Dietary components and plasma insulin responses to fasting and refeeding in genetically obese hyperglycaemic (*ob/ob*) mice

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1. To investigate the role of dietary components in the hyperinsulinaemia of the obese hyperglycaemic (*ob/ob*) syndrome, plasma insulin responses to fasting and refeeding were examined in Aston *ob/ob* mice supplied with standard diet, non-digestible-carbohydrate test food, and isoenergetic test foods from which either carbohydrate, protein or fat was omitted.

2. During fasting, plasma insulin concentrations fell more rapidly and to a greater extent than plasma glucose. Refeeding the standard diet raised insulin concentrations above normal, associated with a 25% compensatory increase in food intake over 24 h.

3. Test foods supplied to previously fed or fasted mice produced glucose responses consistent with the available carbohydrate content. Carbohydrate-free food (protein and fat) provided a small insulinotropic stimulus; the effect of protein-free food (carbohydrate and fat) was greater; and the combination of carbohydrate with protein (fat-free food) evoked a marked insulin response. In contrast, insulin concentrations declined in mice given the non-digestible-carbohydrate food.

4. Consumption of the standard diet was increased after 24 h feeding non-digestible-carbohydrate food, but was unaffected by a 30 h fast initiated 54 h previously.

5. These results demonstrate that hyperinsulinaemia in *ob/ob* mice is not merely triggered by the ingestion of bulk, but depends on the type of nutrient ingested. Dietary carbohydrate appears to be the major stimulus to the hyperinsulinaemia, with an important augmentation in the presence of protein. Since direct glucose stimulation of insulin release is defective in *ob/ob* mice, the hyperinsulinaemia must be mediated by increased activity of the enteroinsular axis.

The *ob/ob* syndrome in mice is inherited as an autosomal recessive trait characterized in the homozygous condition by an age-related pattern of hyperphagia, obesity, hyperglycaemia and hyperinsulinaemia (Herberg & Coleman, 1977; Bray & York, 1979; Flatt & Bailey, 1981*b*; Bailey *et al.* 1982). Raised insulin concentrations promote metabolic abnormalities such as excessive lipogenesis and hyperglycaemia through insulin resistance and pancreatic A-cell dysfunction (Assimacopoulos-Jeannet & Jeanrenaud, 1976; Flatt & Bailey, 1981*b*; Flatt *et al.* 1982). The factors which initiate increased insulin secretion in young *ob/ob* mice are not established (Dubuc, 1976; Bray & York, 1979) but there is considerable evidence that pathways triggered by feeding are instrumental in the maintenance of hyperinsulinaemia in later life (Flatt & Bailey, 1982*a*, 1983*a*). More than a casual relationship exists between insulin concentrations and food intake. For example, there are similar age changes in hyperinsulinaemia and hyperphagia (Bailey *et al.* 1982), and plasma insulin concentrations decrease markedly when food is withheld (Cuendet *et al.* 1975; Flatt & Bailey, 1981*b*). Furthermore, conditioning to intensify the neuro-endocrine pathways evoked by feeding augments the insulin response to food ingestion (Flatt & Bailey, 1983*a*). The severe hyperinsulinaemia in fed adult *ob/ob* mice does not result from a direct stimulatory effect of hyperglycaemia on the pancreatic B-cells, since parenterally administered glucose does not stimulate insulin release in these mice (Flatt & Bailey, 1981*b*, 1982*b*). However, enterally

Table 1. *Composition of the test foods (g/kg)*

Component	Fat-free food	Protein-free food	Carbohydrate-free food	Non-digestible-carbohydrate food
Casein*	200	—	200	—
Maize oil	—	100	220	—
Liquid paraffin	50	—	—	50
Petroleum jelly	50	—	—	50
Glucose	500	500	—	—
Cellulose powder	100	300	480	800
Vitamin–mineral premix†	100	100	100	100
Digestible energy (MJ/kg)	10·7	11·5	10·8	Negligible

* (g/kg): Crude protein (nitrogen × 6·25) 850, moisture 120, ash 20, fat plus carbohydrate ⩽ 10.

