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Soybean oil treatment impairs glucose-stimulated insulin secretion and changes fatty acid composition of normal and diabetic islets

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Abstract We investigated the effect of sub-chronic soybean oil (SO) treatment on the insulin secretion and fatty acid composition of islets of Langerhans obtained from Goto-Kakizaki (GK), a model of type 2 diabetes, and normal Wistar rats. We observed that soybean-treated Wistar rats present insulin resistance and defective islet insulin secretion when compared with untreated Wistar rats. The decrease in insulin secretion occurred at all concentrations of glucose and arginine tested. Furthermore we observed that soybean-treated normal islets present a significant decrease in two saturated fatty acids, myristic and heneicosanoic acids, and one monounsaturated eicosenoic acid, and the appearance of the monounsaturated erucic acid. Concerning diabetic animals, we observed that soybean-treated diabetic rats, when compared with untreated GK rats, present an increase in plasma non-fasting free fatty acids, an exacerbation of islet insulin secretion impairment in all conditions tested and a significant decrease in the monounsaturated palmitoleic acid. Altogether our results show that SO treatment results in a decrease of insulin secretion and alterations on fatty acid composition in normal and diabetic islets. Furthermore, the impairment of insulin secretion, islet erucic acid and fasting plasma insulin levels are similar in treated normal and untreated diabetic rats, suggesting that SO could have a deleterious effect on β-cell function and insulin sensitivity.

Key words Insulin secretion • Fatty acids • Islets of Langerhans • GK rats • Diabetes mellitus

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Introduction

Type 2 diabetes is one of the most common metabolic diseases. Chronic hyperglycaemia is a consequence of the interplay between β-cell dysfunction, insulin resistance and elevated hepatic glucose production [1]. Changes in dietetic habits and the increase in sedentary life are the main causes of the rise of the prevalence of type 2 diabetes [1, 2].

The similarity between fatty acids' effects on the insulin secretory response of isolated islets and the mechanisms underlying type 2 diabetes has been described [3, 4]. The influence of free fatty acid (FFA) on insulin secretion is associated with the suppression of peripheral glucose uptake, stimulation of hepatic gluconeogenesis, defect in islet glucose recognition and fatty acid oxidation, as stated in the fuel concept and the Randle cycle hypothesis, the latter also known as the glucose–fatty acid cycle [5–10]. More recently, the action of fatty acids on

insulin secretion has been related to a mechanism involving modulation of uncoupling protein-2 (UCP-2) expression [11]. In cells, fatty acids are mainly localised in membrane phospholipids and are a major component of intracellular diacylglycerol, phosphatidic acid and triacylglycerol [12, 13]. Thus, it is clear that changes in the contents of fatty acids can influence membrane structure and function of islet cells, namely the activities of β-cell channels and receptors such as K^+, Ca^{2+} and voltage-sensitive channels, glucose transporters, and adenylate cyclase-coupled and phospholipase C-coupled receptors [14]. Furthermore, phospholipids, diacylglycerol, phosphatidic acid and triacylglycerol are implicated in different cellular mechanisms, as they can be precursors or generators of important molecules such as prostaglandins and ceramides and they are involved also in gene expression, influencing a range of glucose and lipid signalling pathways [8, 10, 13–17].

Accumulating evidence suggests that chain length, spatial conformation and degree of saturation of FFAs profoundly influence the insulinotropic potency and the stimulation of K_{ATP} channel activity [7, 16, 18–20]. Studies focussed on fatty acids chain length reported that the increase of the number of carbons has a negative effect on insulin release by increasing β-cell K_{ATP} channel activity and induces uncoupling of β-cell oxidative phosphorylation [7, 16, 18]. Concerning the spatial conformation, *trans* fatty acids induce better results in insulin release than the corresponding *cis* isoforms [21]. However, it has been shown that *cis* monounsaturated fatty acids (MUFAs) have a positive effect on glycaemic control [16, 22].

Recently, it has been reported that diets based on polyunsaturated fatty acids have a higher protective effect in diabetic patients at cardiovascular risk when compared with those based on MUFAs or saturated fatty acids [22]. Previous studies suggested that a MUFA-enriched diet may be useful in the treatment of patients with glucose intolerance or type 2 diabetes [23]. However, studies showing a positive association between saturated fatty acids and a higher risk of type 2 diabetes are more consistent [18, 20, 24]. It has been shown that palmitic acid (C16:0) is toxic to β-cells, even at moderate levels [19, 25]. This fatty acid affects glucose-induced insulin gene expression, ceramide synthesis and activates mitochondrial apoptotic pathways [13, 15, 26–28]. In contrast, the monounsaturated palmitoleic acid (C16:1) promotes β-cell proliferation at low glucose concentrations, improves β-cell function and counteracts the negative effects of palmitic acid [27]. Along the same line, it has been shown that the saturated stearic acid (C18:0) has proapoptotic properties unlike equivalent concentrations of the polyunsaturated linoleic acid (C18:2n6c) [15, 29]. Concerning the monounsaturated oleic acid (C18:1n9c), contradictory results about its proapoptotic properties have been obtained [15, 30].

There is controversial evidence concerning the effects of soybean (oil, diet, protein) treatment. Lipids based on soybean oil (SO) are traditionally used as nutritional support for surgical or critical patients [31, 32]. Some studies with normal rats on a SO diet reported an increase in body weight and fat gain as well as an impairment of glucose tolerance [33] and insulin resistance [34, 35]. However, a rise of glucose-stimulated insulin secretion (GSIS) was also observed [36]. Dietary soybean has positive effects on lipid and glucose levels and cardiovascular risk markers [37]. Furthermore, SO is a vegetal oil rich in antioxidants. This property has been explored and discussed in studies of oxidative stress [35].

Previous studies performed on Goto-Kakizaki (GK) rats, a spontaneously type 2 diabetic animal model, on a high-fat diet, demonstrated impaired GSIS [11] or an enhancement of insulin secretion during oral glucose tolerance test [38]. It has been shown that in Wistar (W) normal rats, high-fat feeding has no effect on islet insulin secretion, insulin content and insulin mRNA level [11], but induces insulin resistance [38].

