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The effects of a cafeteria diet on insulin production and clearance in rats

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Abstract

The aim of the present study was to determine the effects of a cafeteria diet on the function and apoptosis of the pancreas, and the activity and expression of the insulin-degrading enzyme (IDE). Female Wistar rats were fed either with a cafeteria diet or a control diet for 17 weeks, and blood and tissues were then collected for analysis. The cafeteria diet-treated rats had higher plasma insulin and C-peptide levels ($P<0·05$), showing increased insulin secretion by the pancreas. Insulin protein and gene expression levels were higher in the pancreas of obese rats, as was its transcriptional controller, pancreatic duodenal homeobox 1 ($P<0·05$). Feeding a cafeteria diet down-regulated the gene expression of the anti-apoptotic marker B-cell/lymphoma 2 (BCL2), and up-regulated the protein levels of BCL2-associated X protein, a pro-apoptotic marker ($P<0·05$). The cafeteria diet caused lipid accumulation in the pancreas and modified the expression of key genes that control lipid metabolism. To assay whether insulin clearance was also modified, we checked the activity of the IDE, one of the enzymes responsible for insulin clearance. We found increased liver IDE activity ($P<0·05$) in the cafeteria diet-fed animals, which could, in part, be due to an up-regulation of its gene expression. Conversely, IDE gene expression was unmodified in the kidney and adipose tissue; although when the adipose tissue weight was considered, the insulin clearance potential was higher in the cafeteria diet-treated rats. In conclusion, treatment with a cafeteria diet for 17 weeks in rats mimicked a pre-diabetic state, with ectopic lipid accumulation in the pancreas, and increased the IDE-mediated insulin clearance capability.

Key words: Cafeteria diet: Pancreas: Insulin-degrading enzyme: Apoptosis: Insulin

The prevalence of overweight and obesity is quickly increasing to epidemic proportions around the world[1]. Obesity is associated with a higher incidence of a number of diseases, including CVD, cancer[2] and diabetes[3]. There are several causes of obesity, some of which are genetically defined, but some others are related to environmental factors. To study obesity, several animal models have been used, including both genetic and diet-induced obesity models. Among them, the cafeteria diet has been used as a robust model because it is a good reproduction of the diet in Western society[4].

The cafeteria diet model has been described in terms of the effects of the diet on increasing body weight and modulating adipogenesis and inflammation[4–6]. The diet consists of feeding animals with a substantial amount of salt, sugar and fat; thus, mimicking the diet consumed by Western cultures[4]. The diet promotes voluntary hyperphagia that results in rapid weight gain, increases fat pad mass and leads to a pre-diabetic state[4,7]. Different studies have shown that the cafeteria diet increases plasma insulin levels and alters glucose metabolism[4,8–11]. Despite the key role of the pancreas in glucose metabolism, scarce work has been performed to study the chronic effects of this type of high-fat diet on this organ.

Insulin resistance induced by the diet might ultimately lead to the impairment of β-cell function and reduced β-cell mass, in part due to an increase in the apoptosis of these cells, and thus might lead to diabetes[12]. The impairment of β-cell function partly results from the accumulation of TAG in the pancreas, as in other non-adipose tissues, when a positive net energy balance occurs[13]. Studies in human islets have confirmed that insulin secretion at high glucose concentrations is impaired in a time-dependent fashion by exposure to NEFA, and NEFA also produce a decrease in insulin biosynthesis[14]. Yet, few *in vivo* studies concerning the effects of the cafeteria diet on the pancreas are available in the literature. In female rats, feeding the cafeteria diet for 14 weeks has been reported to diminish the glucose (and other depolarising agent)-stimulated insulin secretion of isolated islets, probably

Abbreviations: BAX, B-cell/lymphoma 2-associated X protein; BCL2, B-cell/lymphoma 2; CPT1a, carnitine palmitoyltransferase-1a; DM2, type 2 diabetes mellitus; FASn, fatty acid synthase; HOMA-IR, homeostatic model assessment for insulin resistance; IDE, insulin-degrading enzyme; PDX1, pancreatic duodenal homeobox 1; TTBS, Tris-buffered saline (TBS) plus 0·5% (v/v) Tween-20; UCP2, uncoupling protein 2.

