

Interactions between effects of adrenalectomy and diet on insulin secretion in *fa/fa* Zucker rats

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Abstract: Our objective was to determine if a cafeteria-type diet with increased fat content would block the decrease in insulin secretion induced by adrenalectomy in obese rats. Five week old Zucker (*fa/fa*) rats were adrenalectomized. One week later, half of the adrenalectomized groups, and age-matched, sham-operated animals were given a diet of 16% fat and 44% carbohydrate. Control animals were maintained on standard rat chow (4.6% fat and 49% carbohydrate). After 4 weeks on the diets, in vivo measurements included caloric intake, weight gain, plasma corticosterone, triglyceride, free fatty acids, and oral glucose tolerance tests. In vitro measurements included glucose-stimulated insulin secretion, glucose phosphorylating activity, islet triglyceride content, and fatty acid oxidizing activity of cultured islets. Generally, the cafeteria diet did not block the effects of adrenalectomy on in vitro insulin secretion parameters, even though in sham-operated animals weight gain and insulin resistance was induced by the diet in vivo. Adrenalectomy and the diet exerted independent effects on glucose phosphorylation and fatty acid oxidation in islets. In conclusion, adrenalectomy decreased the elevated insulin secretion in *fa/fa* rats. The failure of a cafeteria diet enriched in fat to block the adrenalectomy-mediated changes in B-cell function indicates the importance of glucocorticoids and centrally-mediated effects on insulin secretion and other metabolic parameters.

Key words: obesity, insulin secretion, islets of Langerhans, adrenalectomy, high fat diet.

Résumé : L'objectif a été de déterminer si un régime de type cafétéria à forte teneur en gras bloquerait la diminution de la sécrétion d'insuline provoquée par une surrénalectomie chez des rats obèses. Des rats Zucker (*fa/fa*) âgés de cinq semaines ont été surrénalectomisés. Une semaine plus tard, la moitié des groupes surrénalectomisés, ainsi que des animaux du même âge opérés de manière fictive, ont été soumis à un régime contenant 16 % de matière grasse et 44 % de glucides. Les animaux témoins ont continué à suivre le régime standard pour rats (4,6 % de matière grasse et 49 % de glucides). Après quatre semaines, les mesures in vivo suivantes ont été effectuées : apport calorique, gain de poids, corticostérone plasmatique, triglycérides, acides gras libres et épreuves d'hyperglycémie provoquée par voie orale. Les mesures in vitro suivantes ont aussi été effectuées : sécrétion d'insuline stimulée par le glucose, activité de phosphorylation du glucose, teneur en triglycérides des îlots et activité d'oxydation des acides gras des îlots cultivés. Règle générale, le régime de type cafétéria n'a pas bloqué les effets de la surrénalectomie sur les paramètres de sécrétion de l'insuline in vitro même si, chez les animaux opérés de manière fictive, un gain de poids et une résistance insulinaire ont été provoqués par le régime in vivo. La surrénalectomie et le régime ont eu des effets indépendants sur la phosphorylation du glucose et l'oxydation des acides gras dans les îlots. En conclusion, la surrénalectomie diminue l'augmentation de sécrétion insulinaire chez les rats *fa/fa*. L'incapacité du régime de type cafétéria à forte teneur en gras à bloquer les variations de la fonction des cellules B véhiculées par la surrénalectomie montre l'importance des effets d'origine centrale et des glucocorticoïdes sur la sécrétion insulinaire et sur d'autres paramètres métaboliques.

Mots clés : obésité, sécrétion d'insuline, îlots de Langherans, surrénalectomie, régime à forte teneur en gras.

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Introduction

Obesity in humans and rodent models is associated with insulin resistance, fasting hyperinsulinemia, and hyperlipidemia (Bray et al. 1988). One cause of fasting hyperinsulinemia may be increased sensitivity of pancreatic islets to glucose due to activity changes in the B-cell glucose sens-

ing enzymes glucokinase and hexokinase (Chan 1995). Islets from neonatal genetically obese Zucker (*fa/fa*) rats exhibit increased sensitivity to glucose (Rohner-Jeanraud et al. 1983), leading to the development of fasting hyperinsulinemia in weanling *fa/fa* rats (Chan et al. 1985). B-cell insensitivity to mannoheptulose, an indicator of glucokinase (GK) dysfunction, is also observed by 5 weeks of age in these animals (Kibenge and Chan 1995).