† Composition (per kg) of test food: retinol 1500 μg, cholecalciferol 12·5 μg, thiamin 4 mg, riboflavin 5 mg, pyridoxine 6 mg, cyanocobalamin 5 μg, α-tocopherol 60 mg, menadione 3 mg, folic acid 1 mg, nicotinic acid 10 mg, pantothenic acid 12 mg, choline 800 mg, inositol 500 mg, calcium 6 g, phosphorus 5 g, sodium chloride 6 g, magnesium 500 mg, potassium 5 g, iron 50 mg, copper 5 mg, manganese 50 mg, cobalt 500 μg, zinc 25 mg, iodine 500 μg, fluorine 50 μg, selenium 50 μg.

administered glucose markedly increases insulin release (Flatt & Bailey, 1981 c), illustrating the importance of insulinotropic factors generated by the enteral presence and absorption of nutrients.

It was recently observed in adult *ob/ob* mice that the acute plasma insulin response to meals of different composition was greatly diminished in the absence of carbohydrate (Flatt & Bailey, 1982 a). The present study further investigates the role of dietary macronutrients in the hyperinsulinaemia of adult Aston *ob/ob* mice. Plasma insulin responses to fasting and refeeding were examined in mice supplied with standard diet, non-digestible-carbohydrate, or isoenergetic test foods from which either carbohydrate, protein or fat was omitted.

MATERIALS AND METHODS

Animals and standard diet

Obese hyperglycaemic (*ob/ob*) mice from the colony maintained at the University of Aston in Birmingham were used at 20 weeks of age. The origin and characteristics of Aston *ob/ob* mice have been described in detail elsewhere (Flatt & Bailey, 1981 a; Bailey *et al.* 1982). Mice were housed in an air-conditioned room at 22 ± 2° with a lighting schedule of 9·5 h light (08.00–17.30 hours) and 14·5 h dark. A standard pellet diet (Mouse Breeding Diet; Heygate and Sons Ltd, Northampton) and tap water were supplied *ad lib*. The standard diet comprised (g/kg) fat 25, protein 176, carbohydrate 468 (digestible energy 15·3 MJ/kg) with vitamins and minerals as described elsewhere (Flatt & Bailey, 1982 a).

Test foods

Fat-free, protein-free, carbohydrate-free foods and a non-digestible-carbohydrate (cellulose based) food were obtained from BP Nutrition Ltd, Witham, Essex (Table 1). The amounts (g/kg) of fat, protein and carbohydrate in the foods were 0, 200 and 500 for fat-free; 100, 0 and 500 for protein-free; and 220, 200 and 0 for carbohydrate-free respectively. These foods were approximately isoenergetic and texture was standardized using liquid paraffin and petroleum jelly.

Experimental procedure

During experiments the mice were housed individually in metabolic cages. To minimize stress, the mice were adapted to the experimental procedures for 2–3 weeks before the study as described previously (Flatt *et al.* 1982). Blood samples (50 μl) for analysis of plasma glucose and insulin were taken from the tail-tip of conscious mice at the times indicated in the tables and figures. The times at which blood samples were taken were staggered to ensure that no more than three samples were taken from each mouse in 24 h.

Fasting and refeeding standard diet

Plasma glucose and insulin were measured at intervals during a 24 h fast and during the subsequent 24 h period of refeeding *ad lib.* with standard diet. Food intake was monitored during the latter period. For comparison, the normal circadian variations in plasma glucose and plasma insulin concentrations, and food intake were measured in *ob/ob* mice when the standard diet was supplied *ad lib.*

Transfer to test foods. Groups of mice were familiarized with the appropriate test food (fat-free, protein-free, carbohydrate-free or non-digestible-carbohydrate) by including the food with the standard diet for 24 h on four occasions during the 2 weeks before the study. On the day of the experiment, the test food was introduced at 10.00 hours to groups of mice which had been fasted previously for 30 h or given standard diet *ad lib*. The test food was the only food available on this occasion and the mice were allowed to feed *ad lib.* for 24 h. As shown in the figures, the mice readily consumed each of the foods provided. Blood samples were taken for plasma glucose and insulin analysis and food intake was monitored. After consuming the test food for 24 h, the mice in each treatment group were either fasted or returned to standard diet. Blood was obtained and food intake was recorded after 6 h.

