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Antihyperglycaemic effect of saccharin in diabetic *ob*/*ob* mice

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1 The effect of chronic saccharin (benzosulphimide) consumption on glucose homeostasis was examined in normal lean +/+ mice and genetically obese hyperglycaemic insulin-resistant *ob*/*ob* mice.

2 Consumption of a 5% (w/v) sodium saccharin solution for 7 weeks prevented the development of hyperglycaemia, improved glucose tolerance (area under curve decreased by 51%), reduced the extent of hyperinsulinaemia (by 21%), and reduced excessive weight gain (by 18%) in *ob*/*ob* mice.

3 Consumption of 5% (w/v) sodium saccharin temporarily decreased hyperphagia at the beginning of treatment, decreased hepatic glycogen content (by 47%), increased abdominal muscle glycogen content (by 82%), but did not significantly alter the hypoglycaemic response to exogenous insulin in *ob*/*ob* mice.

4 Consumption of a 1% (w/v) sodium saccharin solution did not prevent the development of hyperglycaemia in *ob*/*ob* mice.

5 Normal lean +/+ mice consuming 5% (w/v) sodium saccharin solution showed a marginal decrease (by 8%) in glycaemia, and glucose tolerance was improved (area under curve decreased by 30%) without a significant change in the insulin response to glucose or the hypoglycaemic effect of exogenous insulin.

6 The results suggest that chronic consumption of saccharin can defer the development of hyperglycaemia and improve glucose homeostasis in insulin-resistant *ob*/*ob* mice through a mechanism that is independent of insulin.

Keywords: Saccharin; antihyperglycaemic; glucose tolerance; insulin; glycogen; obese-diabetic *ob*/*ob* mice

Introduction

The non-nutritive sweetener saccharin (benzosulphimide) is widely used in soft drinks, foods and speciality dietary products for the management of diabetes mellitus (Ministry of Agriculture, Fisheries and Food, 1990; Nutrition Subcommittee, British Diabetic Association, 1992). Normal dietary amounts of saccharin do not acutely affect basal glycaemia in non-diabetic and diabetic man (Goldfine *et al*., 1969; Proels *et al*., 1973; Horwitz *et al*., 1988). However, animal studies have suggested that chronic consumption of larger amounts of saccharin might reduce blood glucose concentrations (Purdom *et al*., 1973), but this has not been systematically studied. Indeed, such an effect could be overlooked in diabetic states, because saccharin is usually consumed as a substitute for dietary sucrose, glucose and fructose.

Herein we present evidence that chronic consumption of sodium saccharin solution (5% w/v) as drinking fluid improves glucose homeostasis in the *ob*/*ob* mouse – a murine model of obesity and non-insulin dependent diabetes with severe insulin resistance (Bailey & Flatt, 1991).

Methods

Five-week old homozygous lean (+/+) and obese-hyperglycaemic (*ob*/*ob*) mice from the Aston colony (Birmingham) were used. The origin and characteristics of these mice have been described previously (Flatt & Bailey, 1981a; Bailey *et al*., 1982). The *ob* gene is an autosomal recessive mutation, and *ob*/*ob* mice were obtained from a breeding colony of heterozygous lean (*ob*/+) mice. A separate colony of homozygous lean (+/+) mice was established by six back-crossings without any *ob*/*ob* offspring. Mice were housed in groups of five and maintained at $22 \pm 2^{\circ}C$ with a fixed 12 h light-dark cycle and supplied a standard pellet diet (Mouse breeding diet, Heygates, Northampton) and tap water. The diet comprised (g kg^{-1}) fat 25, protein 176, carbohydrate 468 (digestible energy 15.3 MJ kg^{-1}) with vitamins and minerals (Flatt & Bailey, 1982).

Two groups of each genotype were studied. After a 1 week run-in period, the test group received a 5% (w/v) solution of sodium saccharin (Sigma Chemical Company, Poole) as drinking fluid, while the control group continued to receive ordinary tap water. Treatment was continued for 7 weeks, and animals were monitored for a further 4 weeks after treatment was stopped. Food and fluid were available *ad libitum*, and food and fluid consumption, body weight, plasma glucose and plasma insulin were measured at weekly intervals as previously described (Flatt & Bailey, 1981a; 1984). Blood samples (80 μl) for plasma glucose and insulin assay were taken from the tail tip of fed animals at 1000 h.