Our recent study demonstrated that adrenalectomy (ADX) normalized B-cell glucose sensitivity, glucokinase kinetics, and mannoheptulose sensitivity in *fa/fa* rats (Kibenge and Chan 1996), implicating the hypothalamo-pituitary-adrenal axis as a regulator of GK function. The actions of ADX on islet biochemistry are likely caused by decreased vagal tone (Stubbs and York 1991) but may also be influenced by

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changes in food intake or other metabolic effects of ADX (Rohner-Jeanrenaud et al. 1983; Vander Tuig et al. 1984).

ADX-mediated reduction of plasma lipids may play an important role in the normalization of plasma insulin levels (Bray et al. 1988; Castonguay et al. 1986). Short term exposure of pancreatic islets to high levels of free fatty acids (FFA) enhances both basal- and glucose- stimulated insulin secretion (GSIS) (Sako and Grill 1990; Stein et al. 1996). Prolonged FFA exposure decreases insulin secretion at high glucose levels as a result of inhibition of glucose metabolism by B-cells (Zhou and Grill 1994), but increases hexokinase V_{max} and elevates the insulin response to low glucose levels (Milburn et al. 1995). McGarry (1992) postulated that the abnormal plasma lipid profiles may contribute to the changes seen in the B-cell response to glucose stimulation because plasma FFA and triglycerides (TG) are elevated in simple obesity.

We hypothesized that post-surgical feeding of an elevated fat diet would negate the benefits of ADX on B-cell function in obese *fa/fa* rats. This idea was based on: (i) the importance of lipids in the regulation of B-cell function (Prentki and Corkey 1996), (ii) the ability of ADX to normalize several insulin secretion parameters in prepubertal *fa/fa* rats (Kibenge and Chan 1996), and (iii) the observation that ADX reduced plasma TG (Castonguay et al. 1986). The results were compared with those from control (sham-operated) rats fed a diet with elevated fat content.

Materials and methods

Animals

Zucker obese 5-week old rats were obtained from Charles River Laboratories (St. Constant, Que.) or bred from Charles River stock. Animals born in-house were routinely weaned and sexed at 21 days of age. All animals were fed Purina rat chow (St. Louis, Mo.) and tap water ad libitum prior to surgery. Rats were either bilaterally adrenalectomized (ADX) or sham-operated (SH) through a ventral incision after anesthesia with a mixture 2 mL of sodium pentobarbital (65 mg/ml) and 2 mL diazepam (5 mg/mL) in 6 mL of saline (0.9% NaCl) (Kibenge and Chan 1996). After surgery the animals were housed individually, in an artificially lit room with a 12 h dark : light cycle at a temperature of 22–25°C. For the first week after surgery animals were fed regular rat chow and allowed access to tap water ad libitum.

One week after surgery, animals were either fed a cafeteria-style diet of sweetened condensed milk diet (CMD) moderately enriched in fat (3.96 kcal/g) or regular rat chow (3.74 kcal/g) for 4 weeks. The enriched fat diet was composed of rat chow, Mazola corn oil, and Eagle's brand sweetened condensed milk made according to Triscari et al. (1985). Diet compositions are shown in Table 1. The groups were as follows: SH-CH, sham-operated rats fed regular chow; SH-CMD, sham-operated rats fed the condensed milk diet; ADX-CH, adrenalectomized rats fed regular chow; and ADX-CMD, adrenalectomized rats fed the condensed milk diet. All animals had access to tap water and saline-sucrose (0.9% : 4%) solution ad libitum to prevent side effects of mineralocorticoid or glucocorticoid deficiencies in the ADX groups. Although sucrose has the potential to alter glucose tolerance, we did not observe any differences between the control *fa/fa* rats in this study and *fa/fa* rats from a separate study in which sucrose was not given (T. Kibenge and C. Chan 1998, unpublished data). Sucrose intake was not quantified and therefore was not included in the calculation of caloric intake. Body weights were measured weekly, and food intake was measured on 3 consecutive days each week.

Table 1. Diet composition^a.

Component	Chow	Condensed milk diet
Crude protein (%) ^b	23.4	14.7
Carbohydrate (%)	49	44.2
Lipid (%)	4.5	15.8
Fibre (%)	5.8	2.5
Vitamin mix / ash (%)	7.3	1.2
Water (%)	10	19

^aFrom Triscari et al. 1985.