Analyses

Food intake was measured as the difference between the amount provided and the amount remaining in the delivery hopper, taking care to account for spillage. Plasma was separated and stored at $-20°$ until assayed for glucose (Stevens, 1971) and insulin (Flatt & Bailey, 1981 *a*). Values within and between individual groups of mice were compared using Student's paired and unpaired *t* tests respectively. Differences were considered to be significant for $P < 0.05$.

RESULTS

Fasting and refeeding standard diet

Fig. 1 (*a*) shows food intake and plasma glucose and insulin concentrations of *ob/ob* mice at intervals during a 24 h fast and during a 24 h period of refeeding with standard diet *ad lib.* During the fast, plasma insulin concentration fell rapidly to achieve less than 20% of the value in the fed state. In contrast, plasma glucose concentrations declined slowly to 60% of concentrations in the fed state. When the mice were refed (Fig. 1 (*a*)), food intake was greater than that during normal feeding (Fig. 1 (*b*)). Mean food intake during the first 10 h of refeeding (24–34 h) was 74·8 (SE 7·4) g/kg body-weight (*n* 8) compared with 37·0 (SE 9·9) g/kg body-weight ($P < 0.01$) for the corresponding period (10.00–20.00 hours) of normal feeding. Over 24 h, food intake of refed mice was 123·8 (SE 15·3) g/24 h per kg body-weight compared with 98·3 (SE 7·8) g/24 h per kg body-weight ($P < 0.05$) during normal feeding. Thus, all the additional food consumed during the 24 h period of refeeding was consumed during the first 10 h. After 10 h of refeeding, plasma glucose (13·8 (SE 1·4) mmol/l) and plasma insulin (59·0 (SE 17·0) ng/ml) concentrations were higher ($P < 0.05$) than values at the same time (20.00 hours) during normal feeding (10·9
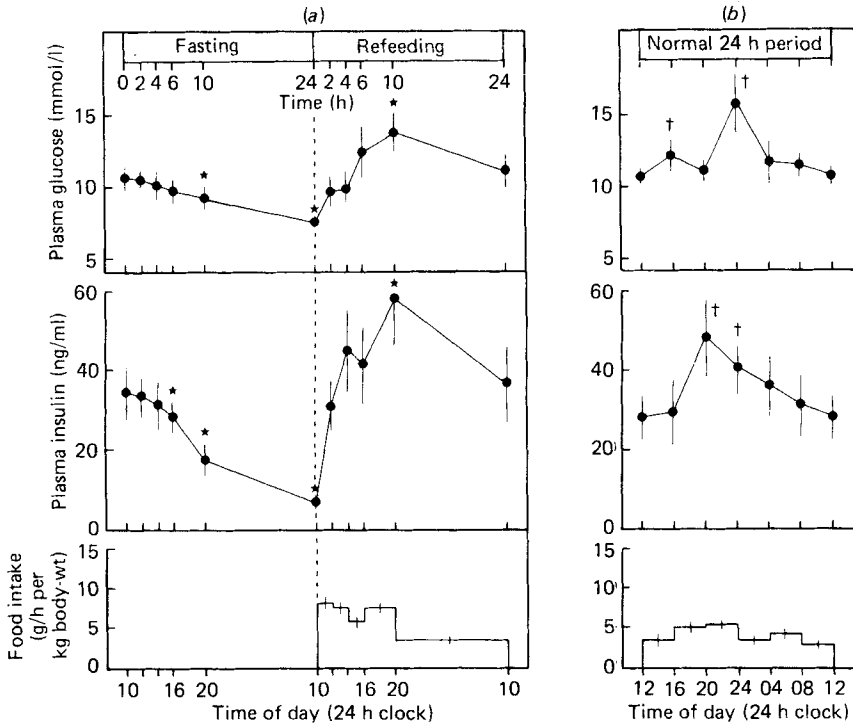
Fig. 1. Plasma glucose, plasma insulin and food intake in 20-week-old *ob/ob* mice (*a*) during a 24 h fast followed by refeeding with standard diet *ad lib.*, and (*b*) during a normal 24 h period with standard diet supplied *ad lib.* Points are mean values with their standard errors, represented by vertical bars, for eight mice. (*a*) * Values when compared with the value at time zero were significantly different (*P* < 0·05); all values were significantly different (*P* < 0·05) compared with 24 h. (*b*) † Values when compared with the value at initial 12.00 hours were significantly different (*P* < 0·05).

