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Characterization of the Spontaneous Diabetes Obesity Syndrome in Mature Male CBA/Ca Mice

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CONNELLY, D. M. AND P. V. TABERNER. *Characterization of the spontaneous diabetes obesity syndrome in mature male CBA/Ca mice*. PHARMACOL BIOCHEM BEHAV **34**(2) 255–259, 1989. — A spontaneous maturity onset diabetes obesity syndrome occurs in a small proportion (10–20%) of male CBA/Ca mice. Inbreeding can increase the incidence to 80%. It occurs at 12–16 weeks of age, and is characterized by hyperphagia, obesity, hyperglycaemia, hypertriglyceridaemia, hyperinsulinaemia, and an impaired glucose tolerance. The mice are also resistant to exogenous insulin. Female mice remain normal except for a slight increase in serum insulin. The male obese diabetic mice have a normal life expectancy. It is proposed that CBA/Ca mice can provide examples of a useful model for investigating the aetiology of type 2 diabetes and obesity, and the effectiveness of antidiabetic and antiobesity drugs.

Obesity Diabetes CBA/Ca mice Plasma glucose Serum insulin Serum triglycerides

THE study of the aetiology of human diabetes and obesity has been much aided by the use of laboratory rodents which possess hereditary hyperglycaemic or obesity syndromes. These can be either single-gene mutants such as diabetes (db) or obese (ob) in the C57BL mouse (12), multiple gene phenotypes as in the Chinese hamster and New Zealand obese (NZO) mouse, or environmentally induced by transferring a desert-living animal such as the spiny mouse or sand rat to an unrestricted diet (15,16).

However, no single animal model exhibits all the characteristics of the human disease, and there is no entirely satisfactory model available for the human maturity onset noninsulin-dependent diabetes mellitus (type 2, NIDDM). The NZO mouse exhibits a progressive hyperglycaemia and hyperinsulinaemia, but tends to recover to normal with age (3). The C57BL/KsJ-dbdb mouse also tends to be insulin resistant, but like the diabetic KK mouse, the syndrome is evident early in life and the animals have a shortened life expectancy (9).

A brief report in 1982 (4) indicated that a small proportion of males of the CBA/Ca mouse, which are widely used in research, exhibited a maturity onset diabetic syndrome consisting of hyperphagia, obesity, and hyperglycaemia. An inbred colony has been raised from these mice, which now show a high penetration of the syndrome in the males, the females appearing normal. A preliminary account of some of these findings has already been reported (7).

METHOD

Animals

The diabetic CBA mice used in this study were derived from six breeding pairs from the London Hospital Medical College CBA/Ca colony. Their mandible shape corresponded to the MRC classification of authentic CBA/Ca strain (Campbell, personal communication). The mice were brother-sister mated and cross-fostered for 24 generations before the study commenced. Mice from inbred strains of C57BL/10 ScSn (C57) and outbred Lac: LACGFCFW (LacG) raised in Bristol Medical School were also used. Outbred CBA/Ca mice were obtained from a commercial source (Olac Supplies Ltd). The colonies were housed at 20–22° in single-sex cages of 4–6 mice with a 12-hr light-dark cycle. CRM-pelleted diet (Labsure, Cambs.) and water were provided ad lib. The diet contained 17.9% protein, 57% carbohydrate, 3.6% crude fibre, 2.4% crude oil, and a balance of trace elements and vitamins. In the experiments to measure food consumption, powdered diet was provided in preweighed hoppers. The amount of food spilt could be calculated after removal of faecal pellets from the sawdust, which had been preweighed. Fluid consumption was estimated by daily weighing of the drinking bottle.

Plasma Glucose Assay

Mice were lightly anaesthetized with diethyl ether and the tip of

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the tail removed with a sterile scalpel. Preliminary experiments indicated that the ether did not significantly affect the plasma glucose level (PGL), and, since ether was necessary for the cardiac puncture procedure, all blood samples were taken from etherized mice. The first 20 μl of blood was discarded and 100 μl collected into heparinized glass haematocrit tubes. The tail was dipped into sulphanilamide (Sigma) and alum to prevent infection and aid healing. The blood was transferred to sodium fluoride-treated tubes and the plasma separated by centrifugation. Samples of plasma (10 μl) were then assayed in duplicate using a Beckman Glucose Analyzer 2. The tail bleedings were performed between 10.00 and 13.00 hr and at intervals of not less than 48 hr.