An intraperitoneal (i.p.) glucose tolerance test was conducted on 24 h fasted mice on day 30 of treatment. Blood samples were taken immediately before and at 30, 60 and 120 min after an i.p. injection of glucose (2 g kg^{-1} body wt. in a 40% w/v solution). Area under the curve above the zero value was calculated (Brichard *et al*., 1990).

An insulin hypoglycaemia test was conducted on 24 h fasted mice on day 44 of treatment. Blood samples were taken immediately before and at 20, 40, and 60 min after i.p. injection of porcine insulin (Novo-Nordisk, Crawley; 0.5 u kg^{-1} to +/+ mice and 10 u kg^{-1} to *ob*/*ob* mice).

Plasma glucose was measured by a glucose oxidase method with a Beckman glucose analyser (Stevens, 1971). Plasma insulin was measured by double antibody radioimmunoassay (Bailey & Ahmed-Sorour, 1980) with rat insulin as standard.

In a separate study, the glycogen content of liver and abdominal muscle was determined in non-fasted *ob*/*ob* mice after 5 weeks consumption of a 5% (w/v) solution of sodium saccharin as drinking fluid. Tissue was digested in 1 M KOH, glycogen was precipitated with ethanol, hydrolysed with H_2SO_4 and neutralized with NaOH. Glucose was assayed by a manual glucose oxidase procedure, and glycogen was expressed as μmol glucose equivalents g^{-1} of tissue (Bailey *et al*., 1992).

The effect of consuming a 1% (w/v) sodium saccharin solution on body weight, food intake and plasma glucose was determined in *ob*/*ob* mice by use of the same procedure as for 5% saccharin. Consumption of the 1% (w/v) and 5% (w/v)

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solutions of sodium saccharin by the *ob/ob* mice provide approximately 100 times and 500 times (respectively) more saccharin per unit body mass than consumed by a diabetic patient using diabetic speciality foods.

Results are presented as means ± s.e.mean, $n = 5$. Data were analysed by one-way ANOVA and Student's unpaired t test. Differences were considered to be significant if $P < 0.05$.

Results

Lean (+/+) mice

Consumption of saccharin (5% w/v) as drinking fluid for 7 weeks did not significantly affect body weight gain or food consumption in lean mice (Figure 1). Mean fluid intake was 25% higher ($P < 0.05$) in mice consuming saccharin. Mean plasma glucose concentrations were 8% lower ($P < 0.05$) during consumption of saccharin, but plasma insulin concentrations were not significantly altered.

Fasted plasma glucose and insulin concentrations measured on day 30 before glucose tolerance tests were similar in the control and saccharin-treated groups (Figure 2). However, glucose tolerance was improved by saccharin (area under response curves 835 ± 52 vs 1212 ± 40 mmol^{-1} 120 min, $P < 0.01$), although insulin concentrations were not significantly altered.