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through a defect in the Ca^{2+} mobilisation by these islets[11]. Additionally, in male rats, treatment with the cafeteria diet for 15 weeks has been shown to induce changes in pancreatic islet morphology[4]. More studies concerning the effects of this diet in the pancreas would be beneficial for the description of this widely used obesity model, which allows the analysis of the environmental effects of the diet, free from possible genetic effects.

Plasma insulin levels are highly dependent on pancreas functionality and the number of islet β-cells, but insulin clearance is also of importance in determining the levels in plasma. *In vivo*, a major role in the clearance and degradation of insulin is played by the metalloproteinase insulin-degrading enzyme (IDE)[15], and the IDE has been identified as a candidate gene for diabetes susceptibility in the Goto–Kakizaki rat, a genetic model of non-insulin-dependent diabetes[16]. These animals exhibit elevated blood glucose and insulin levels[16] due to a mutated form of the IDE, which provokes reduced insulin degradation and causes symptoms typical of the human type 2 diabetes mellitus (DM2) syndrome[17]. The evidence for a putative influence of the IDE on the pathogenesis of DM2 has been confirmed with human genetic studies that have linked polymorphisms in the IDE gene to an increased risk for insulin resistance and DM2[18–20]. Furthermore, genome-wide association studies in human subjects have revealed that the IDE region of chromosome 10q contains a variant that confers DM2 risk[21]. Lastly, IDE knockout mice are both glucose-intolerant and hyperinsulinaemic, supporting the concept that the IDE is important in the maintenance of normal blood glucose and insulin levels[22].

Despite the data provided above, the specific effects of the cafeteria diet on insulin production and clearance remain unclear. Given the high prevalence of diet-induced obesity and the importance of this model of study, we aimed to investigate the effects of the cafeteria diet by analysing the pancreas functionality and apoptosis. Moreover, we also evaluated the effects of this diet on the activity and expression of the IDE, which, to our knowledge, have not been studied previously.

Materials and methods

Animal experimental procedures

Wistar female rats (Charles River Laboratories), weighing between 160 and 175 g, were housed in animal quarters at 22°C with a 12 h light–12 h dark cycle, and after 1 week in quarantine, the animals were treated as described previously[23]. Briefly, twelve rats were divided in two groups (n 6): a control group fed with a standard diet and a group fed with a cafeteria diet (Table 1) and water in addition to the standard diet. The animals were fed *ad libitum*, and the food was renewed daily. At the end of the treatment (17 weeks), the food was removed and 3 h later, the animals were killed by beheading. Blood was collected using heparin, and animal tissues were excised, frozen immediately in liquid N_2 and stored at −80°C until analysis. All procedures were approved by the Experimental Animals Ethics Committee of the Rovira i Virgili University.

Table 1. Cafeteria diet composition

Components	Quantity per rat
Bacon	15–20 g
Sweets	1/2
Biscuit with pâté	1
Cheese	1–2 g
Muffins	1/2
Carrots	3 g
Milk with sugar (220 g/l)	50 ml
Water	*Ad libitum*
Standard diet	*Ad libitum*

Intraperitoneal glucose tolerance test and plasma parameters

Intraperitoneal glucose tolerance tests were carried out (2 g glucose/kg body weight) after overnight fasting at week 15 and also 3 d before killing at week 17. Glucose was measured with a glucometer after blood samples had been collected by tail bleeding (Menarini).

Insulin and C-peptide plasma levels at killing were assayed using ELISA methodology (Mercodia) following the manufacturer's instructions. Glucose plasma levels were determined using an enzymatic colorimetric kit (QCA).

Homeostatic model assessment for insulin resistance (HOMA-IR) and the HOMA-β index were calculated using the fasting values of glucose and insulin with the following formulas:

$$\text{HOMA-IR} = \frac{\text{insulin}\,(\mu\text{U/ml}) \times \text{glucose}\,(\text{mM})}{22{\cdot}5},$$

$$\text{HOMA-}\beta = \frac{20 \times \text{insulin}\,(\mu\text{U/ml})}{\text{glucose}\,(\text{mM}) - 3{\cdot}5}.$$

Insulin content in the pancreas

For insulin extraction, the pancreas was homogenised with an acid–ethanol solution (75 % ethanol, 1·0 M-glacial acetic acid and 0·1 M-HCl), and the extracts were kept overnight at 4°C and then centrifuged. The insulin levels from the extracts were measured using ELISA methodology (Mercodia).