^bAll percents by weight.

All protocols were approved by the local Animal Care Committee and met the guidelines of the Canadian Council on Animal Care.

Oral glucose tolerance test

After 4 weeks on the diets, the animals were fasted overnight and the saline-sucrose solution was replaced with saline. The animals were weighed at 0830h the next day and 0.5 mL blood samples were collected in heparinized tubes from the tail vein. Glucose (1 g/kg body weight) was then given by oral gavage, and blood samples were collected after 10, 20, 30, 40, and 60 min. The samples were centrifuged and the plasma obtained was stored at -20°C for insulin, glucose and corticosterone analyses, and at -70°C for FFA and TG determination. Plasma glucose was measured using a glucose analyzer II (Beckman, Fullerton, Calif.). Plasma TG and FFA levels were determined spectrophotometrically with commercially available kits (Sigma Diagnostics, St. Louis, Mo., and Boehringer Mannheim, Laval, Que., respectively). A radioimmunoassay (RIA) specific for rat insulin was used to determine the plasma insulin (Chan et al. 1985). Corticosterone was determined by RIA following the kit manufacturers's manual (ICN Biomedical, Costa Mesa, Calif.). Only ADX animals with corticosterone levels less than the assay detection limit (25 ng/mL) were included in the analysis.

Islet isolation and insulin release

Immediately following completion of the oral glucose tolerance test, the rats were anesthetized with sodium pentobarbital (65 mg/kg body weight ip). The pancreas was exposed by laparotomy and 5 mL collagenase was introduced via a catheter into the pancreobiliary duct to distend the pancreas. The pancreas was excised and finely chopped with scissors. Pancreatic islets were isolated by sequential collagenase digestion and dextran step-density gradient and cultured as previously described (Kibenge and Chan 1995; Chan et al. 1993). After overnight incubation, the culture medium was replaced with 1.0 mL of fresh medium containing various glucose concentrations (0–25 mM) and 0.1% gelatin. Mannoheptulose (1–100 mM) was added to islet samples in media containing 16.5 mM glucose and all samples were then statically incubated for 90 min at 37°C (95% air–5% CO₂, saturated with water vapour). Although culture conditions may alter insulin secretion patterns, our previous work demonstrated that the effects of ADX persisted in 24 h cultured islets (Kibenge and Chan 1996). Insulin release and islet insulin content were measured as previously described by Chan et al. (1993).

Glucose phosphorylating activity

Islets were isolated and cultured as described for the insulin release protocol. Hexokinase and glucokinase activities were determined by measuring phosphorylation of [¹⁴C]glucose (Chan et al. 1995). Hexokinase activity was determined by using glucose concentrations from 0.05–0.5 mM, while 6–16 mM glucose was used for glucokinase activity measurements. Velocities were calculated after correcting for specific activity of [¹⁴C]glucose and normalized

Table 2. The effects of ADX and diet composition on food intake and weight gain.

Treatment	<i>n</i>	Initial food intake (kcal/d)	Mean daily food intake (kcal/d)	Mean daily fat intake (g ± SE)	Initial weight (g ± SE)	Weight gain (g ± SE)	% gain vs. SH-CH
ADX-CH	10	42.1 ± 1.3*	60.2 ± 3.4*	0.72 ± 0.04*	154.1 ± 6.7	122.2 ± 10.8*	-44.7
ADX-CMD	10	50.8 ± 5.6*	80.4 ± 2.6*†	3.2 ± 0.10*†	149.6 ± 7.8	139.2 ± 8.6*	-37
SH-CH	16	89.2 ± 5.5	105.0 ± 3.7	1.20 ± 0.04	145.6 ± 4.1	221.0 ± 7.0	0
SH-CMD	16	87.1 ± 6.2	117.2 ± 6.0	4.68 ± 0.23†	142.4 ± 5.3	262.9 ± 15.6†	19

Note: Initial food intake is that during week 5–6, the week post-surgery when all rats were fed chow. Mean daily food intake is calculated for weeks 6–10 to evaluate the effects of the two diets. Values are means ± SE, for the number of rats in each group (*n*). Rats were adrenalectomized (ADX) or sham operated (SH) at 5 weeks and were fed either chow or CMD from 6 week of age until they were killed at 10 weeks.