Serum Immunoreactive Insulin Assay

Serum immunoreactive insulin (SIRI) was assayed by the method of Herbert *et al.* (11) using dextran-treated charcoal to separate bound from unbound hormone. Mice were bled by cardiac puncture under light ether anaesthesia and serum prepared from a 1–1.5 ml sample of blood as follows: whole blood was centrifuged at 1000 × g for 4 minutes, the plasma was removed and left to clot for 20 minutes in a glass tube. The serum was obtained by a further centrifugation and physical compression of the clotted material. Freeze-dried guinea pig anti-human insulin (Wellcome Laboratories) was used as the anti-serum. [^{125}I]-Insulin was purchased from Amersham International plc. For control assays, bulk serum was prepared from fresh bovine blood, and twice treated with activated charcoal before use. Serum prepared this way contained no detectable insulin. All assays were performed in duplicate and 3 control samples were included in each group of 6 assays.

Serum Triglyceride Assay

Serum was obtained by cardiac puncture as described above. Aliquots of 20 μl were diluted to 80 μl and assayed by the lipase-glycerol-oxidase-peroxidase method of Uwajima et al. (17) using a Technicon RA 1000 autoanalyzer.

Glucose Tolerance Testing

Mice were fasted for 20–24 hr prior to testing. They were lightly anaesthetized with ether and an 0.75 mm outside diameter intravenous cannula (Portex Ltd.) passed down the oesophagus for administration of the glucose. The dose of glucose was 6 g·kg^{-1}, dissolved in distilled water at a concentration of 0.667 g·ml^{-1}. Control mice were given an equivalent volume of water. The mice were tail bled for plasma glucose assay at 0, 30, 60, 120 and 240 min postglucose. Mice were randomly allocated to a single time point.

Insulin Sensitivity

Insulin zinc protamine (Wellcome Laboratories) was diluted in isotonic sodium phosphate pH 7.4 and administered by subcutaneous injection at a dose of 67 IU/kg body weight. The PGL was determined 30 minutes prior to injection and 1–6 hr postinjection. Preliminary studies of the time course of the action of insulin indicated that the peak fall in PGL occurred between 4 and 5 hours postinjection. The 5-hr time point was used in the subsequent experiments.

Statistical Analyses

The statistical significance of the differences between groups of data were determined by two-way analysis of variance (ANOVA), paired *t*-test, or by the Mann-Whitney U-test where appropriate.

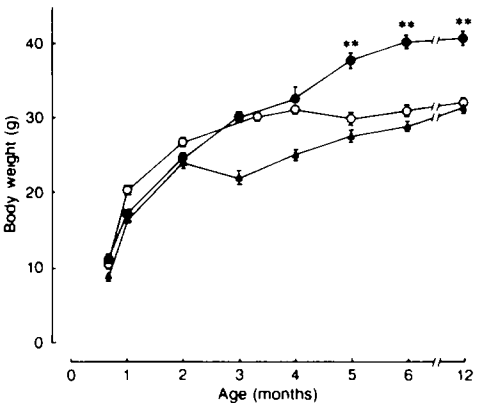

FIG. 1. Change in body weight with age in CBA mice. Results are means ± SEM of n = 6–8. Inbred males: ●; outbred normal males: ○; inbred females: ▲. **Inbred males > control males, $F(7,58) = 12.6$, $p<0.001$, ANOVA.

RESULTS

The weight gain of the inbred CBA/Ca mice from weaning to 12 months of age, compared to normal male and female CBA/Ca mice on an unrestricted diet, is shown in Fig. 1. The normal males achieved their maximum body weight by 3 months, but the diabetic males continued to gain weight up to 6 months, and were significantly heavier ($p<0.001$) than normal males from 5 to 12 months of age. The percentage weight gain of the normal CBA males from 8 to 25 weeks (26.5 ± 2.4%, n = 16) was similar to that observed in normal LacG males (27.2% ± 1.1%, n = 16). Obese male mice have survived for 18–24 months, and their life expectancy appears to be no shorter than that of lean controls.