TAG content in the pancreas

TAG from the pancreas were extracted using PBS containing 0·1 % Triton X-100 (Sigma-Aldrich), and the levels of TAG were determined using an enzymatic colorimetric kit (QCA). The protein content of each sample was measured using the Bradford method[24] and was used to normalise the TAG values.

Insulin-degrading enzyme activity assay

Liver extracts were prepared by homogenising the tissue in Cytobuster Protein Extraction Reagent (Novagen) according to the manufacturer's recommended protocol. IDE activity was assessed with the InnoZyme Insulysin/IDE Immunocapture Activity Assay Kit (Calbiochem/Merck) and is expressed as relative fluorescent units.

Western blot

Protein was extracted from the whole frozen pancreas using RIPA (radio-immunoprecipitation assay) 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 (phenylmethanesulfonyl fluoride solution). Total protein levels of the lysate were determined using the Bradford method[24]. After boiling for 5 min, 100 μg of protein were loaded and electrophoresed through a 4–15 % SDS-polyacrylamide gel. The samples were then transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories) and blocked at room temperature for 1 h using 5 % (w/v) non-fat milk in TTBS buffer (Tris-buffered saline (TBS) plus 0·5 % (v/v) Tween-20). The membranes were incubated overnight at 4°C with rabbit polyclonal B-cell/lymphoma 2 (BCL2)-associated X protein (BAX) primary antibody (Cell Signaling Technology) at a 1:1000 dilution in blocking solution or rabbit anti-β-actin antibody (diluted 1:750; Sigma-Aldrich). After washing with TTBS, the blots were incubated with peroxidase-conjugated monoclonal anti-rabbit secondary antibody (Sigma-Aldrich) at a 1:10 000 dilution at room temperature for 2 h. The blots were then washed thoroughly in TTBS, followed by TBS. Immunoreactive proteins were visualised with the ECL Plus Western Blotting Detection System (GE Healthcare) using a FluorChem system (Alpha Innotech) and software version 6.0.2. Densitometric analysis of the immunoblots was performed using ImageJ 1.44p software (National Institutes of Health); all of the proteins were quantified relative to the loading control.

Quantitative RT-PCR

Total RNA from the pancreas, liver and mesenteric adipose tissue was extracted using the TRIzol reagent, following the manufacturer's instructions. Complementary DNA was generated using the High-Capacity complementary DNA Reverse Transcription Kit (Applied Biosystems), and it was subjected to quantitative RT-PCR amplification using the Taqman Master Mix (Applied Biosystems). Specific Taqman probes (Applied Biosystems) were used for different genes, as follows: Rn01774648-g1 for insulin; Rn00565839-m1 for IDE; Rn00755591_m1 for pancreatic duodenal homeobox 1 (PDX1); Rn01754856-m1 for mitochondrial uncoupling protein 2 (UCP2); Rn00569117_m1 for fatty acid synthase (FASn); Rn00580702_m1 for carnitine palmitoyltransferase-1a (CPT1a); Rn99999125_m1 for BCL2; Rn01480160_g1 for BAX. β-Actin was used as the reference gene (Rn00667869-m1). The reactions were performed using a quantitative RT-PCR 7300 System (Applied Biosystems) according to the manufacturer's instructions. The relative mRNA expression levels were calculated using the $\Delta\Delta C_t$ method.

Calculations and statistical analysis

The results are expressed as means with their standard errors, and the effects were assessed by Student's *t* test. All of the calculations were performed using SPSS software (SPSS, Inc.).