(SE 0·8) mmol/l and 49·6 (SE 10·3) ng/ml respectively). However, after 24 h of refeeding, values were not significantly different from those observed during normal feeding.

Transfer to test foods

Test foods in fasted mice

Groups of 30 h fasted *ob/ob* mice were refed for 24 h with either fat-free, protein-free, carbohydrate-free or non-digestible food supplied *ad lib.* Food intake and plasma glucose and insulin concentrations are shown in Fig. 2 and the results are summarized in Table 2. Food intake was higher in mice receiving the carbohydrate-free food. The other foods were consumed in similar amounts. There was a rapid and marked increase in the plasma glucose concentrations of mice consuming the test foods which contained glucose (fat-free and protein-free foods), but concentrations declined slowly in mice consuming foods without glucose (carbohydrate-free and non-digestible-carbohydrate). The decline of glucose was smaller with carbohydrate-free food. This suggests a glucose-sparing effect due to preferential usage of non-carbohydrate energy sources (dietary fat) and greater production of glucose from dietary protein via gluconeogenesis. The fat-free food (containing glucose and protein) produced particularly marked elevations of both plasma glucose and insulin concentrations. The protein-free (glucose and fat) and carbohydrate-free (protein and fat) foods produced smaller insulin responses, even after correction for differences in food intake by dividing by the amounts eaten in 24 h. Although the mice receiving the non-digestible-carbohydrate
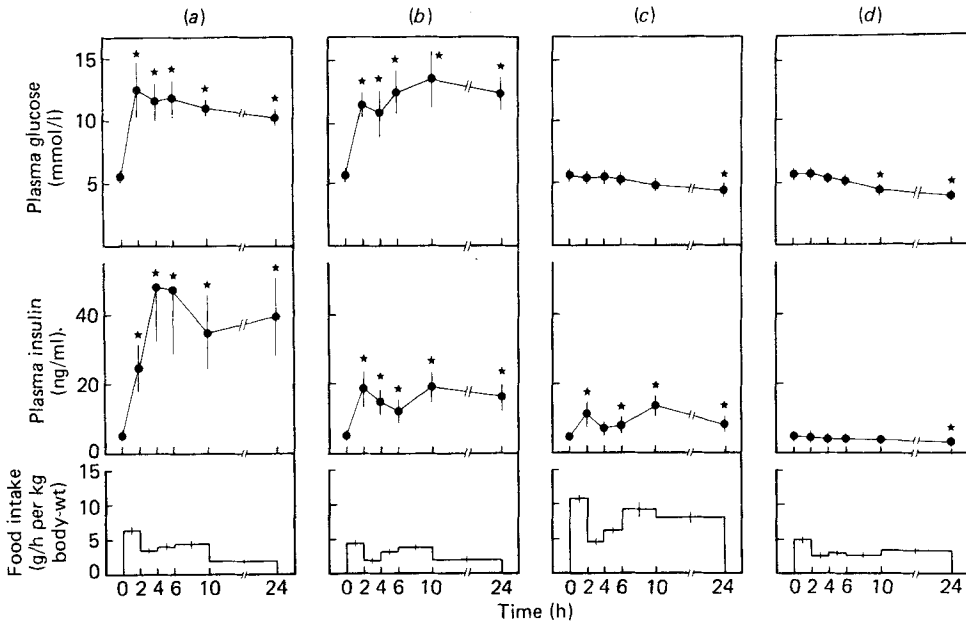
Fig. 2. Plasma glucose, plasma insulin and food intake in 30 h fasted 20-week-old *ob/ob* mice refed with (*a*) fat-free, (*b*) protein-free, (*c*) carbohydate-free or (*d*) non-digestible-carbohydrate test foods *ad lib.* (for details, see Table 1). Points are mean values, with their standard errors represented by vertical bars, for eight mice. * Values when compared with the value at time zero were significantly different ($P < 0.05$).

food consumed a substantial quantity during the test period, a small decrease in plasma insulin concentrations was observed.