The calorific value of the diet of the mice was 13.3 kJ·g^{-1}, making it possible to calculate the energy intake of the mice in terms of kJ·g^{-1} body weight per week (Table 1). At 8 weeks of age the inbred CBA males consumed almost twice the calories of the LacG or normal CBA, which were similar. At 25 weeks all 3 groups consumed less calories and the diabetic CBA ate only slightly more than the normal CBA or LacG mice.

The plasma glucose levels (10.00 a.m.) of fed mice are shown

TABLE 1

CALORIFIC VALUE OF WEEKLY DIETARY INTAKE (kJ·g^{-1})

	Age	
	7–8 Weeks	24–25 Weeks
LacG (male) n = 3	8.00 (7.44–8.20)	5.22 (5.06–5.30)
CBA (outbred male) n = 3	7.84 (7.56–7.96)	5.05 (4.98–5.40)
CBA (inbred obese) n = 4	13.80* (9.96–15.12)	6.65* (6.58–7.88)

Results are the mean values (and ranges) from cages of 6 mice measured over 7 days.

*CBA obese > other groups at same age, $p = 0.028$ (Mann-Whitney U-test).

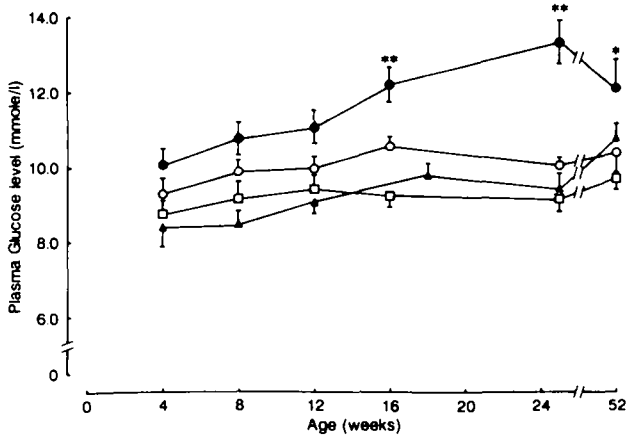

FIG. 2. Influence of age on the plasma glucose levels of LacG and CBA mice. Results are means ± SEM of n = 8–10. Inbred CBA male: ●; inbred CBA female: ▲; outbred control male: ○; outbred control female: □. Effect of age on plasma glucose: control males, females, NS, ANOVA; obese CBA males, $F(5,50) = 9.4$, $p<0.01$. CBA inbred males > other groups at same age: $*p<0.05$, $**p<0.01$.

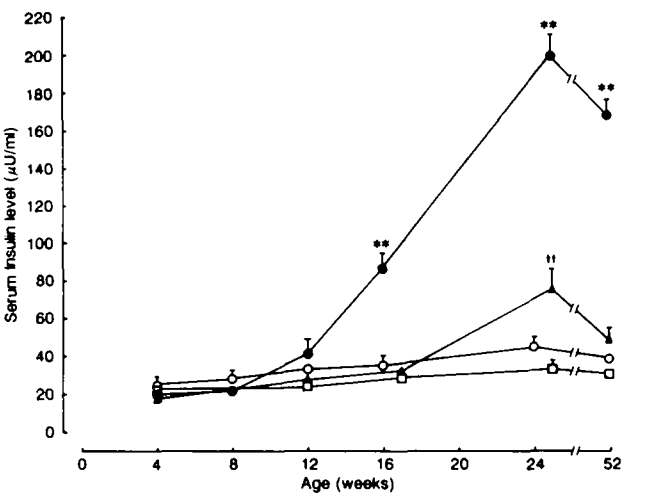

FIG. 3. Influence of age on the plasma immunoreactive insulin levels of CBA mice. For key, see Fig. 2. Effect of age on serum insulin: control males, females, NS, ANOVA; obese CBA males, $F(5,41) = 11.8$, $p<0.01$. **Male CBA > other groups, $p<0.01$, t-test. ††Inbred female CBA > outbred CBA, $p<0.01$, t-test.

in Fig. 2. The LacG males and CBA females have similar plasma glucose levels which do not change with age. Both the normal and diabetic CBA males, however, showed a higher glucose level at 8 weeks compared to the females at 12 weeks, but the diabetic males had significantly higher glucose levels ($p<0.01$) than all other groups at 25 weeks. The plasma glucose peaked at about 25 weeks, but was still elevated at 50 weeks (see control values in Fig. 5).