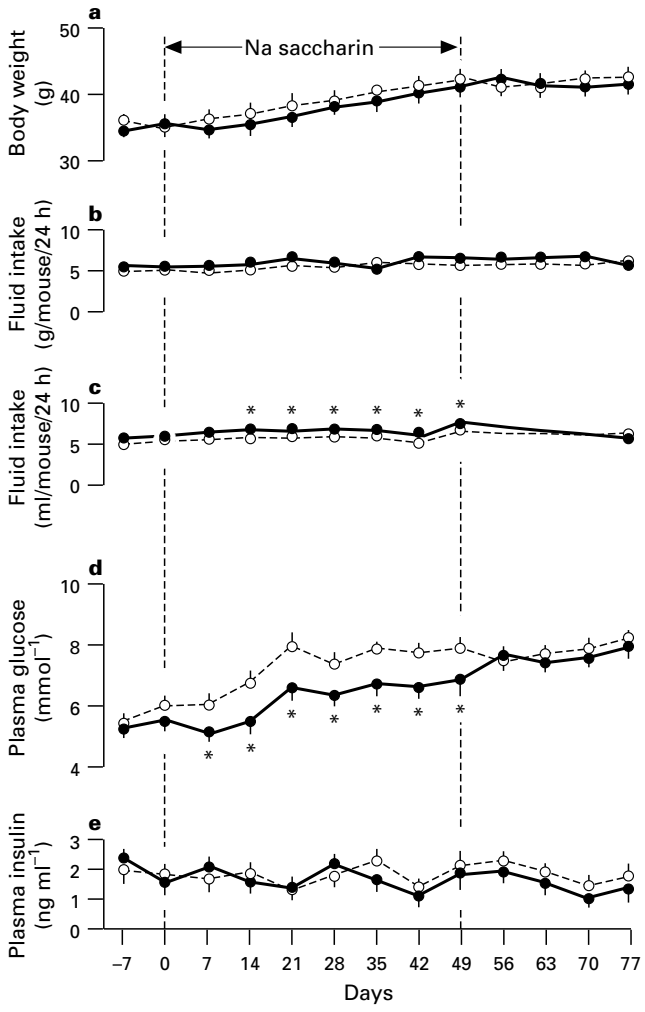

Figure 1 Effect of saccharin consumption (5% as drinking fluid) on (a) body weight, (b) food and (c) fluid intake, (d) plasma glucose and (e) plasma insulin concentrations in young lean (+/+) mice. Saccharin (●); control (○). Values are mean and vertical lines s.e.mean, $n = 5$. *$P < 0.05$ versus control.

Insulin hypoglycaemia tests on day 44 showed no significant difference in the disappearance of plasma glucose by mice consuming saccharin (Figure 3): the percentage decrease (0–60 min) was 61.2 ± 5.4% versus 68.7 ± 5.2%, $P > 0.1$, for the control and saccharin groups, respectively.

Obese (ob/ob) *mice*

The untreated *ob/ob* mice were characteristically obese, hyperphagic and hyperinsulinaemic, and became hyperglycaemic at about 7 weeks of age (Figure 4). Commencement of saccharin consumption (5% w/v) as drinking fluid produced an initial reduction in food consumption compared with control *ob/ob* mice (week 1). Thereafter food consumption was usually slightly below that of controls, but the overall mean food consumption (8.0 ± 0.3 g/mouse/day) was

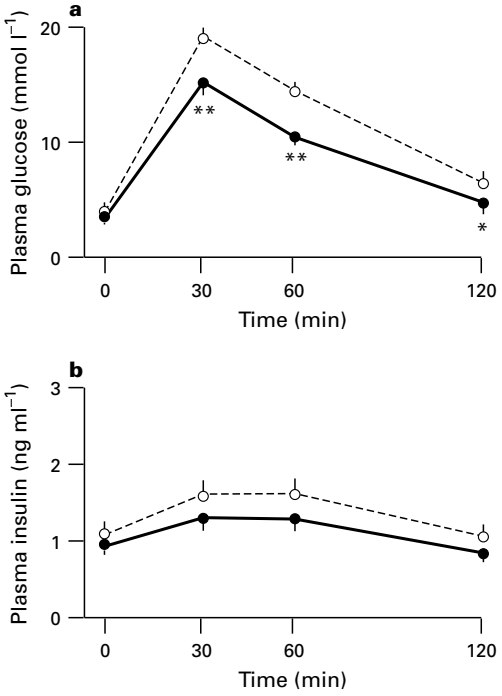

Figure 2 Effect of saccharin consumption (5% as drinking fluid) for 30 days on intraperitoneal glucose tolerance in fasted lean (+/+) mice. Saccharin (●); control (○). Values are mean and vertical lines show s.e.mean, $n = 5$. *$P < 0.05$, **$P < 0.01$ versus control.

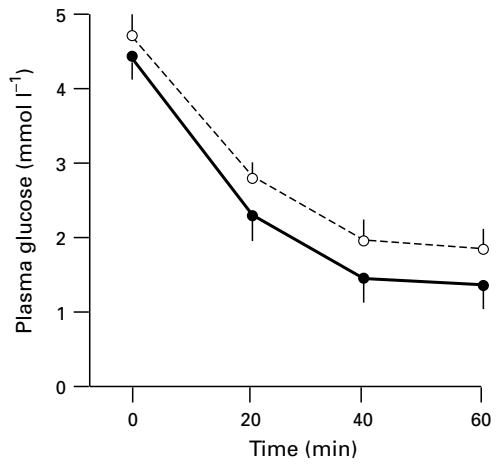

Figure 3 Effect of saccharin consumption (5% as drinking fluid) for 44 days on intraperitoneal insulin-induced hypoglycaemia in fasted lean (+/+) mice. Saccharin (●); control (○). Values are mean and vertical lines show s.e.mean, $n = 5$.