This study was designed to examine and clarify the effects of sub-chronic (7 days) SO treatment on insulin secretion and fatty acid composition of islets of Langerhans obtained from diabetic GK and normal W rats.

Research design and methods

Materials

Liberase was supplied by Roche Diagnostic GmbH, Mannheim, Germany. Guinea pig-rat insulin serum was obtained from Biotrend Chemikalien, GmbH, Germany and rat insulin standard from Linco Research Inc, USA. All the other chemicals were supplied by Sigma Chemicals Co., USA or Merck Darmstad, Germany and were of the highest grade of purity commercially available.

Animals

Female spontaneously diabetic GK rats (aged 12 weeks) were obtained from our local breeding colony (Laboratory Research Center, University Hospital, Coimbra, Portugal). Non-diabetic female Wistar rats, of similar age, were also obtained from our local colony. They were kept under controlled light (12 h/12 h day/night cycle), temperature (22–24°C) and humidity (50–60%) conditions and had free access to water and powdered rodent chow (diet AO4-Panlab, Spain). W and GK rats were divided in two groups [untreated (W, GK) and a soybean oil treated (Ws, GKs) for 7 days (2 ml/kg/day, i.p.)]. Experiments were conducted 24 h after the last injection. Adhering to procedures approved by the Institutional Animal Care and Use Committee, the animals were sacrificed by cervical displacement.

Body weight and blood glucose determination

Body weight was measured before sacrifice, in fed animals. Blood glucose levels were determined through the glucose-oxidase reaction using a glucometer and compatible reactive tests (glucometer-Elite-Bayer, Portugal) after an overnight fast and 2 h after intraperitoneal glucose load (1.8 g/kg body weight).

Plasma insulin and free fatty acid determinations

Blood samples (1 ml) were taken by cardiac puncture from anaesthetised animals (non-fasting and overnight fasting) with ketamine chloride (75 mg/kg, i.m.; Parke Davis, Ann Arbor, MI, USA) and chlorpromazine chloride (2.65 mg/kg, i.m., Laboratórios Victoria, Portugal). Plasma was immediately stored at -20°C for the measurement of plasma insulin and FFAs.

Plasma insulin levels were determinated by competitive enzyme-linked immunosorbent assay (ELISA; see 'Insulin assay' section) and FFA levels by Half-Micro test (Roche Applied Science, Penzberg, Germany).

Insulin sensitivity

Insulin sensitivity was measured through two different indexes: homeostasis model assessment (HOMA) calculated as (G0)x(I0)/22.5, where G0 is fasting glucose (mmol/l) and I0 is fasting insulin (μU/ml) and quantitative insulin-sensitivity check index (QUICKI), calculated as 1/[log(G0)+log(I0)], where G0 is fasting glucose (mg/dl) and I0 is fasting insulin (μU/ml) [39].

Islet isolation

Rat pancreatic islets were isolated from fed animals by liberase digestion, as previously described [40]. For fatty acid analysis, isolated islets (n=200/aliquot) were stored at -20°C.

Islet insulin release samples preparation

Isolated islets were pre-incubated for 60 min in Krebs medium supplemented with 8 mM glucose and 30 mg/ml bovine serum albumin (BSA) protease free. The medium was replaced with Krebs solution (in mM: 120 NaCl; 5 KCl; 25 $NaHCO_3$; 1.13 $MgCl_2 \cdot 6H_2O$; 2.56 $CaCl_2 \cdot 2H_2O$; gassed with 95% O_2–5% CO_2; pH 7.4) containing 2 mM glucose and 1 mg/ml BSA, for 30 min. Then, insulin released from isolated islets was measured under static incubation conditions using 5 islets/well plated on a 12-well cluster incubated with a particular secretagogue [2 ml Krebs solution containing glucose (2, 8 or 11 mM) or arginine (20 mM) plus glucose (11 mM) and 1 mg/ml BSA]. After 30 min, samples were collected and stored at -20°C for subsequent insulin measurement.

Insulin assay

The levels of plasma insulin and insulin released from islets were measured by competitive ELISA with the insulin-capturing antibody immobilised directly in the solid phase as previously described [40, 41]. Immunoglobulin G-certified microtitre plates (96 wells, Nunc, Roskilde, Denmark) were incubated overnight at 4°C, with guinea pig-rat insulin serum and rat insulin used as standard. Plasma and islet insulin samples were diluted in PBS and Hepes buffers, respectively, and supplemented with BSA. Each condition was run in duplicate. 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system (Sigma Chemicals Co., USA) was used as substrate for the insulin-peroxidase conjugate and the reaction product was assayed spectrophotometrically at 450 nm (Microplate reader – Model 550, Bio-Rad), after a 30-min incubation period with TMB at room temperature (the reaction was stopped with sulphuric acid).

Insulin released from islets was expressed as fmol/5 islets/30 min and plasma insulin levels as pmol/l.

Islet fatty acid analysis

Fatty acid composition was analysed in isolated islets obtained from untreated and treated W and GK rats. Fatty acid composition in SO was also measured.

We prepared fatty acid methyl esters (FAMEs) according to a procedure previously described [42], with slight modifications [43]. Briefly, lipids were extracted from Langerhans islet with methanol/chloroform (1:2). The mixture was centrifugated at 4°C. The upper layer was removed by aspiration and the interphase by filtration. The filtrate was evaporated to dryness under a N_2 flow and the residue was redissolved in chloroform. N_2 was blown over the samples to avoid fatty acid oxidation. The FAME was prepared using 5% HCl in methanol that was used as a derivatising reagent, at 60°C for 3 h. FAMEs were analysed with a Perkin–Elmer gas chromatograph adapted for capillary columns with helium as the carrier gas. The linear velocity was 35 cm/s with a flow rate of 4.0 ml/min. We used an Alltech capillary column (AT-SILAR 30 mx0.53 mm IDx0.5 μ) with a temperature programme as follows: 5 min at 140°C; 4°C/min to 210°C; 10 min at the upper temperature. The results were calculated using appropriate integration software (JCL 6000 chromatography data system).