Results

Cafeteria diet increases insulin production in the pancreas

We first examined the effects of the cafeteria diet on pancreatic insulin production after 17 weeks of the treatment. The cafeteria diet-fed rats showed significantly higher plasma insulin (cafeteria: 0·94 (SEM 0·2) nM *v.* control: 0·18 (SEM 0·0) nM) and C-peptide levels (cafeteria: 2·54 (SEM 0·4) nM *v.* control: 0·77 (SEM 0·0) nM), demonstrating an increase in insulin secretion by the pancreas. After the intraperitoneal glucose tolerance tests at 15 and 17 weeks of the treatment, HOMA-IR and the HOMA-β index were calculated (Table 2). After 15 weeks of cafeteria-diet feeding, the HOMA-IR index indicated that the animals had peripheral insulin resistance, as we mentioned above, and the HOMA-IR index was even higher after two additional weeks of the treatment. In contrast, the values of the HOMA-β index at 15 weeks indicated that there were no significant differences in pancreas functionality, despite peripheral insulin resistance. However, after two more weeks of the treatment (at week 17), the HOMA-β values were significantly increased in the cafeteria-diet treatment group, suggesting that pancreas functionality in terms of the response to glucose was higher than that in the controls to counteract peripheral insulin resistance. We also determined insulin content in the pancreas, which showed that there was significantly more insulin accumulation in the pancreata of the cafeteria-fed rats (cafeteria: 69·90 (SEM 17·2) ng/mg tissue *v.* control: 8·76 (SEM 0·9) ng/mg tissue). The increased insulin secretion and pancreatic insulin content agree with the observed effect on insulin gene expression, which was significantly higher in the obese animals (Table 3). In addition,

Table 2. Effects of the cafeteria diet on homeostatic model assessment for insulin resistance (HOMA-IR) and the homeostatic model assessment-β (HOMA-β) index

(Mean values with their standard errors)

	HOMA-IR				HOMA-β			
	Control		Cafeteria		Control		Cafeteria	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
15 weeks	0·14	0·0	6·24*	2·2	−33·04	34·6	781·11	812·5
17 weeks	0·27	0·6	9·06*	3·6	858·42	1107·4	7009·43*	1058·5

* Mean values were significantly different between the treatments ($P \leq 0{\cdot}05$).

Table 3. Gene expression in the pancreas
(Mean values with their standard errors)

	Control		Cafeteria	
Gene	Mean	SEM	Mean	SEM
Insulin	1·50	0·6	5·11*	1·4
PDX1	1·43	0·5	4·07*	1·0
UCP2	0·89	0·1	1·64**	0·3
BCL2	1·00	0·0	0·72*	0·1
BAX	1·07	0·2	0·86	0·1
CPT1a	1·03	0·1	1·63**	0·2
FASn	1·08	0·2	0·75	0·1

PDX1, pancreatic duodenal homeobox 1; *UCP2*, uncoupling protein 2; *BCL2*, B-cell/lymphoma 2; *BAX*, BCL2-associated X protein; *CPT1a*, carnitine palmitoyltransferase-1a; *FASn*, fatty acid synthase.
Mean values were significantly different between the treatments: *$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}1$.

the cafeteria diet increased the expression of PDX1, an important regulator of insulin transcription (Table 3).

Cafeteria diet activates apoptosis biomarkers

To examine the effects of the cafeteria diet on apoptosis in the pancreas, we analysed the anti-apoptotic marker BCL2 and the pro-apoptotic marker BAX. The cafeteria-fed rats showed a decrease in the expression of BCL2 (Table 3). Concerning BAX expression, although the mRNA levels of this gene were not altered (Table 3), we did observe an increase in the protein levels of BAX in the cafeteria diet-fed group (Fig. 1).

Cafeteria diet increases pancreatic TAG content

Feeding a cafeteria diet leads to a higher amount of TAG and NEFA in the plasma. Under physiological conditions, most

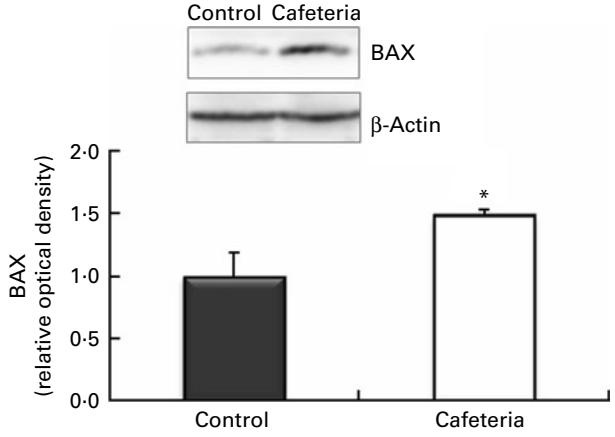

Fig. 1. Effects of the cafeteria diet on B-cell/lymphoma 2-associated X protein (BAX) protein levels in the pancreas. BAX protein levels were quantified by Western blot analysis. A representative Western blot is provided. Protein expression was quantified relative to the β-actin loading control using ImageJ 1.44p software (National Institutes of Health). Values are means of six animals, with their standard errors represented by vertical bars. *Mean values were significantly different from the control group ($P < 0{\cdot}05$).