Test foods in fed mice

Values presented in Fig. 3 and summarized in Table 2 show the food intake and plasma glucose and insulin concentrations of fed *ob/ob* mice transferred from standard diet to fat-free, protein-free, carbohydrate-free or non-digestible-carbohydrate foods for 24 h. The effects were qualitatively comparable with those observed after supplying the test foods to 30 h fasted *ob/ob* mice. Carbohydrate-free food was consumed in greater quantities than the other test foods, which were consumed in similar amounts. The extent of hyperglycaemia was maintained in mice receiving foods containing glucose (fat-free and protein-free), whereas plasma glucose concentrations declined in mice receiving the carbohydrate-free and non-digestible-carbohydrate foods. Plasma insulin concentrations were well maintained by the fat-free food (glucose and protein), and a small decrease was observed in mice consuming the protein-free food (glucose and fat). Mice receiving the foods without glucose (carbohydrate-free and non-digestible-carbohydrate foods) showed a rapid fall in plasma insulin concentrations, although substantial quantities of the foods were consumed. Indeed the consumption of carbohydrate-free food was 81 % greater than the normal consumption of standard pellet diet during 24 h.

Withdrawal of test foods and return to normal diet

After consuming test food for 24 h, half the mice in each of the groups represented in Figs 2 and 3 were fasted and half were returned to standard diet *ad lib.* Food intake and plasma glucose and insulin concentrations were noted after 6 h (Table 3).

Effect of fasting. Plasma glucose concentrations fell during the 6 h fast in all mice

Table 2. *Body-weight, food intake and the plasma glucose and insulin responses to feeding test foods for 24 h in groups of ob/ob mice previously fasted for 30 h or fed ad lib.*

(Mean values with their standard errors for eight mice)

Group	Body-wt (g)		Food intake (g/24 h per kg body-wt)		Plasma glucose response				Plasma insulin response			
					Δ Glucose (mmol/l)†		Δ Glucose* food intake		Δ Insulin (ng/ml)†		Δ Insulin* food intake	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fasted 30 h												
Fat-free	83·8	3·5	72^c	9	52·4^{bcd}	1·6	0·72^{cd}	0·06	174·6^{bcd}	25·2	2·40^{bcd}	0·32
Protein-free	88·8	2·4	61^c	7	43·4^{acd}	1·6	0·70^{cd}	0·05	57·1^{acd}	4·6	0·92^{acd}	0·08
Carbohydrate-free	81·8	7·3	162^{abd}	22	−2·7^{ab}	0·3	−0·04^{ab}	0·01	26·1^{abd}	2·8	0·16^{abd}	0·02
Non-digestible-carbohydrate	81·7	3·1	83^c	8	−4·3^{ab}	0·7	−0·05^{ab}	0·01	−7·9^{abc}	0·8	−0·08^{abc}	0·01
Fed ad lib.												
Fat-free	85·3	3·2	73^c	7	0·4^{cd}	1·7	0·01^{cd}	0·01	63·7^{bcd}	14·4	0·86^{bcd}	0·13
Protein-free	89·3	3·2	66^c	9	1·6^{cd}	1·5	0·02^{cd}	0·01	−33·3^{acd}	14·4	−0·50^{ad}	0·11
Carbohydrate-free	85·2	4·7	178^{abd}	26	−13·3^{ab}	0·8	−0·07^{ab}	0·01	−72·1^{ab}	9·1	−0·40^{ad}	0·05
Non-digestible-carbohydrate	88·0	3·3	63^c	4	−12·7^{ab}	1·6	−0·20^{ab}	0·06	−71·9^{ab}	4·5	−1·12^{abc}	0·07

† Change in plasma glucose (mmol/l) or plasma insulin (ng/ml) calculated as the sum of the values at 2, 4, 6, 10 and 24 h minus five times the value at time zero.

* Δ Plasma glucose or the Δ plasma insulin value divided by the food intake (g/24 h per kg body-weight).