Unsaturation index (UI) was calculated according to the formula, UI=Σ% of unsaturated fatty acidsxnumber of double bonds of each unsaturated fatty acid [43].

Statistical analysis

Data were expressed as means±SEM of the indicated number of experiments. Statistical significance was tested using unpaired Student's t-test; $p<0.05$ was considered statistically significant.

Results

Body weight, blood glucose and plasma insulin levels

As shown in Table 1, body weight of diabetic rats is significantly lower, while blood glucose levels 2 h after glucose load are significantly higher when compared with normal animals. With SO treatment, normal and diabetic rats did not present significant alterations in body weight and blood glucose levels 2 h after glucose load (Table 1) when compared with normal and diabetic untreated rats, respectively.

It was also observed that fasting plasma insulin (113.6%), blood glucose (72.2%) levels and HOMA index (120.0%) are significantly higher and QUICKI (18.2%) significantly lower in untreated GK rats when compared with untreated W rats (Fig. 1). In normal rats, SO treatment did not change fasting blood glucose levels but significantly increased fasting insulinaemia (146%; Fig. 1b) and altered HOMA and QUICKIs (Fig. 1c and d). In diabetic rats, SO significantly decreased fasting blood glucose levels (34%; Fig. 1a). In these animals, fasting plasma insulin levels and HOMA and QUICKIs did not present any statistical difference (Fig. 1b, c and d).

Table 1 Body weight, glucose levels measured 2 h after glucose load and non-fasting and fasting plasma FFAs in treated and untreated non-diabetic and diabetic rats

	Non-diabetic rats		Diabetic rats	
	W	Ws	GK	GKs
Body weight (g)	247±5.2	267±4.8	203±4.5***	194±3.7
Glucose level 2 h after glucose load (mmol/l)	87±4.3	90±3.8	318.4±16.9***	295±16.8
Non-fasting plasma FFA (mM)	0.28±0.08	0.21±0.07	0.21±0.05	0.42±0.06§
Fasting plasma FFA (mM)	0.84±0.04	0.64±0.1	0.71±0.1	0.61±0.1

W, untreated Wistar rats; *Ws*, SO-treated Wistar rats; *GK*, untreated Goto-Kakizaki rats; *GKs*, SO-treated Goto-Kakizaki rats. The values shown represent the means±SEM of n=8–20 animals in each group. ***p<0.005, *vs.* untreated Wistar. §p<0.05, *vs.* untreated GK

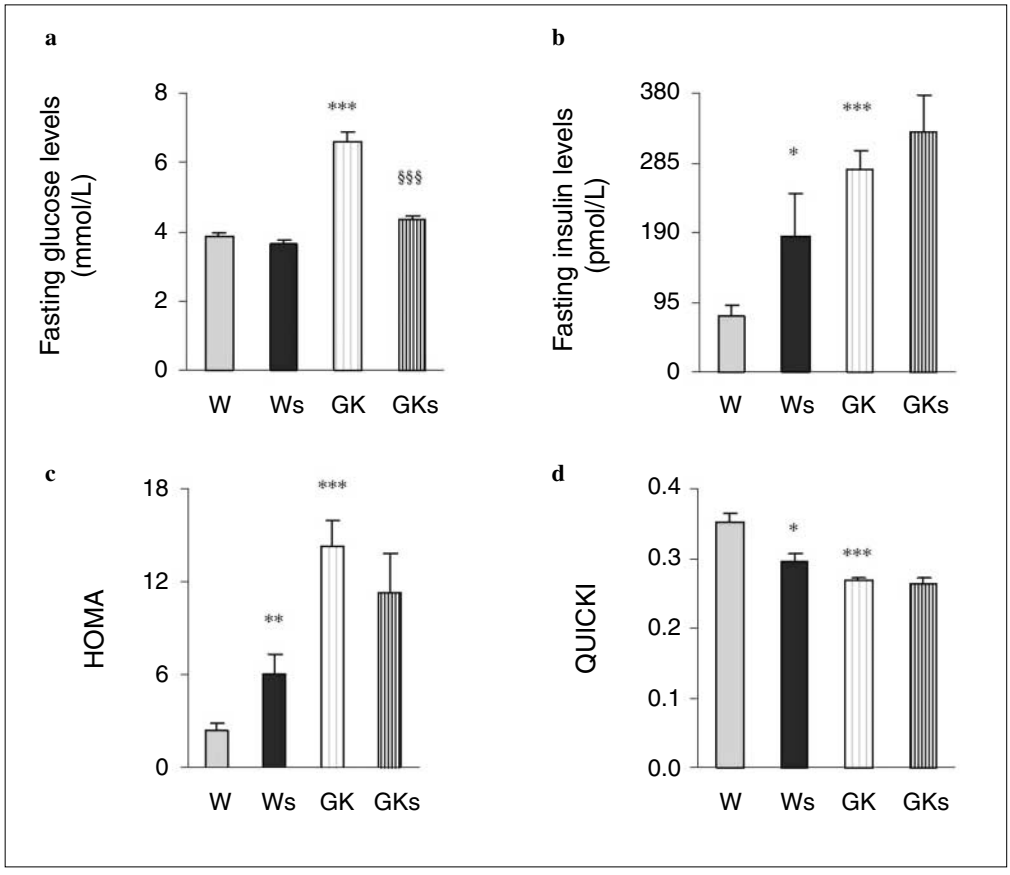

Fig. 1 Fasting blood glucose (**a**), fasting plasma insulin (**b**), HOMA index (**c**) and QUICKI (**d**) in non-diabetic and diabetic rats. *W*, untreated Wistar rats; *Ws*, SO-treated Wistar rats; *GK*, untreated Goto Kakizaki rats; *GKs*, SO-treated Goto Kakizaki rats. The values shown represent the means±SEM of n=8–17 animals in each group. *p<0.05; **p<0.01; ***p<0.005, *vs.* untreated Wistar. §§§p<0.005, *vs.* untreated GK

Plasma free fatty acids levels

Diabetes *per se* and SO treatment in normal rats did not induce a significant alteration in plasma fasting and non-fasting FFAs levels (Table 1). However, in diabetic rats, SO treatment significantly increased (100%) non-fasting FFA levels (Table 1).