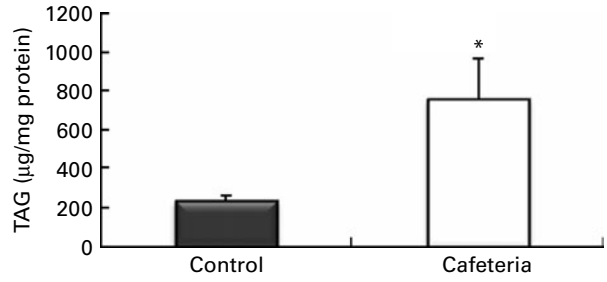

Fig. 2. Effects of the cafeteria diet on pancreatic TAG content. After 17 weeks of the cafeteria diet, the animals were killed, and the pancreas was obtained. The TAG content in the pancreas was measured using an enzymatic colorimetric kit. Values are means of six animals, with their standard errors represented by vertical bars. *Mean values were significantly different from the control group ($P < 0{\cdot}05$).

TAG are stored in adipocytes, but in animals with obesity, increased stores of TAG are detectable in other tissues. To determine whether the cafeteria diet treatment led to an accumulation of lipids in other tissues, we examined the TAG content in the pancreas, and found that it was four times higher in the obese animals than in the control group (Fig. 2). To better understand lipid metabolism in the pancreas, we also analysed the expression of key regulatory genes. We selected the *FASn* gene, the key enzyme of *de novo* fatty acid synthesis[25], and the *CPT1a* gene, the key controller of NEFA oxidation[26]. The cafeteria-fed rats showed a slight increase in CPT1a mRNA levels (Table 3). In contrast, the cafeteria diet tended to reduce the mRNA levels of FASn (Table 3).

It has previously been reported that UCP2 expression is regulated in tandem with the level of NEFA[27,28]; similarly, we observed that the cafeteria-fed rats showed a slight increase in the levels of UCP2 mRNA in the pancreas (Table 3).

Cafeteria diet modifies the activity and expression of insulin-degrading enzyme

The cafeteria diet-fed animals showed higher levels of plasma insulin due to a higher insulin production and secretion, but we speculated that insulin clearance could also have contributed to this effect. Thus, we analysed the gene expression and activity of one of the main factors responsible for insulin clearance: the IDE. Because the liver is an organ with high IDE mRNA and protein levels[29–31], we determined IDE gene expression and activity in this tissue. Fig. 3 shows that IDE enzyme activity in the liver was increased by the cafeteria diet, and IDE mRNA levels were also slightly increased. This effect was stronger when the whole tissue weight was considered, which better correlates with the real capacity of liver IDE to remove insulin (Table 4). To analyse further the capacity of the cafeteria-fed rats to degrade insulin, we also determined IDE gene expression in the kidney, an important site of insulin clearance from the systemic circulation. The results did not show any effect of the cafeteria diet on renal IDE or when considering the total tissue weight. Furthermore, we did not observe any effect due to the cafeteria diet in white

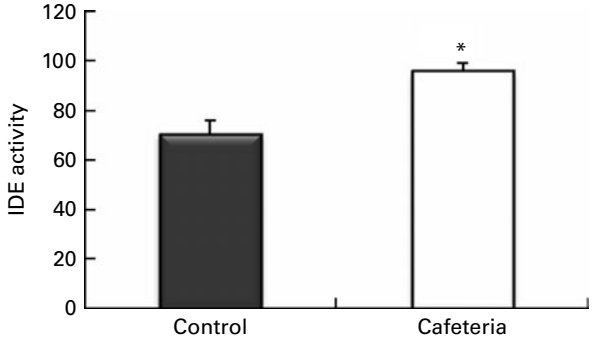

Fig. 3. Effects of the cafeteria diet on insulin-degrading enzyme (IDE) activity in the liver. IDE activity from liver samples was determined with an immunocapture-based fluorometric assay. Values are means of six animals, with their standard errors represented by vertical bars. * Mean values were significantly different from the control group ($P<0·05$).

adipose tissue, where the IDE is also expressed (Table 4). However, when considering the total tissue weight, the cafeteria-fed rats had a higher capacity to remove insulin due to the IDE in the adipose tissue.