The serum triglyceride levels are shown in Table 2. Levels in the inbred CBA mice were 100–200% higher than those found in the normal male and female CBA mice at 12 weeks, which, in turn, were significantly higher than the levels in LacG mice at the same age. In all CBA males and females the triglyceride levels increased with age to a peak at 25 weeks; the males remaining consistently higher than the females at all times. In contrast, the LacG mice showed no increase in serum triglyceride levels with age.

The immunoreactive serum insulin levels of both sexes from the inbred and outbred colonies of CBA mice are shown in Fig. 3. All four groups of mice had similar insulin levels at 8 or 12 weeks. However, the CBA males showed a ten-fold increase in insulin by 25 weeks, and there was a smaller, but still significant, 3-fold increase in serum insulin in the females at the same age. The normal CBA mice showed no significant change in serum insulin over the same period.

In order to separate the effect of the obesity from the effect of age on glucose tolerance in inbred CBAs, mice of both sexes were tested at 6–8 weeks and 22–25 weeks of age. The glucose load of 6 $g \cdot kg^{-1}$ was given by gastric intubation and the PGLs monitored over 4 hr. The glucose tolerance curves are shown in Fig. 4. The older male CBA mice had PGLs which were significantly higher than those observed in young (lean) male mice, which were, in turn, significantly higher than the female mice. The glucose tolerance curve of the female CBA mice was virtually identical to that observed in the normoglycaemic LacG mice (data not shown).

The glycaemic response to exogenous insulin in the obese CBA mice was compared with normal CBA mice of either sex (Fig. 5). After a dose of 67 $IU \cdot kg^{-1}$ SC there was a small but not significant fall in the PGL of female CBA mice at both 6 and 50 weeks of age, and a highly significant fall in the PGL in the normal male CBA mice. In the obese males at 50 weeks there was actually a small rise in the PGL 5 hr after insulin.

A small proportion (15–20%) of the outbred CBA/Ca mice obtained commercially also developed the obesity-diabetes syn-

TABLE 2

SERUM TRIGLYCERIDE LEVELS ($mmol \cdot l^{-1}$)

Age (weeks)	CBA Obese Males	CBA Normal Males	CBA Females	LacG Males
8	2.16 ± 0.14(8)	—	1.48 ± 0.10(8)*	—
12/13	2.89 ± 0.19(8)	1.42 ± 0.14(6)§	1.53 ± 0.14(8)†	1.03 ± 0.11(8)‡
25	3.51 ± 0.40(7)	2.34 ± 0.28(6)§	2.81 ± 0.26(8)	0.86 ± 0.07(8)‡
50	3.44 ± 0.46(6)	2.28 ± 0.25(6)§	2.75 ± 0.34(6)	1.08 ± 0.09(6)‡

Results are the mean ± sem of groups of 6–8 mice.
CBA female < CBA obese male, $*p<0.05$; $†p<0.01$.
LacG male < CBA mice of either sex, $‡p<0.01$.
CBA normal male < CBA obese male, $§p<0.01$.

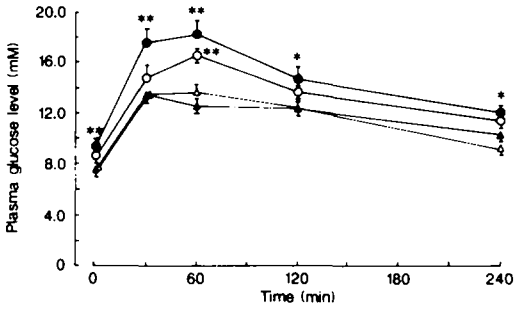

FIG. 4. Plasma glucose levels in inbred CBA mice following an oral dose of glucose (6 g·kg^{-1} body weight) dissolved in distilled water (0.667 g·ml^{-1}). Results are means ± SEM of n = 8–10. Lean male (6–8 weeks old): ○; obese male (22–25 weeks old): ●; female (6–8 weeks old): △; female (22–25 weeks old): ▲. Obese male > normal male, $p<0.05$, ANOVA; obese male > female, $p<0.01$.

drome from the age of 12–16 weeks. These mice were not included in the older groups of normal CBA/Ca, but were found to be similar to the inbred CBA/Ca in terms of all the parameters measured.