Islet insulin secretion

Isolated islets from untreated diabetic rats were not responsive to glucose. Under static incubation conditions we observed an impairment of GSIS (G2, 46%; G8, 74.1%; G11, 81.1%), when compared with untreated normal islets (Fig. 2a). SO treatment induced an impairment of isolated islet insulin secretion from normal rats (G2, 64%; G8, 70%; G11, 76%; G11+arg, 57%; Fig. 2b) and worsened insulin response of diabetic islets (G2, 69%; G8, 68%; G11, 65%; G11+arg 20, 75%; Fig. 2c).

Islet insulin response from SO-treated W rats was similar to that of untreated GK rats (Fig. 2d).

Fatty acid composition of isolated islets

When we compared islet fatty acid composition in both untreated groups of animals (Fig. 3a), we observed that diabetic animals present a decrease in myristic acid (C14:0) (47.8%) and an increase in palmitoleic acid (C16:1) (114.3%) levels. Furthermore, the presence of the monounsaturated erucic acid (C22:1) is observed in diabetic islets (Fig. 3a).

Treated normal rats presented a significant decrease (Fig. 3b) in two saturated fatty acids, myristic (C14:0) (34%) and heneicosanoic (C21:0) (72%) acids and one MUFA, eicosenoic acid (C20:1) (68%). Furthermore, SO treatment induced the appearance of the monounsaturated erucic acid (C22:1) (Fig. 3b and d). In diabetic rats, SO treatment induced a significant decrease in the monounsaturated palmitoleic acid (C16:1) (53%; Fig. 3c). However, SO did not induce a significant alteration in the saturation/unsaturation ratio, unsaturation index and in the percentage of saturated, polyunsaturated, monounsaturated and total unsaturated fatty acids when islets isolated from the four different groups of animals were compared (Table 2).

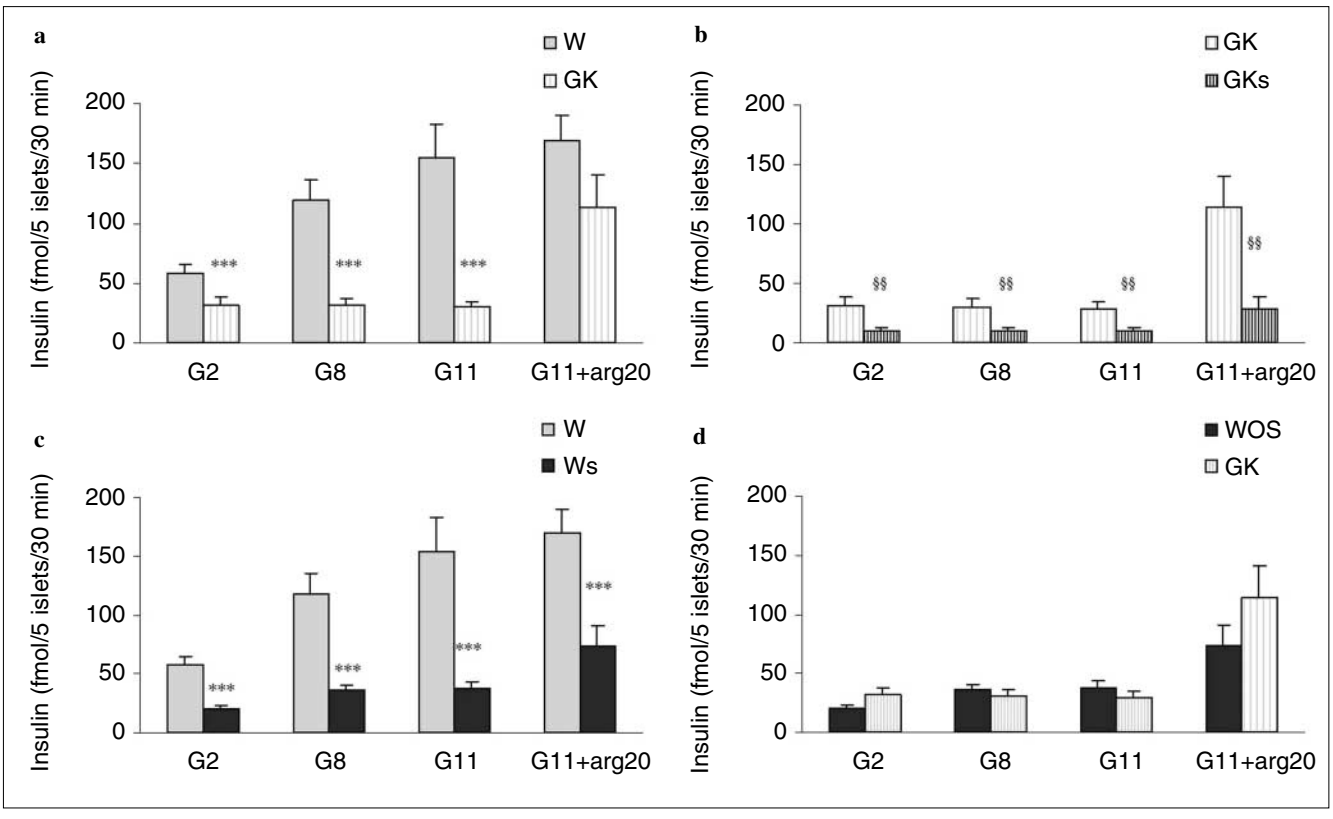

Fig. 2 *The effects of glucose-induced and glucose plus arginine-induced insulin secretion from non-diabetic and diabetic islets isolated from untreated and treated animals*. **a** Comparison between untreated Wistar and GK rats. **b** Comparison between untreated and treated Wistar rats. **c** Comparison between untreated and treated GK rats. **d** Comparison between treated Wistar and untreated GK rats. *W*, untreated Wistar rats; *Ws*, SO-treated Wistar rats; *GK*, untreated Goto Kakizaki rats; *GKs*, SO-treated Goto Kakizaki rats. *G2*, 2 mM glucose; *G8*, 8 mM glucose; *G11*, 11 mM glucose; *G11+arg20*, 11 mM glucose+20 mM arginine. The values shown represent the means±SEM of n=17–28 experiments in each group. ***p<0.005, *vs.* untreated Wistar. §§p<0.01, *vs.* untreated GK