Discussion

Obesity, driven by an excess of fat intake, leads to a toxic lipid accumulation in non-adipose tissues, which is accompanied by insulin resistance. Concurrently, the increased insulin demand promotes a β-cell compensation that involves increased pancreas functionality and/or increased β-cell mass. Insulin resistance may develop into DM2, as driven by β-cell failure[12,32,33].

The present study was designed to examine the effects of the cafeteria diet on insulin production by evaluating pancreas functionality and apoptosis and the activity and expression of the IDE, as an estimation of insulin clearance. We had previously shown that 17 weeks of feeding a cafeteria diet led to insulin resistance[23]. The fact that plasma TAG and fatty acids are increased by the cafeteria diet[4,34,35] indicates a failure in the adipose tissue, which is unable to remove the lipids from the circulation and might not respond to the insulin inhibition of lipolysis. In the present study, we found that TAG content in the pancreas was already increased by 17 weeks. The source of lipids for this increased TAG storage might be plasma NEFA, as under conditions of high NEFA, *de novo* fatty acid synthesis is inhibited, a condition that is in agreement with the present gene expression results for *FASn*. The lack of malonyl-CoA, secondary to the inhibited *de novo* fatty acid synthesis, avoids the main CPT1a-inhibitory regulation, thus allowing the entrance of fatty acids into the mitochondria for oxidation. In fact, we found a higher gene expression of *CPT1a*, suggesting that, in the pancreas, increased fatty acid β-oxidation occurred to counteract lipid accumulation. Increased fatty acid oxidation has also been observed in the rat insulinoma cell line INS-1 treated with oleate and palmitate for 72 h[36], and has been associated with impaired glucose-induced insulin secretion in INS-1 β-cells[36,37] and islets[38]. Defects in glucose-stimulated insulin secretion in INS-1 cells after chronic fatty acid treatment could have been related, in part, to an increase in UCP2 mRNA that is associated with uncoupled mitochondria[39,40]. We observed a tendency for the up-regulation in pancreatic *UCP2* gene expression after feeding the cafeteria diet. Based on the present results, we cannot discern whether the individual β-cell insulin-secretory functionality was modified due to the cafeteria diet; however, we did find that, despite this increased lipid accumulation, the pancreata of the cafeteria diet-treated rats were still able to respond to an acute glucose load, with a similar increment in plasma insulin as the control rats, and, in fact, the HOMA-β index in the cafeteria-fed rats was better. To counteract peripheral insulin resistance, the cafeteria diet-treated animals exhibited increased insulin synthesis and secretion. We found higher insulin protein and gene expression in the pancreata of the cafeteria-treated rats and an up-regulation of *PDX1*, a transcriptional controller of insulin gene expression[41]. Altogether, it is likely that the increased amount of insulin and up-regulated insulin and *PDX1* gene expression in the pancreas is due to an increase in β-cells. In mice, a high-fat diet leads to increased β-cell mass[42,43], and treatment with a cafeteria diet in rats has been shown to lead to larger pancreatic islets, although their functionality was not evaluated in that study[4]. Despite the results showing that pancreas still responded to a glucose load after the cafeteria diet treatment, we also observed that there were signs of apoptosis in the pancreas, specifically the down-regulation of BCL2 expression and increased BAX protein levels. Although we found that the *BAX* gene expression was not altered, changes in BAX protein levels without changes in gene expression have previously been shown to correlate with apoptosis[44]. *In vitro* studies have

Table 4. Insulin-degrading enzyme gene expression in the liver, white adipose tissue (WAT) and kidney
(Mean values with their standard errors)

		Control		Cafeteria	
Tissue		Mean	SEM	Mean	SEM
Liver	Gene expression relative to the control	0·94	0·1	1·23**	0·1
	Relative gene expression × tissue weight	9·74	1·1	16·46*	1·9
WAT	Gene expression relative to the control	1·00	0·0	0·91	0·0
	Relative gene expression × tissue weight	3·99	0·5	18·52*	3·0
Kidney	Gene expression relative to the control	1·01	0·1	0·94	0·1
	Relative gene expression × tissue weight	1·62	0·2	1·95	0·1

Mean values were significantly different between the treatments: *$P\leq0·05$, **$P\leq0·1$.