$^{a, b, c, d}$ Superscript letters indicate a significant difference ($P < 0.05$) compared with group means for ^{a}fat-free, ^{b}protein-free, ^{c}carbohydrate-free, ^{d}non-digestible-carbohydrate.
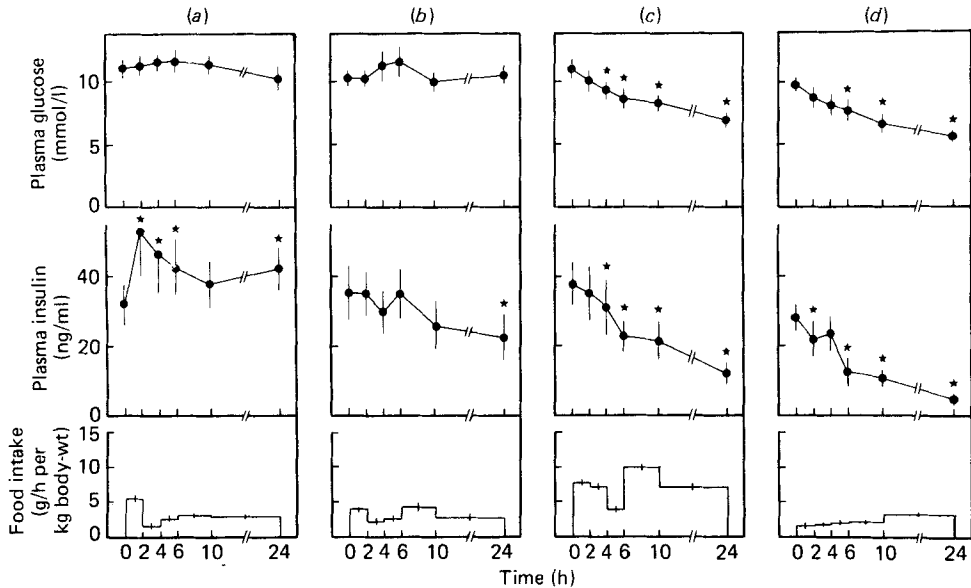
Fig. 3. Plasma glucose, plasma insulin and food intake in fed 20-week-old *ob/ob* mice transferred from standard diet to (*a*) fat-free, (*b*) protein-free, (*c*) carbohydrate-free or (*d*) non-digestible-carbohydrate foods *ad lib.* (for details, see Table 1). Points are mean values, with their standard errors represented by vertical bars, for eight mice. * Values when compared with the value at time zero were significantly different ($P < 0.05$).

transferred to the test foods from the fed state. However, glucose concentrations of mice fasted for 30 h before receiving the foods lacking glucose (carbohydrate-free and non-digestible-carbohydrate) were already low, and did not change during the 6 h test. Thus, *ob/ob* mice can maintain glucose concentrations at 3·5–4·5 mmol/l after 54 h without carbohydrate. The insulin concentrations of mice receiving carbohydrate-free and non-digestible-carbohydrate foods were similarly unchanged by the 6 h fast. However, a decrease in plasma insulin occurred in mice given protein-free (glucose and fat) and especially fat-free (glucose and protein) food in which plasma insulin was initially high.

Effect of refeeding standard diet. When returned to the standard diet, mice previously receiving the non-digestible-carbohydrate food consumed the greatest quantity of diet in 6 h. This presumably reflects a compensation mechanism to correct for the energy deficit incurred during the previous 24 h. Mice previously given the carbohydrate-free food also consumed a substantial quantity of standard diet, despite excessive consumption of test food during the previous 24 h. Indeed, whether the mice were fasted for 30 h or fed *ad lib.* before introduction of the test foods did not affect refeeding with standard diet. The large intake of standard diet by mice previously fed on non-digestible-carbohydrate food was accompanied by a marked rise in plasma insulin. Glucose concentrations were also substantially raised, similar to mice previously given carbohydrate-free food. Before reintroduction of the standard diet, the glucose and insulin concentrations of both groups of mice were low. It is noteworthy that plasma insulin concentrations were raised in all mice except those previously consuming the fat-free food, in which plasma insulin was initially very high.

Table 3. *Food intake and the plasma glucose and insulin responses to fasting or feeding standard diet* ad lib. *for 6 h in mice previously given the test foods**

(Mean values with their standard errors for four mice)