*Significant effect of ADX, $P < 0.0001$.

†Significant effect of diet, $P < 0.05$.

by comparing to islet protein (Lowry method, Sigma Chemical Co., St. Louis, Mo.). For glucokinase, values were corrected for hexokinase activity by subtracting $V_{\text{Hexokinase}}$ at 0.5 mM (Chan et al. 1995).

Fatty acid oxidation and islet triglyceride content

Pancreatic islet FFA oxidation was measured essentially as described by Chen et al. (1994a). Islet TG content was determined as described by Lee et al. (1994).

Statistical analysis

Data are expressed as means ± SE and *n* refers to the number of donor animals in each group. Data were analyzed by analysis of variance (ANOVA) using the general linear model for unbalanced data sets, followed by Student-Newman-Keuls test. All results were considered significant at $P < 0.05$.

Results

Energy intake and weight gain

Caloric intake was significantly influenced by both ADX ($P < 0.0001$, ANOVA) and diet ($P < 0.0001$). ADX reduced caloric intake by 50% in the first week after surgery (animals at 5 weeks of age) when all rats were fed chow ($P < 0.005$) (Table 2). At 6 weeks of age, half of the rats were put on the CMD for the next 4 weeks while the remainder of the animals were maintained on regular chow. ADX significantly reduced food intake in *fa/fa* rats on both diets during weeks 7–10. Diet did not alter the mean daily energy intake in sham rats but ADX-CMD rats ate 20 kcal/d more than ADX-CH rats, ($P < 0.05$, Table 2). Fat intake was greater in the SH-CMD group than in the SH-CH group by 3.9-fold, and 4.4-fold greater in the ADX-CMD group than in the ADX-CH group.

The weight profiles are shown in Table 2. Weight gain was significantly influenced by ADX (ANOVA, $P < 0.0001$). ADX decreased weight gain by 45% in ADX-CH *fa/fa* rats when compared with sham controls and this outcome was not modified by feeding the CMD. At the end of 5 weeks, *fa/fa* rats had gained a similar amount of weight as sham lean rats fed the same diet (not shown). The CMD significantly increased the weight gain of sham rats by 15–20% compared with the SH-CH group ($P < 0.05$).

Plasma levels of corticosterone, triglycerides, and free fatty acids

Corticosterone

The success of the ADX surgery was confirmed by detecting plasma corticosterone levels that were <98% than that of the controls. The *fa/fa* SH-CH rats had higher levels of corticosterone than did the SH-CMD rats ($P < 0.05$, Table 3).

Triglycerides

Obese sham rats exhibited plasma TG levels 4 to 10-fold higher than lean sham rats (not shown), but ADX reduced TG levels by ~85% ($P < 0.0001$, Table 3). The CMD did not block this effect of ADX on plasma TG.

Free fatty acids

ADX decreased the FFA of *fa/fa* rats (Table 3) to levels similar to those in lean rats (not shown). Diet had no effect on plasma FFA in sham control or ADX rats.

Oral glucose tolerance

ADX reduced fasting plasma glucose levels of *fa/fa* rats (Table 3) and plasma glucose during the oral glucose tolerance test, independent of the diet (Fig. 1A). In oral glucose tolerance tests (Fig. 1A) the CMD further impaired glucose tolerance in both sham and ADX animals, as indicated by the increased area under the curve (inset). The stress of oral gavage may have contributed to the difference in fasting glucose levels because ADX rats would lack any adrenal hormone (glucocorticoid or adrenalin) response. Sham *fa/fa* rats were hyperinsulinemic as insulin was 4-fold higher than in lean rats (not shown). ADX decreased both the fasting and glucose-stimulated plasma insulin levels by 40–60% (Table 3). There was no significant effect of dietary intervention in sham rats, but the area under the curve indicated a further 25% suppression of insulin secretion in ADX-CMD rats compared with ADX-CH rats.

In vitro insulin secretion and islet insulin content

Glucose-stimulated insulin secretion (GSIS) was measured by incubating isolated pancreatic islets with various glucose concentrations (0–25 mM). In ADX *fa/fa* islets basal insulin and GSIS were lower than in islets from sham-fed rats, and both were further depressed by feeding the CMD (Fig. 2A). GSIS in SH-CMD obese islets was higher than in SH-CH obese islets. The calculated EC_{50} was ~2-fold lower in CMD obese rats compared with respective controls (Table 4). The

Table 3. Effects of ADX and diet composition on fasting plasma corticosterone, TG, FFA, glucose, and insulin.