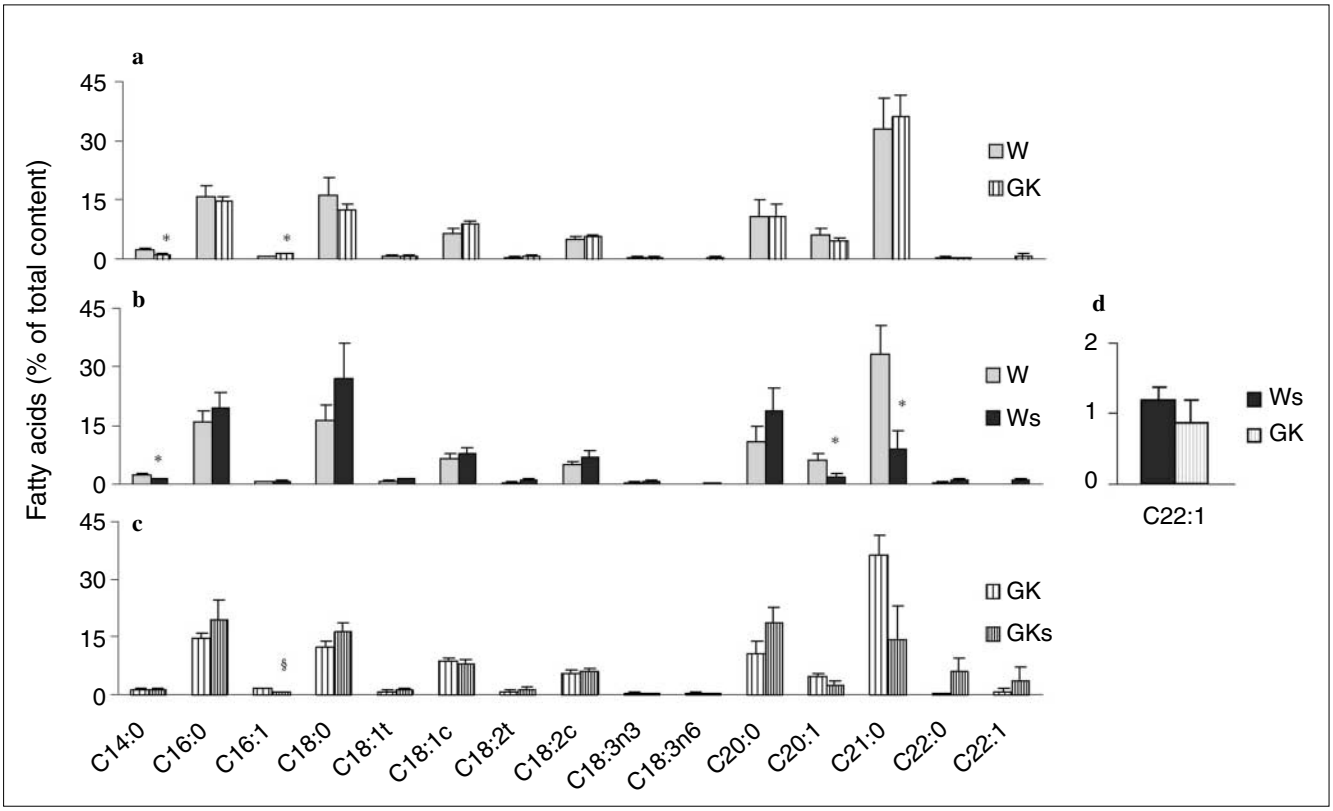

Fig. 3 *Fatty acid composition of non-diabetic and diabetic islets isolated from untreated and treated animals*. **a** Comparison between untreated Wistar and GK rats. **b** Comparison between untreated and treated Wistar rats. **c** Comparison between untreated and treated GK rats. **d** Comparison between treated Wistar and untreated GK rats. *W*, untreated Wistar rats; *Ws*, SO-treated Wistar rats; *GK*, untreated Goto Kakizaki rats; *GKs*, SO-treated Goto Kakizaki rats. The values shown represent the means±SEM of n=6–9 animals in each group. *p<0.05, *vs.* untreated Wistar. §p<0.05, *vs.* untreated GK

Table 2 Islet fatty acids of non-diabetic and diabetic rats untreated and treated with soybean oil

	Non-diabetic rats		Diabetic rats	
	W	Ws	GK	GKs
Saturated (%)	79.2±1.8	77.2±3.2	75.5±0.8	76.1±2.3
Polyunsaturated (%)	6.2±1.3	9.5±1.6	7.6±1.1	8.0±0.8
Monounsaturated (%)	14.6±1.3	13.3±2.5	17.0±1.3	15.9±2.4
Total unsaturated (%)	20.8±1.8	22.8±3.2	24.5±0.8	23.9±2.3
Unsaturated/saturated	0.27±0.03	0.31±0.05	0.33±0.01	0.32±0.04
Unsaturated index	27.7±2.7	33.5±4.4	33.0±1.5	32.4±2.6

W, untreated Wistar rats; *Ws*, SO-treated Wistar rats; *GK*, untreated Goto-Kakizaki rats; *GKs*, SO-treated Goto-Kakizaki rats. The values shown represent the means±SEM of n=6–9 animals in each group

Discussion

The GK rat is a spontaneously non-obese diabetic animal model, produced by selective inbreeding of Wistar rats presenting the highest glucose levels during the oral glucose tolerance test [44]. This animal model is characterised by β-cell mass reduction, β-cell dysfunction and insulin resistance leading to diabetes mellitus [40, 45, 46].

