. The effects of GSPE on insulin production can be explained through its action on lipid metabolism in β-cells

β-Cells of cafeteria animals live in a high-glucose, high-insulin and high-FFA environment that affects their functionality. We reproduced these three effects separately in cultured β-cells to identify where

\[17\], GSPE treatment did not have any effects on insulin clearance (results not shown).

Pancreas insulin content. (A) C-peptide plasma levels. After 30 days of treatment, animals were sacrificed, and blood and pancreas samples were obtained. Pancreas insulin content and C-peptide plasma levels were quantified by the ELISA method. Data are means±S.E.M. Different letters indicate significantly different groups with P<0.05.
slightly but statistically significantly decreases the amount of triglycerides in the cell (observed decrease: $-0.096\pm0.03$). We next checked the triglyceride content of the pancreas of cafeteria-fed animals and found that GSPE reduced it significantly after 30 days of treatment (Fig. 3).

We also analyzed the differential expression of key genes that control lipid metabolism in the pancreas (Table 1). We selected the Cpt1a gene, which is the key controller of free fatty acid oxidation [21], the Fasn gene, which is the key enzyme of de novo fatty acid synthesis [22], and the Srebf1 gene, a transcription factor that activates the expression of several genes involved in FFA and triglyceride synthesis, as well as other components of the regulatory machinery of lipid metabolism [23]. Cafeteria-fed animals showed a slight increase in Cpt1a gene expression [17], suggesting an increase of $\beta$-oxidation, and GSPE treatment caused a higher increase in Cpt1a mRNA levels (Table 1). Fatty acid synthase levels, which were reduced by a cafeteria diet [17], were significantly decreased with GSPE treatment as shown by Fasn gene expression (Table 1). GSPE treatment tended to reduce the mRNA levels of Srebf1 after 30 days of treatment (Table 1). These data agreed with the lipid-mobilization effect attributed to the GSPE.

To better understand how procyanidins modify $\beta$-cell functionality, we assessed whether the effects of GSPE on $\beta$-cells were mediated via AMPK-activated protein kinase (AMPK). When we analyzed AMPK protein levels (Fig. 4), we observed that a cafeteria diet produces a significant decrease in the levels of this protein in the pancreas, which was counteracted by GSPE treatment.

4. Discussion

Procyanidins have clear and well-defined beneficial, protective effects against most risk factors of metabolic syndrome, and they have been shown to have positive effects on glucose metabolism under conditions of slightly disrupted glucose homeostasis [1]. We have
Previously shown that GSPE acts peripherally on adipose cells to improve glycaemia, which leads to lower insulinemia in cafeteria-fed rats [2]. However, there are limited data regarding the effects of procyanidins on β-cells [3]. Taking into account that β-cells are responsible for maintaining glucose homeostasis by synthesizing and secreting insulin, the purpose of this study was to understand the effects of procyanidins on β-cell functionality under an insulin resistance condition.

Our results showed that rats fed with a cafeteria diet for 13 weeks and treated with 25 mg GSPE/kg BW for 30 days had decreased insulin production. Studies with other flavonoids also showed reduced insulinemia. Ihm et al. [24] showed that chronic intake of catechin for 12 weeks in the prediabetic stage significantly reduces insulin plasma levels. This study was performed with the Otsuka Long-Evans Tokushima Fatty (OLETF) rat model, a distinct model of type 2 diabetes that has some characteristic features, such as late onset of hyperglycemia, hyperinsulinemia and obesity [25]. Similar to our results, the phenolic acids chlorogenic acid and caffeic acid administered with high-fat diet significantly lowered plasma insulin levels compared to the high-fat diet group [26]. In a similar way, a 4-week treatment with bitter melon extract, traditionally used as an antidiabetic, is effective for improving insulin resistance in a mouse model of type 2 diabetes (animals fed with a high-fat diet for 12 weeks) by reducing blood glucose and insulin [27]. The authors suggested that the extract regulates the PPAR-mediated pathway, because thiazolidinedione, a synthetic PPAR-γ ligand that significantly increases insulin sensitivity via PPAR-γ, actually causes improved insulin sensitivity in a high-fat diet [28,29]. Insulin sensitivity is highly dependent on the peripheral actions of compounds. GSPE also proved to be effective at working through PPAR-γ in adipose tissue [23], but PPAR-γ also plays a role in pancreas tissue. We measured PPAR-γ expression in the pancreas of cafeteria-fed rats (pancreas: 1.50 ± 0.4; cafeteria + 25: 1.21 ± 0.4; vs. control: 1.15 ± 0.3), and we did not observe changes in PPAR-γ gene expression, but this might be due to the very low levels of expression of this gene in the whole pancreas.