DISCUSSION

The results confirm that male CBA/Ca mice spontaneously develop a maturity onset diabetes-obesity syndrome characterised by hyperphagia, excessive weight gain, hyperglycaemia, hypertriglyceridaemia and hyperinsulinaemia. Between 10 and 20% of the original London colony (Campbell, personal communication) and commercially available CBA/Ca mice are affected, whereas 80% of the males of the inbred Bristol colony now develop the syndrome. The severity of the syndrome varied between animals, particularly in terms of serum insulin levels. For example, the serum insulin of 40-week mice ranged from 75 to 888 μU·ml^{-1}. Despite these symptoms, their life expectancy appears to be normal.

There is good evidence that insulin sensitivity decreases with age and that glucose tolerance is impaired (8). Thus, in comparing young mice with older animals it is important to allow for the effects of ageing. Normal CBA male mice and LacG mice showed no change in PGL, serum insulin, or insulin sensitivity with age. The changes in the obese male CBA mice can therefore be ascribed to a diabetogenic disturbance rather than merely an ageing process.

The CBA mice also provide further evidence for the well-established association between obesity and hyperinsulinaemia (1, 14, 18). High circulating insulin levels are known to result in a down regulation of insulin receptors (12) and consequent insulin resistance. In this study there is clear evidence that the rise in circulating insulin (Fig. 3) precedes the increase in body weight (Fig. 1). If hyperphagia leads to a rise in PGL, provoking increased insulin output from the pancreas, then this could precipitate the observed insulin resistance and subsequent obesity.

Although male mice from several strains which exhibit diabetes-obesity syndromes are more severely afflicted than females (5), there are no reports at present of a strain in which only the males are affected. The mode of inheritance of this syndrome is as yet unknown, and a diallele cross experiment with CBA, db/db, ob/ob and genetically distant mice would be necessary to clarify the background of the mutation.

It has been argued that the ob/ob and db/db mice are not ideal models for the study of human obesity, since the human syndrome is believed to represent a response to environmental factors rather than a familial trait (2). This does not preclude a contributory genetic link, for only some individuals develop obesity under particular circumstances. An environmental basis for the CBA syndrome is unlikely, since the obese male mice are kept under identical conditions as their normal littermates. One possibility is that the diabetic syndrome is related to testosterone levels, but we have found that surgical castration of male CBA mice does not prevent the development or affect the severity of the syndrome (6). Preliminary studies (10) have indicated that the treatment of obese male CBA mice with anorectic drugs alleviates the syndrome. These mice may therefore provide a useful model for the investigation of appetite suppressants.

Since the CBA diabetic syndrome only appears in later life, and the mice have a normal lifespan, they may be a more appropriate model for investigating maturity onset type 2 diabetes and obesity than the currently exploited animal models. CBA/Ca mice are widely available, inbreeding increases the incidence of the syndrome, and the use of normal males or age-matched female littermates as controls is also a considerable practical advantage.

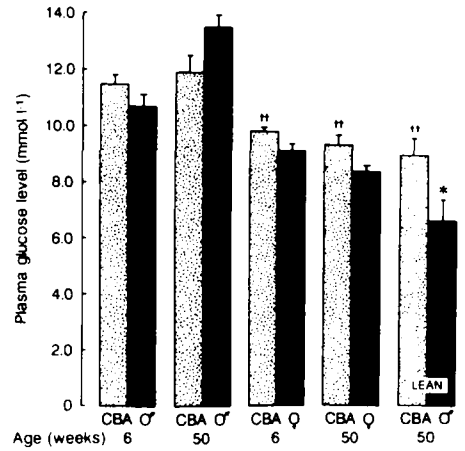

FIG. 5. Effect of exogenous insulin on plasma glucose levels of normal and obese CBA mice. Results are expressed in mmol·l^{-1} and are means ± SEM of groups of 7–8 mice. The plasma glucose was assayed 30 min prior to insulin and 5 hr postinjection. **Postinsulin < preinsulin, $p<0.01$, paired t-test. ††CBA females < CBA obese males of same age, $p<0.01$, t-test.

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