Treatment	Corticosterone (ng/L)	Insulin (pM)	Glucose (mM)	TG (mg/dl)	FFA (mM)
ADX-CH	7.7 ± 2.5 (10)*	222 ± 73 (8)	5.99 ± 0.20 (8) [‡]	4.75 ± 1.12 (8) [‡]	0.15 ± 0.05(6) [‡]
ADX-CMD	8.1 ± 2.0 (10)*	163 ± 37 (9) [‡]	5.30 ± 0.65 (8) [‡]	7.45 ± 1.33 (10) [‡]	0.23 ± 0.05(8) [‡]
SH-CH	763 ± 33 (12) [†]	379 ± 87 (7)	7.03 ± 0.28 (7)	47.57 ± 12.41 (7)	1.05 ± 0.46(8)
SH-CMD	696 ± 43 (14)	398 ± 103 (7)	7.29 ± 0.24 (7)	47.39 ± 5.24 (12)	0.67 ± 0.29 (8)

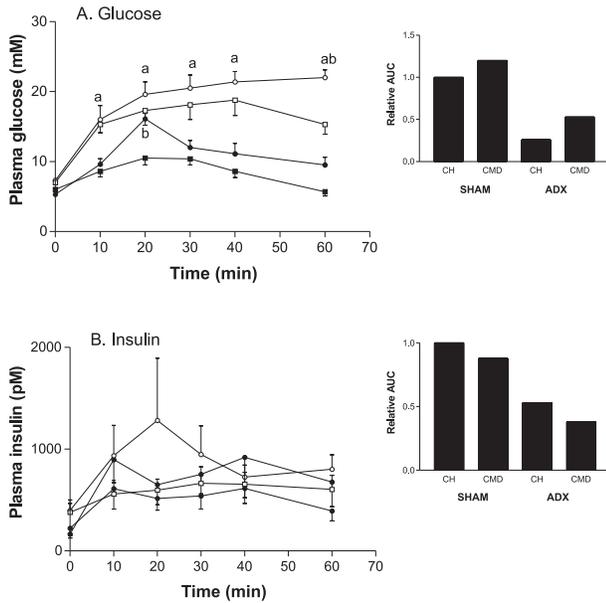
Note: The values are means ± SE, with *n* representing the number of animals for which a sample was obtained for each assay. Some assays could not be performed for individual rats due to insufficient plasma.

*Significant effect of ADX, $P < 0.0001$.

[†]Significant effect of diet, $P < 0.05$.

[‡]Significant effect of ADX, $P < 0.05$.

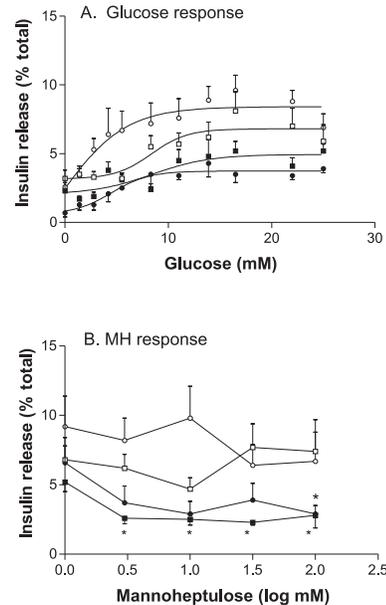
Fig. 1. Effects of ADX and dietary fat on plasma glucose (A) and insulin (B) during an oral glucose and insulin tolerance test in obese rats. Glucose (1 g/kg) was administered after collection of a basal blood sample at $t = 0$. Fasting plasma glucose and insulin levels are reported in Table 3. Data are expressed as means ± SE. Number of animals per groups were as follows: SH-CH, 7 (open squares); SH-CMD, 7 (open circles); ADX-CH, 8 (closed squares); and ADX-CMD, 8 (closed circles). a, Significant effect of ADX effect compared with sham, $P < 0.05$; b, Significant effect of diet within ADX or sham treatment groups, $P < 0.05$. Insets: Area under the curve analysis (expressed as arbitrary units relative to SH-CH for glucose (top) and insulin (bottom)).



insulin content of *fa/fa* rat islets was not significantly affected by ADX or diet ($P > 0.05$, not shown).