	Response to 6 h fasting				Responses to 6 h feeding									
	ΔGlucose (mmol/l)†		ΔInsulin (ng/ml)†		Food intake (g/6 h per kg body-wt)		ΔGlucose (mmol/l)†		ΔGlucose‡ food intake		ΔInsulin (ng/ml)†		ΔInsulin‡ food intake	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fasted 30 h before feeding test foods														
Fat-free	-1.30^{bcd}	0.55	-35.37^{bcd}	1.30	22.2^{bd}	3.2	2.24^{d}	1.46	0.10	0.04	-22.5^{cd}	13.65	-1.02^{bcd}	0.13
Protein-free	-2.84^{acd}	0.22	-10.05^{acd}	2.81	30.6^{ad}	1.8	0.66^{cd}	0.73	0.02^{cd}	0.02	19.34	12.42	0.63^{a}	0.21
Carbohydrate-free	0.19^{ab}	0.39	-0.24^{ab}	2.30	32.4^{d}	6.8	6.01^{b}	1.05	0.19^{b}	0.03	28.13^{a}	6.40	0.87^{a}	0.18
Non-digestible-carbohydrate	0.78^{ab}	0.31	1.47^{ab}	1.75	61.2^{abc}	10.2	6.77^{ab}	1.05	0.11^{b}	0.02	34.20^{a}	6.52	0.56^{a}	0.09
Fed ad lib. **before feeding test foods**														
Fat-free	-2.53	0.89	-28.10^{bcd}	3.34	29.4	9.0	-0.36^{a}	1.03	-0.01	0.02	-9.30^{cd}	5.53	-0.32^{bcd}	0.15
Protein-free	-1.20	0.73	-7.73^{a}	4.83	22.8	6.0	0.89	1.82	0.04	0.03	9.60	11.43	0.42^{a}	0.30
Carbohydrate-free	-1.15^{a}	0.65	0.50^{a}	9.17	38.4	4.9	3.98	1.63	0.10^{a}	0.03	24.00^{a}	9.34	0.63^{a}	0.16
Non-digestible-carbohydrate	-1.27	0.19	-0.62^{a}	0.20	69.6	8.4	2.42^{a}	0.66	0.03^{c}	0.01	35.28^{a}	11.85	0.51^{a}	0.11

* After consuming the test foods for 24 h, the *ob/ob* mice used in the experiments shown in Figs 2 and 3 were either fasted or given standard diet *ad lib.* for 6 h.

† Change in plasma glucose (mmol/l) or plasma insulin (ng/ml) between the beginning and end of the 6 h period.

‡ Δ Plasma glucose or Δ plasma insulin value divided by the food intake (g/6 h per kg body-weight).

$^{a, b, c, d}$ Superscript letters indicate a significant difference ($P < 0.05$) compared with group means for ^{a}fat-free, ^{b}protein-free, ^{c}carbohydrate-free, ^{d}non-digestible-carbohydrate.

DISCUSSION

Hyperinsulinaemia is an established early and prominent pathogenic feature of the *ob/ob* syndrome (Herberg & Coleman, 1977; Bray & York, 1979) but attention has only recently been devoted to the factors which promote excessive insulin secretion (Flatt & Bailey, 1983*b*; Flatt *et al.* 1983*c*). It appears that the severe hyperinsulinaemia of fed adult Aston *ob/ob* mice is not due to direct stimulation of the pancreatic B-cells by the raised circulating glucose (Flatt & Bailey, 1981*b*, 1982*b*). Moreover, the onset of hyperinsulinaemia precedes the moderate hyperglycaemia, which develops in a less abrupt age-related pattern (Bailey *et al.* 1982). Further evidence for a dissociation between the hyperinsulinaemia and hyperglycaemia is derived from the present demonstration that fasting produced a rapid and marked fall in plasma insulin before the gradual decline in glucose. These observations collectively indicate that factors other than hyperglycaemia are responsible for the maintenance and presumably the genesis of hyperinsulinaemia in *ob/ob* mice. Such factors may include other nutrient secretogogues (protein and fat) although the augmented insulin responses to oral carbohydrate (Flatt & Bailey, 1981*c*, 1982*a*) and to normal feeding in conditioned *ob/ob* mice (Flatt & Bailey, 1983*a*) suggest the involvement of neural and hormonal components of the enteroinsular axis (Creutzfeldt, 1979). A causal relationship between nutrient intake and hyperinsulinaemia is further illustrated by the large plasma insulin response to refeeding standard diet in 24 h fasted *ob/ob* mice. Interestingly, the changes in food intake and plasma insulin were similar in profile and both varied from the normal diurnal pattern. Consistent with previous observations (Bailey *et al.* 1975), *ob/ob* mice continued to feed actively throughout 24 h. However, after a 24 h fast, *ob/ob* mice given standard diet *ad lib.* only consumed an additional 25% of their normal daily intake. This is not due to a limitation in feeding capacity, since normal amounts of food were consumed after 10 h of refeeding. It might therefore reflect an ability of *ob/ob* mice to utilize their substantial nutrient stores and exploit their high metabolic efficiency to offset food deprivation and hunger motivation (Ramirez & Sprott, 1978).

