# Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals

*Lancet* 2004; 364: 778–85

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## Summary

**Background** **Clinical studies suggest a role for dietary glycaemic index (GI) in bodyweight regulation and diabetes risk. However, partly because manipulation of GI can produce changes in potentially confounding dietary factors such as fibre content, palatability, and energy density, its relevance to human health remains controversial. This study examined the independent effects of GI in animals.**

**Methods** **Partially pancreatectomised male Sprague-Dawley rats were given diets with identical nutrients, except for the type of starch: high-GI (n=11) or low-GI (n=10). The animals were fed in a controlled way to maintain the same mean bodyweight in the two groups for 18 weeks. Further experiments examined the effects of GI in rats in a cross-over design and C57BL/6J mice in a parallel design.**

**Findings** **Despite having similar mean bodyweight (547·9 [SE 13·4] *vs* 549·2 [15·2] g), rats given high-GI food had more body fat (97·8 [13·6] *vs* 57·3 [7·2] g; p=0·0152) and less lean body mass (450·1 [9·6] *vs* 491·9 [11·7] g; p=0·0120) than those given low-GI food. The high-GI group also had greater increases over time in the areas under the curve for blood glucose and plasma insulin after oral glucose, lower plasma adiponectin concentrations, higher plasma triglyceride concentrations, and severe disruption of islet-cell architecture. Mice on the high-GI diet had almost twice the body fat of those on the low-GI diet after 9 weeks.**

**Interpretation** **These findings provide a mechanistic basis for interpretation of studies of GI in human beings.**

**Relevance to practice** **The term GI describes how a food, meal, or diet affects blood sugar during the postprandial period. GI as an independent factor can cause obesity and increase risks of diabetes and heart disease in animals. Use of low-GI diets in prevention and treatment of human disease merits thorough examination.**

## Introduction

Over the past few decades, reduction in dietary fat intake has been widely advocated for the prevention and treatment of obesity. However, the long-term effectiveness of low-fat diets has been called into question,[1,2] and interest in alternative dietary approaches has grown. A dietary factor termed the **glycaemic index** (GI) has been related to risk of obesity and diabetes on experimental and theoretical grounds.[3] Habitual consumption of high-GI meals (ie, causing a large postprandial rise in blood glucose concentration) could initiate a sequence of metabolic events that simulate hunger, promote fat deposition, and place the pancreatic β cell under increased stress. Several clinical trials have found lower bodyweight or adiposity among free-living individuals consuming self-selected low-GI diets compared with those eating high-GI diets.[4,5] However, the clinical outcomes in such studies cannot be attributed solely to GI, because interventions designed to modify this dietary factor unavoidably produce changes in other factors that might also influence bodyweight (eg, fibre content, palatability, energy density). Therefore, we aimed to examine the effects of GI on adiposity and related endpoints in animals by use of diets with identical content of macronutrients, micronutrients, and fibre. To control for confounding that might arise from any differences in palatability or bioavailability of dietary energy, we adjusted food amounts to maintain the same mean bodyweight between groups.

**Glycaemic index**
The index ranks carbohydrate-containing foods according to how they affect postprandial blood glucose concentration. It is defined as the incremental area under the glucose response curve after consumption of a standard amount of carbohydrate from a test food relative to that for a control food (white bread or glucose). Most refined grain products, potato, and the sugars glucose and sucrose have high GI. Most non-starchy vegetables, fruits, legumes, and nuts have low GI.

## Methods

We did three experiments: the first with rats in a parallel design; the second with rats in a cross-over design; and the third with mice in a parallel design. For the rat experiments, we carried out partial pancreatectomy according to the methods of Leahy and colleagues.[6] After this procedure, rats show no gross changes in glucose homoeostasis on standard diets but develop mild hyperglycaemia when given sucrose solution in place of drinking water. The decrease in β-cell mass in this animal model is analogous to that observed in people with prediabetes,[7] a group proposed to be most susceptible to the effects of the GI of their diet.[3,8] For the mouse experiment, no partial pancreatectomy was done. Approval for the study was obtained from the Children's Hospital Boston Animal Care and Use Committee.

### Parallel experiment in rats

2 weeks after 60% pancreatectomy (week 0), rats were randomly assigned high-GI or low-GI diets with the same nutrient and energy content. Oral glucose tolerance tests were done at weeks 0, 2, 5, 8, and 14, and an insulin tolerance test was done at week 16. Body

composition was measured by the tritiated water method at week 17. The animals were killed at week 18, and fat pads were excised and weighed.