We observed that diabetic GK rats have lower body weight, increased fasting insulinaemia, higher levels of glucose, fasting and 2 h after glucose load, and insulin resistance (Table 1, Fig. 1), when compared with normal W rats. These results are in agreement with previous studies [40, 45, 46]. However, there is no significant difference in non-fasting and fasting FFA levels between normal and diabetic rats (Table 1), suggesting that the cycle Randle hypothesis is not involved in the diabetic syn-

drome at this age. The cycle Randle hypothesis, also known as the glucose–fatty acid cycle, postulates that the increase of fatty acid oxidation causes a commensurate decrease in glucose oxidation, leading to a decrease in glucose uptake and hyperglycaemia [6]. We also observed that in GK rats GSIS is drastically inhibited when isolated islets were exposed to 2, 8 and 11 mM of glucose (Fig. 2a), which is in accordance with previous studies from our laboratory [40].

Analysing the fatty acid composition of untreated diabetic islets, we observed a significant decrease in saturated myristic acid (C14:0), an increase in monounsaturated palmitoleic acid (C16:1) and the presence of the monounsaturated erucic acid (C22:1) (Fig. 3a) when compared with untreated normal animals. It has been reported that impaired GSIS observed in GK rats is associated with alterations in β-cell glucose metabolism, protein kinase C pathway, modulation of UCP-2 expression, acetylcholine and oxidative stress [11, 26, 47–49]. However, it can also be associated with fatty acid content alterations. Fatty acids are key components of cellular membrane phospholipids and are involved in intracellular mechanisms, as previously described [12, 13]. Previous studies reported the stimulatory effect of the monounsaturated palmitoleic acid (C16:1) on K_{ATP} channel activity [16] and promotion of β-cell function and proliferation at low glucose concentrations [27]. Although we observed a significant increase in palmitoleic acid (C16:1) in untreated diabetic islets (Fig. 3a), this increase is not sufficient to recover the secretory capacity of islets. However, this may represent an attempt of diabetic islets to recover their function. This idea is also supported by the appearance of the C22:1 fatty acid in untreated GK rats. The decrease in insulin secretion in the presence of long-chain fatty acids, like C22:1 fatty acid, is in agreement with previous studies [7, 16].

SO is one of the most highly consumed oils worldwide. In humans, increased fat consumption is closely related to type 2 diabetes mellitus. Thus, our major goal was to investigate the effects of SO sub-chronic (7 days) treatment on insulin response of glucose-stimulated islets isolated from diabetic GK and normal W rats as well as its effect on islet fatty acid composition. In normal rats treated with SO, the increase in fasting plasma insulin and maintenance of fasting glucose levels results in a significant increase of HOMA and significant decrease of QUICKIs (Fig. 1), indicating an increase in insulin resistance. Shang and colleagues [38] demonstrated that Wistar rats fed with a high-fat diet for 2 months did not present alterations in glucose tolerance although enhanced insulin secretion during oral glucose tolerance test was observed. Wistar rats did not change body weight and plasma FFAs levels under SO treatment (Table 1), indicating a normal FFA uptake [50]. We also observed that GSIS from pancreatic islets isolated from treated Wistar rats was drastically inhibited. The impairment of insulin response was observed at all concentrations of glucose and arginine tested (Fig. 2b). Although GSIS decreased with SO treatment, plasma FFA levels did not increase. Therefore, we excluded the glucose–fatty acid cycle hypothesis, which implies the increase of plasma FFAs and the impairment of β-cell responsiveness to glucose [5]. Tinahones and colleagues [12] suggested that secretion failure could be related to alterations of islet fatty acids. Concerning this issue we observed that treated Wistar rats present a significant decrease in two saturated fatty acids, myristic (C14:0) and heneicosanoic (C21:0) acids, and one MUFA, eicosenoic acid (C20:1), and the appearance of the monounsaturated erucic acid (C22:1) (Fig. 3b). The appearance of the long-chain C22:1 fatty acid and the impairment of insulin secretion were consistent with previous studies reporting that the increase of the number of carbons has a negative effect on insulin release by increasing β-cell K_{ATP} channel activity and inducing the uncoupling of β-cell oxidative phosphorylation [7, 16]. Other studies show that saturated fatty acids are associated with a higher risk of type 2 diabetes [18, 20, 24]. In our study, the decrease in saturated fatty acids observed in islets isolated from treated W rats may represent an attempt of animals to circumvent the β-cell secretory deficiency. Furthermore, our results indicate that islet fatty acid alterations are positively associated with GSIS impairment (Figs. 2b and 3b).

In summary, we observed that in normal Wistar rats, SO treatment induces insulin resistance and islet insulin secretion failure, which is in accordance with previous studies [2, 20, 34, 35]. Furthermore, the impairment of GSIS observed (Fig. 2b) could be the result of islet fatty acid alterations. This suggestion is supported by previous studies stating that besides the involvement of FFAs in membrane phospholipids, they also act as lipid-signalling molecules [12, 14, 17].

SO treatment in GK rats significantly decreased fasting glucose levels but did not induce significant alterations in HOMA, QUICKIs and glucose intolerance (Fig. 1). Previous studies showed similar results in a type 2 diabetic rat model. Those studies reported that three months of SO diet did not induce a statistical change in body weight, glucose tolerance or insulin resistance [33, 34]. However, we also observed that SO treatment significantly increased non-fasting plasma FFAs up to fasting levels. The impairment of GSIS observed in GK rats is exacerbated by SO treatment (Fig. 2c). Similar to SO-treated normal rats, the decrease in insulin secretion occurred at all concentrations of glucose and arginine tested. The regulation of insulin secretion *in vivo* involves the interaction of several factors besides glucose levels. Others nutrients as well as neural and hormonal influences are responsible for insulin stimulation *in vivo*. Furthermore, the glucagon/insulin ratio is particularly related to fasting glucose levels. Altogether, these can explain the discrepancy between the reduction of

Table 3 Fatty acid composition of soybean oil

Fatty acids	C14:0	C16:0	C18:0	C18:1t	C18:1c	C18:2t	C18:2c	C18:3n3	C18:3n6	C20:0	C20:1	C21:0	C22:0
Total contents (%)	0.2	13.2	4.0	19.2	0.5	0.3	53.3	0.6	6.9	0.8	0.2	0.2	0.5

fasting blood glucose and the impairment of islet insulin secretion. The increase of plasma FFA levels may be directly involved in inhibition of GSIS, as supported by the glucose–fatty acid cycle or by changes in UCP-2 levels [6, 11]. Previous studies demonstrated that 48-h exposure of pancreatic β-cells to high glucose and FFA levels results in a 49% desensitisation and suppression of insulin secretion [5, 6, 13]. We observed that SO treatment only induces a significant decrease of palmitoleic acid (C16:1) (Fig. 3c). Previous studies reported that the increase of the monounsaturated palmitoleic acid (C16:1) is associated with β-cell proliferation at low glucose concentrations [27] and β-cell function improvement [12, 17]. Accordingly, the failure of SO-treated GK islet insulin response observed in our study could be related to a decrease in islet palmitoleic acid.