We previously showed that the inhibitory action of mannoheptulose on GSIS in isolated islets from ADX *fa/fa* rats normalized within 2 weeks post surgery (Kibenge and Chan 1996). In this study we investigated whether the ADX effects on mannoheptulose sensitivity would be antagonized by chronic feeding of CMD. Glucose (16.5 mM)-stimulated insulin release was measured in the absence or presence of mannoheptulose (1–10 mM). As predicted, mannoheptulose did not have a significant inhibitory effect on GSIS in obese sham rat islets on either diet ($P > 0.05$, Fig. 2B). In the

Fig. 2. (A) Effects of ADX and dietary fat on in vitro glucose-stimulated insulin secretion in obese rats. Islets were exposed to glucose concentrations as indicated for 90 min. The total islet insulin content were not different between groups (not shown) and the EC_{50} for glucose and *n* for each group are reported in Table 4. Symbols are the same as for the lower panel. (B) Effects of ADX and dietary fat on mannoheptulose sensitivity in obese rat islets. Insulin secretion was stimulated by 16.5 mM glucose in the absence (0) and presence of mannoheptulose. The number of animals in each group were as follows: SH-CH, 5 (open squares); SH-CMD, 6 (open circles); ADX-CH, 8 (closed squares); and ADX-CMD, 4 (closed circles). *Significant effect compared with respective glucose controls, $P < 0.05$. Data are expressed as means ± SE.



obese ADX groups, GSIS in ADX.CH rat islets was significantly inhibited by mannoheptulose (3 or 100 mM, $P = 0.044$; 10 mM, $P = 0.051$). In obese ADX-CMD islets, significant results were observed only at the highest mannoheptulose concentration but GSIS was inhibited up to 40% by lower concentrations.

Glucokinase activity in isolated islets

Glucose phosphorylating activity was measured in disrupted islet preparations and the results are presented in Fig. 3. Both ADX and diet had significant effects on glucose

Table 4. Effects of ADX and diet composition on glucose sensitivity (EC_{50}) and insulin content in isolated pancreatic islets.

Treatment	<i>n</i>	EC_{50} (mM)
ADX-CH	6	7.15 ± 3.76
ADX-CMD	4	4.83 ± 0.62
SH-CH	6	8.34 ± 1.28
SH-CMD	6	$3.32 \pm 0.91^{\S}$

Note: The data are expressed as means \pm SE.

§ Significant effect of diet, $P < 0.05$.

phosphorylation in *fa/fa* rats. ADX significantly ($P < 0.05$) reduced glucose phosphorylation by glucokinase in ADX-CH islets ($V_{max} = 489 \pm 102$ pmol $\cdot\mu\text{g protein}^{-1}\cdot\text{h}^{-1}$) but not in ADX-CMD islets ($V_{max} = 1314 \pm 417$ pmol $\cdot\mu\text{g protein}^{-1}\cdot\text{h}^{-1}$) compared with their respective controls ($V_{max} = 1144 \pm 100$ and 1327 ± 168 pmol $\cdot\mu\text{g protein}^{-1}\cdot\text{h}^{-1}$ for SH-CH and SH-CMD, respectively). Hexokinase activity was not different among *fa/fa* rat islet groups (data not shown).

Fatty acid oxidation in isolated islets

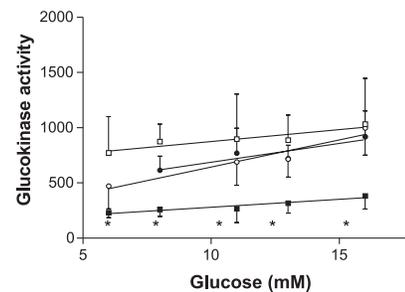
Increasing dietary fat may alter the balance between glucose and FFA utilization in islets. We investigated whether ADX and (or) diet would increase FFA oxidation in isolated islets. Oxidation of 0.5 mM of [^{14}C]palmitic acid by isolated islets from Zucker rats was measured in the presence of low (3 mM) or high (25 mM) glucose concentrations. Results are shown in Table 5. Islet FFA oxidation was significantly influenced by diet composition and ADX ($P < 0.05$, ANOVA). SH-CH islets had the highest rate of FFA oxidation at both high and low glucose concentrations. At the high glucose concentration, FFA oxidation was significantly reduced in SH-CMD islets, whereas FFA oxidation was reduced at the low glucose concentration in ADX-CH islets.