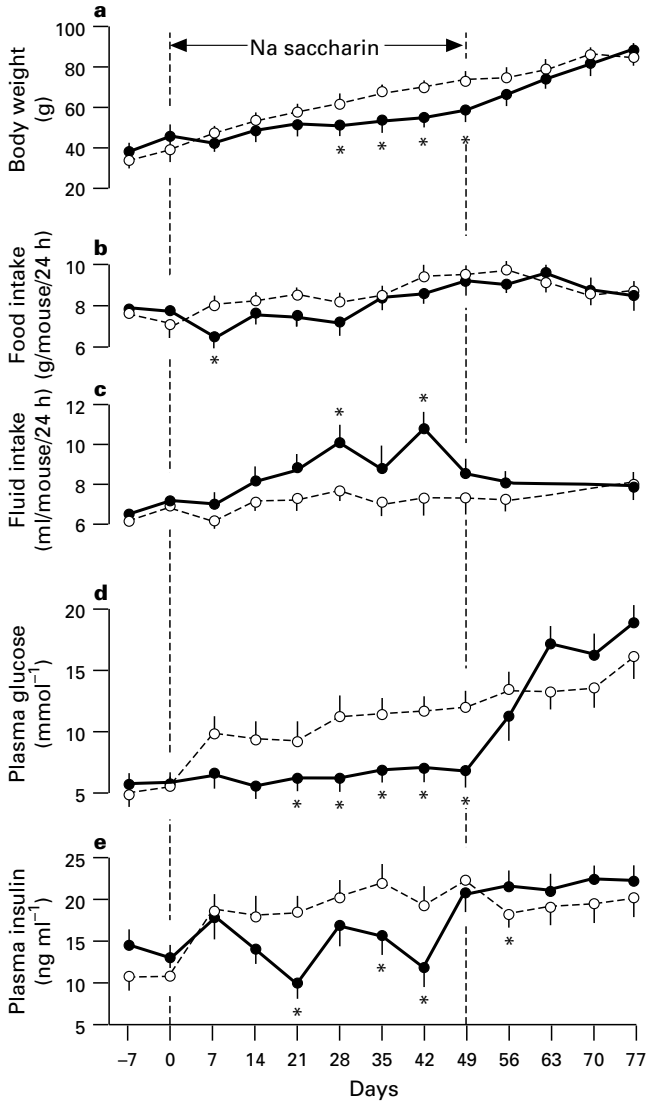

Figure 4 Effect of saccharin consumption (5% as drinking fluid) on (a) body weight, (b) food and (c) fluid intake, (d) plasma glucose and (e) plasma insulin concentrations in young obese (*ob*/*ob*) mice. Saccharin (●); control (○). Values are mean and vertical lines show s.e.mean, $n=5$. *$P<0.05$ versus control.

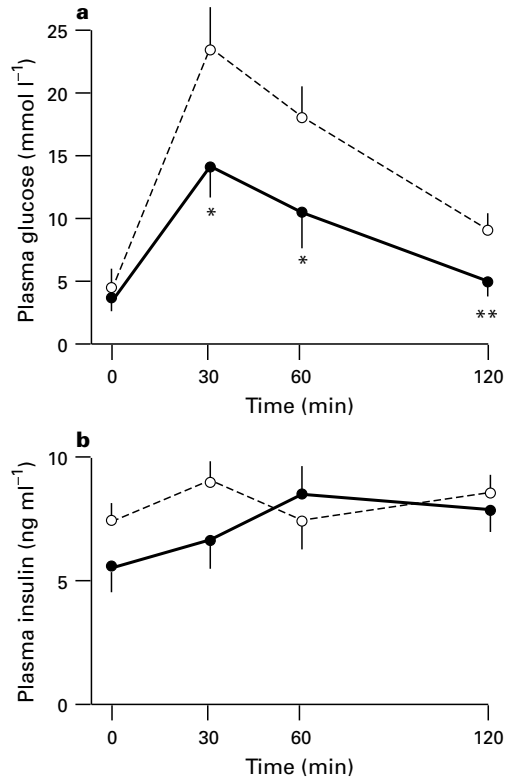

Figure 5 Effect of saccharin consumption (5% as drinking fluid) for 30 days on intraperitoneal glucose tolerance in fasted obese (*ob*/*ob*) mice. Saccharin (●); control (○). Values are mean and vertical lines show s.e.mean, $n=5$. *$P<0.05$, **$P<0.01$ versus control.