In summary, in treated GK rats, the increase in non-fasting FFA levels and the potentiation of GSIS decrease could be related to the interference of SO on the Randle cycle or UCP-2 expression [6, 11, 13], as previously discussed. Furthermore, our results show that the exacerbation of GSIS impairment could also be associated with the decrease in palmitoleic acid (C16:1) levels.

In both groups of rats, SO treatment interferes with islet fatty acid composition, this effect being independent of SO fatty acid content. As shown in Table 3, islet fatty acids that were significantly altered with SO treatment correspond to fatty acids that are absent or present at lower percentage in SO. It should be noted that the positive correlation between GSIS impairment (Fig. 2d) and the levels of the erucic acid (C22:1, Fig. 3d) observed in SO-treated W rats is similar to that found in untreated GK rats, suggesting an association between C22:1 fatty acid levels and insulin secretion. Furthermore, we observed a similarity in fasting plasma insulin levels between SO-treated normal and untreated diabetic rats (Fig. 1b). Moreover, the insulin sensitivity index values of treated normal rats approach those of untreated diabetic animals (Fig. 1c, d).

Furthermore our results show that both groups of treated animals present different responses to SO treatment. In normal Wistar rats, the treatment induces insulin resistance and decreases GSIS similar to diabetic GK rats. This could be justified by the changes of fatty acid composition of islets of Langerhans (myristic, eicosanoic, heneicosanoic, erucic acids) that could interfere with cell channels and receptor activities or lipid-signalling molecules. In diabetic islet secretion, treatment interference appears to be related with palmitoleic acid decrease and the involvement of the Randle cycle or changes in UCP-2 levels. More studies should be performed to clarify the exact role of different fatty acids on β-cell function.

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References

1. Zimmet P, Cowie C, Ekoe J, Shan JE (2004) Classification of diabetes mellitus and other categories of glucose intolerance. In: DeFronzo RA, Ferrannini E, Keen H, Zimmet P (eds) International textbook of diabetes mellitus, 3rd Edn. John Wiley & Sons Ltd, England, pp 3–14
2. Golay A, Ybarra J (2005) Link between obesity and type 2 diabetes. Best Pract Res Clin Endocrinol Metab 19:649–663
3. Capito K, Hansen SE, Hedeskov CJ, Islin H, Thams P (1992) Fat-induced changes in mouse pancreatic islet insulin secretion, insulin biosynthesis and glucose metabolism. Acta Diabetol 8:193–198
4. Mason TM, Goh T, Tchipashvili V, Sandhu H, Gupta N, Lewis GF, Giacca A (1999) Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. Diabetes 48:524–530
5. Zhou YP, Grill VE (1994) Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93:870–876
6. Randle PJ (1998) Regulatory interaction between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev 14:263–283
7. Carlsson C, Borg LAH, Welsh N (1999) Sodium palmitate induces partial mitochondrial uncoupling and reactive oxygen species in rat pancreatic islets in vitro. Endocrinology 140:3422–3428
8. Garvey WT (2004) Mechanisms of insulin signal transduction. In DeFronzo RA, Ferrannini E, Keen H, Zimmet P (eds) International textbook of diabetes mellitus, 3rd Edn. John Wiley & Sons Ltd, England, pp 227–252
9. Kishore P, Tonelli J, Koppaka S (2006) Time-dependent effects of free Fatty acids on glucose effectiveness in type 2 diabetes. Diabetes 55:1761–1768
10. Winzell MS, Strom K, Holm C, Ahren B (2006) Glucose-stimulated insulin secretion correlates with beta-cell lipolysis. Nutr Metab Cardiovasc Dis 16[Suppl 1]:S11–S16
11. Briaud I, Kelpe CL, Johnson LM, Tran POT, Poitout V (2002) Differential effects of hyperlipidemia on insulin secretion in islets of Langerhans from hyperglycaemic versus normoglycemic rats. Diabetes 51:662–668