Pancreatic islet triglycerides

Elevation of pancreatic islet TG is reported to precede the loss of GSIS in animal models of diabetes (Lee et al. 1994). The increase in islet TG mirrors the high plasma FFA that is present in these animals. We measured islet TG in sham and ADX Zucker rats and compared the effects of different diets. In the obese rats, TG content was 30% higher in SH-CH than SH-CMD islets. TG was reduced by 85% in ADX-CMD islets ($P < 0.05$), but not in the ADX-CH islets ($P > 0.05$). The islet TG content in sham lean rats was 2-fold lower than in sham *fa/fa* rats (not shown).

Discussion

This study was undertaken to determine if the ability of ADX to decrease the elevated insulin secretion in *fa/fa* rats would be negated by eating a cafeteria-type diet. Because ADX is known to reduce plasma TG levels (Castonguay et al. 1986), the fat content of the diet was increased. However, the elevation in fat was only moderate and this diet was also enriched in carbohydrate and decreased in protein and fibre, which may also have affected insulin secretion. To ensure that the diet was not overtly detrimental to the health of normal animals, studies in lean rats were also conducted; the data are not reported in detail here. Body weight gain in the lean rats fed the CMD (136.5 ± 6.9 g) were not different

Fig. 3. Effects of ADX and dietary fat on total glucose phosphorylating activity (fmol $\cdot\mu\text{g protein}^{-1}\cdot\text{h}^{-1}$) in obese rat islets. Data are expressed as means \pm SE and *n* for each group was as follows: SH-CH, 9 (open squares); SH-CMD, 9 (open circles); ADX-CH, 8 (closed squares); and ADX-CMD, 4 (closed circles). *Significant effect compared with SH-CH, $P < 0.05$.

than those fed regular chow (118.4 ± 4.4 g, $P > 0.05$), suggesting that the low protein content was still sufficient to support normal growth in young rats.

The hyperinsulinemia observed in *fa/fa* rats has been attributed in part to increased glucose phosphorylating activity by glucokinase at low glucose concentrations (Chan et al. 1995; Chan 1993). Altered sensitivity to a competitive inhibitor of glucokinase, mannoheptulose, was also reported (Chan et al. 1993). Previously, we demonstrated that, insulin secretion from isolated islets of *fa/fa* rats was partially normalized at two weeks following ADX, coincident with an increase in the glucokinase K_m (Kibenge and Chan 1996). Obese ADX rats also had normal sensitivity to the competitive inhibitor mannoheptulose (Kibenge and Chan 1996). In the current study conducted 5 weeks following ADX, mannoheptulose sensitivity of the islets was similarly improved. This was accompanied by a reduction in the glucokinase activity and insulin secretion that correlated with a reduction in the V_{max} , without alteration in the K_m . Thus, a time-dependent adaptation in glucokinase function may occur after ADX in which there is first a decrease in glucose sensitivity of the existing enzyme followed by a reduction in enzyme expression. Consistent with the glucokinase kinetic data, the EC_{50} for glucose of insulin secretion was unaltered by ADX but there was a decrease in the maximal insulin response. ADX was previously shown to depress islet glucose metabolism (Borelli et al. 1982), a key determinant of insulin secretion. The reduction in fasting glycemia and improved glucose tolerance induced by ADX was the probable mechanism by which glucokinase expression was decreased. The glucose concentration to which islets are exposed largely determines glucokinase activity and expression (Liang et al. 1992; Chen et al. 1994b; Iynedjian et al. 1989).

The ability of ADX to reduce plasma TG and FFA is unlikely to account for the reduced insulin secretion and glucokinase activity, because chronic elevation of these agents suppresses GSIS (Sako and Grill 1990; Zhou and Grill 1994). Moreover, ADX decreased palmitate oxidation in *fa/fa* rat islets and this would be expected to elevate rather than suppress GSIS (Zhou and Grill 1994; Milburn et al. 1995). A third possibility is that reduction of the corticosterone-regulated parasympathetic drive to the pancreatic islets normalizes

Table 5. The effects of ADX and diet composition on FFA oxidation and TG content in isolated islets.