The role of the individual dietary constituents in hyperinsulinaemia was assessed by examining plasma insulin responses to isoenergetic test foods lacking either carbohydrate, protein or fat. Mice fasted for 30 h and refed carbohydrate-free food showed only a small increase of insulin above the low basal concentration, although a considerable quantity of the food was consumed. Plasma glucose declined slowly, reflecting both the absence of dietary carbohydrate and the metabolic effects of the small insulin response. This indicates that a mixture of protein and fat is a poor insulinotropic stimulus in *ob/ob* mice. However, combination of fat with carbohydrate in the protein-free diet evoked prominent increases of insulin and glucose, the latter clearly illustrating glucose intolerance (Flatt & Bailey, 1981*b*). Further evidence for a powerful insulin-releasing action of dietary carbohydrate was obtained by refeeding the fat-free food. In this instance, the marked glucose response was marginally less than after the protein-free food, but the insulin response to the carbohydrate–protein combination was substantially raised. This enhancement is not attributable to differences in food intake, and might therefore result from changes in nutrient availability due to the restraining effect of fat on gastric motility (Hunt & Knox, 1968) and glucose potentiation of the insulin releasing effect of dietary protein. Over-all, these findings support the view that dietary carbohydrate is the major stimulus to hyperinsulinaemia in *ob/ob* mice, with an important augmentation in the presence of protein. This is substantiated by the responses of fed hyperinsulinaemic *ob/ob* mice transferred from standard diet to the test foods. Indeed the hyperinsulinaemic effect of the carbohydrate–protein combination exceeded that of the standard diet, as illustrated by the fall in plasma insulin after the standard diet was reintroduced.

Studies with the non-digestible-carbohydrate test food further show that the hyperinsulinaemia of *ob/ob* mice is primarily dependent on the type rather than the amount of nutrient ingested. Thus, although considerable quantities of cellulose were consumed, glucose and insulin fell progressively to attain concentrations only marginally less than in mice given carbohydrate-free food. Since neuro-endocrine reflexes participate in the insulin response to feeding in *ob/ob* mice (Flatt & Bailey, 1983a), signal generation must be nutrient specific, and not merely triggered by ingesting bulk. Furthermore, feeding with non-digestible-carbohydrate food did not preclude the subsequent consumption of increased amounts of standard diet, thereby confirming that *ob/ob* mice recognize and at least partially compensate for an immediate deficit in energy intake (Parsons *et al.* 1954; Fuller & Jacoby, 1955; Ramirez & Sprott, 1978). Although the long-term efficiency of compensation was not studied, energy balance in the 30 h period preceding the consumption of the various test foods did not alter refeeding with standard diet.

The present study extends previous observations (Chlouverakis, 1971; Lemonnier *et al.* 1971; Genuth, 1976; Flatt & Bailey, 1982a) that available dietary carbohydrate is especially important in the hyperinsulinaemia of *ob/ob* mice, and additionally indicates that this action is augmented by combination with protein. The glucose-dependent effect of amino acids involves direct stimulation of pancreatic B-cells and indirect mediation presumably via neural pathways and the secretion of insulinotropic hormones from the gut and pancreatic A-cells (Flatt & Bailey, 1982c, 1983a; Flatt *et al.* 1982). In contrast, the insulinotropic effect of dietary carbohydrate cannot be attributed to a direct stimulatory action of glucose and must, therefore, be generated through the enteroinsular axis (Flatt & Bailey, 1981b, c). Recent studies have implicated pathologically raised concentrations of glucose-dependent insulinotropic polypeptide or gastric inhibitory polypeptide (GIP) and possibly low molecular weight enteroglucagon in the hyperinsulinaemia of *ob/ob* mice (Flatt *et al.* 1983a, b). However, GIP is only one component of the enteroinsular axis (Creutzfeldt, 1979; Ebert & Creutzfeldt, 1982) and the combined effects of this and other lesser known incretins dependent on dietary carbohydrate for their insulinotropic action are likely to promote the hyperinsulinaemia of Aston *ob/ob* mice.

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