reported that chronic hyperglycaemia and high NEFA can induce β-cell apoptosis[45,46]. In Zucker diabetic fatty rats, obesity, chronic hyperglycaemia and worsening insulin resistance have been shown to lead ultimately to β-cell apoptosis[47,48]. In addition, male C57BL/6J mice fed a high-fat diet (60 % fat) for 8[42] or 12 weeks[43] have shown increased islet β-cell apoptosis. However, different results have also been reported, as a 60 % high-fat diet did not induce pancreatic apoptosis in mice[49]. In the present study, we showed that in rats, hyperglycaemia, increased fatty acids and/or pancreatic lipid accumulation derived from a 17-week cafeteria diet triggered the apoptosis process in the pancreata of rats, although this pancreatic damage was not reflected in insulin plasma concentrations.

We also focused on another important step for the regulation of glucose metabolism, the insulin clearance, specifically, one of its main regulators, the IDE. Alterations in gene coding for this enzyme have been linked with diabetes susceptibility[16–21]. In adipocytes isolated from human patients, insulin degradation (probably mediated by the IDE) has been demonstrated to be reduced in pre-diabetic and diabetic states[50]. In mice, the lack of the IDE leads to glucose intolerance and hyperinsulinaemia[22]. The effects of the diet on the expression of the IDE in the brain have been studied in the context of insulin resistance as an underlying mechanism that is responsible for an increased risk of Alzheimer's disease and, thus, of the possible involvement of cerebral IDE in the development of β-amyloidosis. In APP/PS1 double transgenic mice (which develop memory deficits and amyloid plaques), providing 10 % sucrose-sweetened water *ad libitum* has been reported to not statistically modify brain IDE protein levels[51]. In another model of Alzheimer-disease-like neuropathology, the feeding of Tg2576 mice with a high-fat diet for 9 months led to insulin resistance and decreased IDE activity and protein expression in the brain[52]. However, the effects of a high-fat diet on the IDE in tissues responsible for insulin clearance and degradation have, to our knowledge, not been studied thus far. The present results showed that the liver activity of the IDE was increased in the cafeteria-fed animals, which could have been partially due to an up-regulation of its gene expression. We did not find the same effects in other tissues. Despite the role of the kidney in degrading insulin[15], we did not observe any effects of the cafeteria diet on renal IDE gene expression, nor did we find any modification of IDE gene expression in adipose tissue. Because we are studying obesity, we must also consider the tissue size, which is mainly relevant for the adipose tissue. When the adipose tissue weight is taken into consideration, the insulin clearance potential is higher in cafeteria diet-treated rats[53]. This has been described in fa/fa genetically obese animals and in obese human subjects, where the potential of insulin cleavage by adipose tissue in obese patients was higher than that in the controls, implying that both insulin secretion and turnover are increased in obese individuals[54]. When considering the whole amount of tissue, the cafeteria diet-fed rats in the present study exhibited a higher capacity to degrade insulin. This fact suggests that feeding a high-fat diet is accompanied by a mechanism to eliminate high plasma insulin levels, and at least with the experimental procedure used in the present study (cafeteria-diet for 17 weeks), it appears that hyperinsulinaemia was not due to impaired insulin clearance but that it was counteracted by enhanced insulin degradation. The present results are not in agreement with previous studies suggesting that hepatic insulin degradation may be reduced as an adaptive mechanism to relieve stress on pancreatic β-cells imposed by insulin resistance that is induced by a high-fat diet[55,56]. Remarkably, the experimental models differ vastly, suggesting that there could be different stages in diet-induced obesity and insulin resistance with different degrees of involvement of hepatic glucose clearance. Thus, the cafeteria diet acts on IDE expression and activity in the liver; it does not directly modulate IDE activity in adipose tissue. However, the increased amount of such tissue induced by the feeding of a cafeteria diet would contribute to the amount of insulin clearance.

In conclusion, we showed that the cafeteria diet treatment for 17 weeks in rats mimicked a pre-diabetic state with ectopic lipid accumulation in the pancreas. At this time point, the insulin content and gene expression in the pancreas were higher in the cafeteria diet-treated group than in the control rats, a condition that leads to hyperinsulinaemia. In addition, initial signs of apoptosis appeared in the pancreas. We also showed that IDE-mediated insulin clearance capability was higher in the cafeteria diet-treated rats than in the controls.

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