Treatment	TG, $\mu\text{g}/\text{islet}$ (n)	CO ₂ production (fmol-islet ⁻¹ ·h ⁻¹)	
		3 mM glucose (n)	25 mM glucose (n)
ADX-CH	1.96 ± 0.07 (4)	7.60 ± 2.37 (6)*	6.65 ± 3.50 (6)
ADX-CMD	0.29 ± 0.05 (5)*§	4.51 ± 1.1 (4)	4.83 ± 0.62 (4)
SH-CH	2.14 ± 0.13 (4)	14.5 ± 3.3 (9)	15.1 ± 4.6 (9)*§
SH-CMD	1.44 ± 0.10 (7)§	5.83 ± 0.93 (7)	4.19 ± 0.32 (7)§

Note: FFA oxidation was assessed by measuring ¹⁴CO₂ production from [¹⁴C]-palmitic acid. Values are means ± SE, with n representing the number of islet donors.

*Significant effect of ADX, $P < 0.05$.

§Significant effect of diet, $P < 0.05$.

insulin secretion (Stubbs and York 1991) and modulates glucokinase function, as previously proposed by us (Kibenge and Chan 1996). These experiments were not designed to test that possibility but chronic exposure of islets to cholinergic agonists did not alter glucokinase kinetics in *fa/fa* or normal rat islets (M.T. Kibenge and C.B. Chan 1995, unpublished data).

Several studies have shown that dietary factors such as weight gain and glucose tolerance can affect the efficacy of ADX in ameliorating metabolic parameters. For example, a high fat diet tended to block the weight reduction and adipocyte hypertrophy induced by ADX in *fa/fa* rats (Bray et al. 1992). Similarly, increasing either fat and carbohydrate content of diets negated the ADX-induced effects on glucose tolerance and energy balance in *ob/ob* mice (Romsos 1991). The diet used in this study was elevated in fat at the expense of protein (Triscari et al. 1985). After eating this diet for 4 weeks, control *fa/fa* rats were heavier and had impaired glucose tolerance compared with chow fed control rats. The diet also had significant effects on isolated islet function; glucose sensitivity was increased, resulting in increased insulin secretion at low glucose levels. These effects could not be attributed to any change in glucokinase kinetics but were correlated with reductions in both islet triglyceride content and palmitic acid oxidation.

Contrary to our expectations, feeding the CMD did not result in marked interactions between ADX and pancreatic islet function in *fa/fa* rats. Glucose-stimulated insulin secretion was similar in the ADX-CH and ADX-CMD groups even though glucokinase activity was higher in the ADX-CMD group. There was a statistically significant decrease in the ADX-induced normalization of mannoheptulose sensitivity in the ADX-CMD rat islets, but the concentration-response curves appeared to be parallel, suggesting that the diet had only a minor effect on this parameter. Likewise, islet triglyceride content was even lower in the ADX-CMD than ADX-CH group, but this was not reflected by any change in palmitic acid oxidation levels. Therefore, the *in vitro* effects of ADX on islet insulin secretion were dominant over the effects of the diet. This provides indirect support for the idea that the HPA axis, via the autonomic nervous system, exerts a direct and important influence on pancreatic islets (Stubbs and York 1991; Mizuno et al. 1998). *In vivo*, the cafeteria-type diet did tend to block some of the beneficial effects of ADX on glucose tolerance. There was a 65% decrease in plasma glucose levels during the oral glucose tolerance test in the ADX-C group, but only a 35% decrease was observed in the ADX-CMD group. Other stud-

ies (Tannenbaum et al. 1997) showed that a diet with similar (20%) fat content induced elevated corticosterone levels in intact lean rats but we did not observe such an effect in intact *fa/fa* rats, possibly because of pre-existing hypercorticosteronemia. These conflicting data suggest that further work is required to determine if the relative inability of the cafeteria-type diet to block effects of ADX is directly related to the absence of adrenal corticosteroids.

In conclusion, feeding a cafeteria-type diet enriched in fat (4-fold) did not prevent the decrease in insulin secretion induced by ADX in *fa/fa* rats or the associated changes in islet lipid metabolism. While some of these ADX-induced changes may be due to the reduction in circulating glucose, the direct contribution of autonomic tone ameliorated by the absence of corticosterone cannot be ruled out.

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