12. Tinahones FJ, Pareja A, Soringuer FJ, Gómez-Zumaquero JM, Cardona F, Rojo-Martínez G (2002) Dietary fatty acids modify insulin secretion of rat pancreatic islet cells in vitro. J. Endocrinol Invest 25:436–441
13. Haber EP, Proscopio J, Carvalho CR, Carpinelli AR, Newsholme P, Curi R (2006) New insights into fatty acids modulation of pancreatic beta-cell function. Int Rev Cytol 248:1–41
14. Hulbert AJ, Turner N, Storlien LH, Else PL (2005) Dietary fatty acids and membrane function: implications for metabolism and disease. Biol Rev Camb Philos Soc 80:155–169
15. Mishra R, Simonson MS (2005) Saturated free fatty acids and apoptosis in microvascular mesangial cells: palmitate activates pro-apoptotic signalling involving caspase 9 and mitochondrial release of endonuclease G. Cardiovasc Diabetol 4:2
16. Riedel MJ, Light PE (2005) Saturated and cis/trans unsaturated acyl CoA esters differentially regulate wild-type and polymorphic β-cell ATP-sensitive K+ channels. Diabetes 54:2070–2079
17. Stubhaug I, Tocher DR, Bell JG, Dick JR, Torstensen BE (2005) Fatty acids metabolism in Atlantic salmon (*Salmo salar L.*) hepatocytes and influence of dietary vegetable oil. Biochim Biophys Acta 1734:277–288
18. Dobbins RL, Szczepaniak LS, Myhill J (2002) The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. Diabetes 51:1825–1833
19. Azevedo-Martins AK, Monteiro AP, Lima CL, Lenzen S, Curi R (2006) Fatty acids-induced toxicity and neutral lipid accumulation in insulin-producing RINm5F cells. Toxicol In Vitro 20:1106–1113
20. Xiao C, Giacca A, Carpentier A, Lewis GF (2006) Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. Diabetologia 49:1371–1379
21. Alstrup KK, Brock B, Hermansen K (2004) Long-term exposure of INS-1 cells to cis and trans fatty acids influences insulin release and fatty acids oxidation differentially. Metabolism 53:1158–1165
22. Sudha V, Radhika G, Mohan V (2004) Current dietary trends in the management of diabetes. Indian J Med Res 120:4–8
23. Rocca AS, LaGreca J, Kalitsky J, Brubaker PL (2005) Monounsaturated fatty acid diets improve glycaemia tolerance through increased secretion of glucagons-like peptide-1. Endocrinology 142:1148–1155
24. van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB (2002) Dietary fat and meat intake in relation to risk of type 2 diabetes in men. Diabetes Care 25:417–424
25. Biden TJ, Robinson D, Cordery D, Hughes WE, Busch AK (2004) Chronic effects of fatty acids on pancreatic β-cell function. New insights from functional genomics. Diabetes 53:S159–S165
26. Briaud I, Harmon JS, Kelpe CL, Segu VB, Poitout V (2001) Lipotoxicity of the pancreatic beta-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. Diabetes 50:315–321
27. Maedler K, Spinas GA, Dyntar D, Moritz W, Kaiser N, Donath MY (2001) Distinct effects of saturated and monounsaturated fatty acids on β-cell turnover and function. Diabetes 50:69–76
28. Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY (2003) Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic β-cell turnover and function. Diabetes 52:726–733
29. Nettleton JA, Katz R (2005) n-3 Long-chain polyunsaturated fatty acids in type 2 diabetes: a review. J Am Diet Assoc 105:428–440
30. Wang X, Li H, De Leo D (2004) Gene and protein kinase expression profiling of reactive oxygen species-associated lipotoxicity in the pancreatic β-cell line MIN6. Diabetes 53:129–140
31. Calder PC (2003) Long-chain n-3 fatty acids and inflammation: potential application in surgical and trauma patients. Braz J Med Biol Res 36:433–446
32. Nishimura M, Yamauchi A, Yamaguchi M, Ueda N, Naito S (2005) Soybean oil in total parenteral nutrition maintains albumin and antioxidant enzyme mRNA levels. Biol Pharm Bull 28:1265–1269
33. Stark AH, Timar B, Madar Z (2000) Adaptation of Sprague Dawley rats to long-term feeding of high fat or high fructose diets. Eur J Nutr 39:229–234
34. Pellizzon M, Buison A, Ordiz F Jr, Santana Ana L, Jen KL (2002) Effects of dietary fatty acids and exercise on bodyweight regulation and metabolism in rats. Obes Res 10:947–955
35. Jen KLC, Buison A, Pellizzon M, Ordiz F Jr, Santa Ana L, Brown J (2003) Differential effects of fatty acids and exercise on body weight regulation and metabolism in female Wistar rats. Exp Biol Med 228:843–849
36. Picinato MC, Curi R, Machado UF, Carpinelli AR (1998) Soybean- and olive-oils-enriched diets increase insulin secretion to glucose stimulus in isolated pancreatic rat islets. Physiol Behav 65:289–294
37. Hermansen K, Sondergaard M, Hoie L, Carstensen M, Brock B (2001) Beneficial effects of soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 24:228–233
38. Shang W, Yasuda K, Takahashi A (2002) Effect of high dietary fat on insulin secretion in genetically diabetic Goto-Kakizaki rats. Pancreas 25:393–399
39. Chen H, Sullivan G, Quon MJ (2005) Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. Diabetes 54:1914–1925
40. Seiça RM, Suzuki KI, Santos RM, Rosário LM (2004) Impaired insulin secretion in isolated islets of Goto-Kakizaki rats, an animal model of non obese type 2 diabetes, is a primary event. Acta Med Port 17:42–48 (In Portuguese)
41. Bergsten P, Hellman B (1993) Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. Diabetes 42:670–674
42. Rosa D, Catalá A (1998) Fatty acids profiles and non enzymatic lipid peroxidation of microsomes and mitochondria from bovine liver, kidney, lung and heart. Arch Physiol Biochem 106:33–37
43. Peixoto F, Vicente J, Madeira VMC (2004) A comparative study of plant and animal mitochondria exposed to paraquat reveals that hydrogen peroxide is not related to the observed toxicity. Toxicol in Vitro 18:733–739

44. Goto Y, Kakizaki M (1981) The spontaneous diabetic rat a model of noninsulin dependent diabetes mellitus. Proc Jpn Acad 57:381–384
45. Seiça RM, Martins MJ, Pessa PB (2003) Morphological changes of islet of Langerhans in an animal model of type 2 diabetes. Acta Med Port 16:381–388 (In Portuguese)
46. Portha B (2005) Programmed disorders of β-cell development and function as one cause for type 2 diabetes? The GK rat paradigm. Diabetes Metab Res Rev 21:495–504
47. Ihara Y, Toyokuni S, Uchida K (1999) Hyperglycemia causes oxidative stress in pancreatic β-cells of GK rats, a model of type 2 diabetes. Diabetes 48:927–932
48. Dolz M, Bailbe D, Giroix MH (2005) Restitution of defective glucose-stimulated insulin secretion in diabetic GK rat by acetylcholine uncovers paradoxical stimulatory effect of beta-cell muscarinic receptor activation on cAMP production. Diabetes 54:3229–3237
49. Warwar N, Efendic S, Ostenson C, Haber EP, Cerasi E, Nesher R (2006) Dynamics of glucose-induced localization of PKC isoenzymes in pancreatic β-cells. Diabetes-related changes in the GK rats. Diabetes 55:590–599
50. Bjorntorp P (2004) The relationship between obesity and diabetes – The role of neuroendocrine and autonomic factors. In DeFronzo RA, Ferrannini E, Keen H, Zimmet P (eds) International textbook of diabetes mellitus, 3rd Edn. John Wiley & Sons Ltd, England, pp 565–582