Cafeteria diet is a good model to reproduce most syndrome X human pathologies [5]. It causes the development of an insulin resistance condition, with hyperglycemia and hypertriglyceridemia conditions and hyperinsulinemia. We tested several conditions to reproduce the effects in vitro that were observed in vivo and found that only hyperlipidemia mimicked them. In fact, lipotoxicity is one of the major causes of β-cell dysfunction in type 2 diabetes. Prolonged exposure of β-cells to high levels of fatty acids can cause impairment in the expression of metabolic genes, leading to decreased glucose-stimulated insulin secretion [31,32], as we have shown. In this study, we observed that chronic exposure of INS-1E cells to the fatty acid oleate resulted in impaired mitochondrial response, lipid accumulation in the cells, and GSIS loss. In these sense, Ucp2 mRNA levels were up-regulated by oleate as was expected because Ucp2 expression is regulated in tandem with the level of FFA [33], and in isolated rat islets and INS-1 pancreatic β-cells, long-term treatment with FFAs can increase Ucp2 mRNA [18,34]. GSPE treatment partially reversed the deleterious effects associated with lipid accumulation. Interestingly, the effects observed in vitro are correlated with the GSPE effects on cafeteria-fed rats, in which pancreatic triglyceride accumulation and plasmatic insulin secretion (measured as C-peptide) are significantly reduced by the GSPE treatment vs. cafeteria-fed rats. In these animals, GSPE also significantly decreased the levels of fatty acid synthase, suggesting reduced fatty acid synthesis. Furthermore, GSPE tended to reduce Srebf1. In fact, this effect on Srebf1 gene expression has also been observed in the white adipose tissue of cafeteria-fed rats [2] after 30 days of GSPE treatment and in the liver after 10 days of treatment [35]. Since Srebf1 activates the expression of acetyl-CoA, down-regulation of Srebf1 by GSPE could result in a lower concentration of malonyl-CoA in β-cells and, therefore, an increase of Cpt-1α [36]. We actually found increased gene expression of Cpt-1α, suggesting that the fatty acids that were present in the cytoplasm could be consumed via β-oxidation upon activation of the long fatty acid carrier Cpt1α, which carries the fatty acids through the mitochondrial membrane. A similar effect was observed in HepG2 cells treated with luteolin, one of the most common flavonoids [37]. Liu et al. have shown that luteolin decreases the gene expression of Sreb1f and Fasn and increases Cpt1a gene expression in the absence and presence of palmitate, and it enhances the phosphorylation of AMPK, leading to a decrease in intracellular lipid levels of HepG2 cells. AMPK, which plays a central role in regulating cellular metabolism and energy balance [38], is also activated by several other natural compounds, including resveratrol, epigallocatechin gallate, berberine and quercetin [39]. In MIN6 cells, berberine acutely increased AMPK activity, and in high-fat diet-fed rats treated with berberine for 6 weeks, it decreased plasma glucose and insulin levels and improved the blood lipid profile [40]. We therefore assessed AMPK involvement in the effects of GSPE and found that a cafeteria diet significantly decreased AMPK protein levels in the pancreas, while GSPE treatment increased the levels back to the levels seen in the control group. It must be highlighted that total AMPK protein levels and AMPK phosphorylation levels follow the same tendency [41–43]. Interestingly, islets cultured with the AMPK activator 5-amino-4-imidazolecarboxamide riboside decreased the expression of Sreb1f and cellular triglyceride content, effects that we observed in INS-1E after GSPE treatment [44]. Taken together, these observations show that GSPE promotes lipid mobilization in β-cells, favoring a negative energy balance. This effect is mediated through AMPK, and it causes changes in insulin secretion.

In conclusion, we show that under conditions of insulin resistance, chronic GSPE treatment (25 mg GSPE/kg BW) significantly decreases insulin production. The effects of GSPE on lipid-damaged β-cells can be explained through its lipid-lowering effect because the triglyceride content in the pancreas was reduced, and procyanidin treatment also affected lipid oxidation through the up-regulation of Cpt1α gene expression and through lipogenesis, which down-regulated Fasn and Sreb1f gene expression. Moreover, GSPE treatment prevented the decrease in AMPK protein levels seen after cafeteria treatment.

References