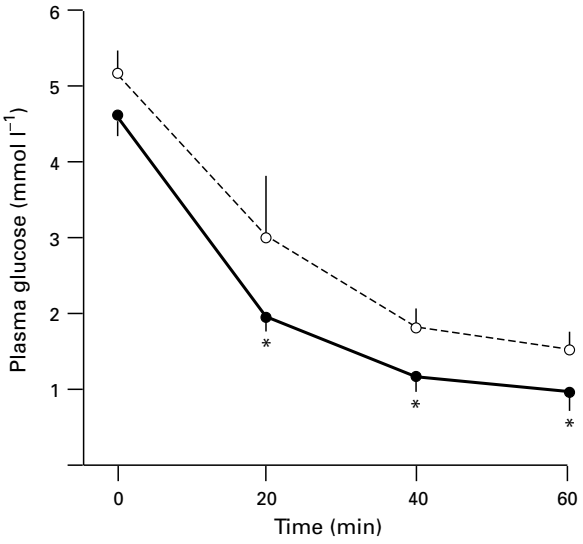

Figure 6 Effect of saccharin consumption (5% as drinking fluid) for 44 days on intraperitoneal insulin-induced hypoglycaemia in fasted obese (*ob*/*ob*) mice. Saccharin (●); control (○). Values are mean and vertical lines show s.e. mean, $n=5$. *$P<0.05$ versus control.

not significantly different from controls (8.3 ± 0.3 g/mouse/day). In contrast, mean fluid intake was 20% greater during saccharin consumption ($P<0.01$). Body weight gain was reduced at 4–7 weeks of saccharin treatment by about 18% ($P<0.05$), but this weight was regained within 4 weeks of stopping saccharin. The development of hyperglycaemia was delayed by saccharin, and after 7 weeks of treatment plasma glucose concentrations were 49% lower than control *ob*/*ob* mice ($P<0.05$), and not significantly different from lean (+/+) mice. Plasma glucose increased to values observed in untreated *ob*/*ob* mice within 1–2 weeks of stopping saccharin. Plasma insulin concentrations fluctuated considerably, averaging 21% lower during the period of saccharin consumption ($P<0.05$), but insulin values were similar to control *ob*/*ob* mice when saccharin was stopped.

Before the glucose tolerance test on day 30, fasting plasma glucose and insulin concentrations were similar in control and saccharin-treated *ob*/*ob* mice (Figure 5). Glucose tolerance was improved by saccharin (area under response curve 744 ± 204 vs 1548 ± 156 mmol^{-1} 120 min, $P<0.05$) without a significant change in insulin concentrations. During insulin hypoglycaemia tests on day 44, the saccharin-treated group achieved lower plasma glucose concentrations (Figure 6), but the percentage decrease (0–60 min) was not significantly different ($76.5\pm2.8\%$ vs $69.8\pm3.1\%$, $P<0.1$, for the saccharin-treated and control groups, respectively).

Liver glycogen content (μmol g^{-1}), measured on day 35, was reduced by 47% in the saccharin-treated group (340 ± 56 vs 647 ± 86, $n=5$, $P<0.05$). Conversely, the abdominal muscle glycogen content (μmol g^{-1}) was almost doubled by the saccharin treatment (51 ± 4 vs 28 ± 2, $n=5$, $P<0.01$).

When *ob*/*ob* mice consumed a 1% (w/v) sodium saccharin solution for 7 weeks there was no significant effect on body weight gain, food intake, or the development of hyperglycaemia (data not shown).

Discussion

Despite extensive use of saccharin in foods and drinks, including those consumed by diabetic patients, there has been little consideration of a chronic effect on glycaemic control beyond that expected when nutritive sugars are substituted with non-nutritive sweeteners (Ministry of Agriculture, Fisheries and Food, 1990; Nutrition Subcommittee, British Diabetic Association, 1992). The present study demonstrates that consumption of a 5% (w/v) saccharin solution as drinking fluid deferred the development of hyperglycaemia and reduced the hyperinsulinaemia and excess weight gain in young *ob*/*ob* mice. Chronic saccharin consumption also produced a small but significant lowering of glycaemia in young lean (+/+) mice.

These effects of saccharin cannot be explained by the temporary decrease of food intake observed in *ob*/*ob* mice. While this will undoubtedly help to improve glucose homeostasis (Flatt & Bailey, 1981a, b), the antihyperglycaemic effect was maintained when hyperphagia returned. Moreover, the small glucose-lowering effect of saccharin in +/+ mice occurred without a change in food intake.

Since saccharin consumption did not significantly lower fasting glucose concentrations in either *ob*/*ob* or +/+ mice, it is possible that saccharin might impinge upon intestinal glucose absorption. A study with chick enterocytes has suggested that saccharin might reduce the rate of intestinal glucose absorption (Kimmich *et al*., 1988), but there is no evidence from *in vivo* observations that overall glucose absorption is incomplete (Purdom *et al*., 1973). Also, saccharin improved glucose tolerance after an intraperitoneal challenge in fasted *ob*/*ob* and +/+ mice.

The inconsistency with which the hyperinsulinaemia was reduced during saccharin consumption in *ob*/*ob* mice suggests that this effect was not essential for the antihyperglycaemic action. While it is known that agents which decrease the hyperinsulinaemia in *ob*/*ob* mice will decrease the insulin resistance and improve glycaemic control (Batchelor *et al*., 1975), the study in lean (+/+) mice substantiates that a glucose-lowering effect is not dependent on a change in insulin concentrations. Also, saccharin improved glucose tolerance without an obvious change in the insulin response in *ob*/*ob* and +/+ mice. Although oral consumption of saccharin (1 ml of a 0.15% solution) activates a cephalic reflex which transiently raises insulin concentrations, this was not sufficient to affect significantly basal glycaemia in lean or obese Zucker *fa*/*fa* rats (Ionescu *et al*., 1988).

If the glucose-lowering effect of saccharin cannot be attributed to changes in insulin concentrations, other mechanisms must operate to decrease hepatic glucose output and/or increase glucose utilization. Acute responses to saccharin consumption in lean and obese Zucker rats involved an increase in hepatic glucose output, possibly due to a cephalically-initiated reflex to increase glycogenolysis (Ionescu *et al*., 1988). This might contribute to a chronic lowering of blood glucose if hepatic glycogen reserves become severely depleted, giving greater capacity for replenishment during periods of feeding and glucose administration. Support for this view was gained from the present observation that the hepatic glycogen content was reduced by 47% in the saccharin-treated *ob*/*ob* mice. Saccharin acutely increased glucose disposal in lean and obese Zucker rats especially the obese rats (Ionescu *et al*., 1988), compatible with the chronically increased muscle glycogen content of the saccharin-treated *ob*/*ob* mice. Improved muscle glycogenesis is also consistent with the chronic improvement in glucose tolerance noted herein.

The mechanism responsible for the increased glucose disposal is not known, but there was no evidence of increased insulin sensitivity during insulin hypoglycaemia tests in lean mice. In *ob*/*ob* mice the insulin hypoglycaemia tests achieved lower glucose concentrations in the saccharin-treated group, but this was no more than would be expected given the generally improved glycemic status (Flatt & Bailey, 1981b), and there was no significant change in the percentage fall in plasma glucose. However, behavioural studies in rats which received insulin injections have noted a greater susceptibility to fatal hypoglycaemia if saccharin has been consumed (Valenstein & Weber, 1965; Deutsch, 1974), a situation consistent with depleted hepatic glycogen reserves.

The daily amount of saccharin consumed as a 5% (w/v) solution in the present study was 500–1000 times greater than that likely to be consumed by a diabetic patient using diabetic speciality foods (5–10 g kg^{-1} body weight by the mice versus about 10 mg kg^{-1} body weight by diabetic patients) (Ministry of Agriculture, Fisheries and Food, 1990). We therefore examined the effect of a lower (1% w/v) saccharin solution in *ob*/*ob* mice, and found this to be ineffective against the development of hyperglycaemia. More extensive dose-response studies in older *ob*/*ob* mice have shown that a chronic antihyperglycaemic effect of saccharin is only evident during consumption of saccharin solutions >2% (w/v) (Day, Turner and Bailey, unpublished observations).

The present studies raise pertinent questions concerning the long-term metabolic effects of large doses of saccharin, and suggest a possible basis for the design of new antihyperglycaemic agents. As saccharin emerges from controversies over safety (Kalkhoff & Levin, 1978; Miller & Frattalli, 1989; Mitchell & Pearson, 1991) and the use of non-nutritive sweeteners becomes an accepted dietary practice (Nutrition Subcommittee, British Diabetic Association, 1992; American Diabetes Association, 1993), the effects of such sweeteners in the management of diabetes require further consideration.

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