Male Sprague-Dawley rats (n=30), aged 6 weeks and weighing 50–55 g, were purchased from Charles River Breeding Laboratories (Wilmington, MA, USA) and maintained on a normal light cycle (0600–1800 h light, 1800–0600 h dark). All animals were housed individually throughout the experiment in cages with wire-mesh floors, equipped with feeders, to facilitate assessment of food intake and potential spillage (accurate to the nearest 0·1 g). At 8 weeks of age, the heaviest 24 rats underwent 60% pancreatectomy as previously described.[6] The rats were anaesthetised with ketamine 60–75 mg/kg intramuscularly and pentobarbital 40–50 mg/kg intraperitoneally. A midline abdominal incision was made, and the splenic lobe of the pancreas was mobilised. Pancreatic tissue was removed between easily recognisable anatomical markers by gentle abrasion with cotton applicators. The excised portion—bordered by the spleen and stomach, extending to but not including the small flap of pancreas attached to the pylorus—constitutes 57% (SE 3) of the weight of the pancreas.[6] At autopsy, the mean pancreatic remnant mass was similar in the low-GI and the high-GI groups (1·04 [SE 0·10] *vs* 1·07 [0·07] g; p=0·8093]). 2 weeks after surgery, animals were grouped into 12 pairs according to similarity in bodyweight, and members of each pair were randomly assigned to the high-GI or the low-GI group. Three rats were withdrawn from the experiment owing to illness not related to diet, at the recommendation of the veterinary surgeon, leaving 11 animals in the high-GI group and ten in the low-GI group. Pathological examination of the three withdrawn animals identified postoperative abscess at day 14 in one and viral pneumonia at days 29 and 31 in two.

High-GI and low-GI diets were designed to resemble standard rodent feed and differed only in the nature of the component starch. Macronutrient composition for both diets was 69% carbohydrate, 20% protein, and 11% fat, as a percentage of total energy. The high-GI diet contained 542 g/kg 100% amylopectin starch (Cerestar USA, Hammond, IN, USA) and the low-GI diet contained 542 g/kg 60% amylose/40% amylopectin starch (Hi-Maize, National Starch and Chemical Company, Bridgewater, NJ, USA). Other ingredients in both diets were: 20 g/kg gelatin, 200 g/kg casein, 85 g/kg sucrose, 56 g/kg soybean oil, 50 g/kg wheat bran, 2 g/kg DL-methionine, 10 g/kg vitamin mix (product number 40060), and 35 g/kg mineral mix (AIN-93G; Harlan-Teklad, Madison, WI, USA). Significant differences in the area under the curve (AUC) in glycaemic response to these two diets was confirmed by feeding identical amounts of high-GI or low-GI food to 12 animals (parallel design) not used in the main study (AUC 125·5 [11·7] *vs* 81·0 [12·2] mmol/L in 120 min; p=0·0229). This difference in GI of 55% is smaller than the difference of 71% between white bread and spaghetti in human beings.[3] Human studies have shown significant effects on clinically relevant endpoints resulting from diets with relative differences in GI in the range of 11%[9] to 61%.[10]

Feed was prepared freshly twice a week and provided to the animals daily. The amount was varied as needed to maintain mean bodyweight between the groups within 3%. Up to and including week 7, rats from the two groups ate similar amounts of food ad libitum and gained similar amounts of weight. At week 8, the mean bodyweight of the high-GI group exceeded that of the low-GI group by 3%, so moderate food restriction was applied. The amount of food provided daily for each animal in the high-GI group was initially decreased by

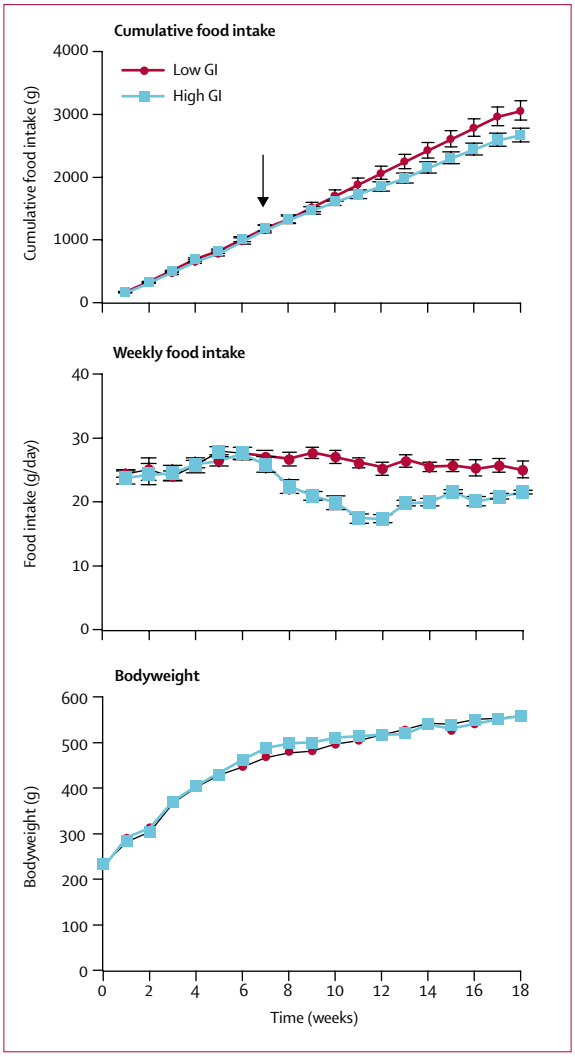


***Figure 1:*** **Food intake and bodyweight among rats in the parallel experiment eating the low-GI and high-GI diets**
Arrow indicates initiation of a moderate food restriction in the high-GI group to prevent these animals from gaining more weight than those in the low-GI group. Error bars=SE.

5% relative to the amount that animal had consumed on each of the previous 3 days. Further adjustments in the amount of food provided were made in this way until the end of the experiment (figure 1). The maximum difference in mean bodyweight between groups was 3·4% (at week 8).

For oral glucose tolerance tests, 2 weeks after surgery (week 0) rats were fasted overnight for 16 h and gavage fed 50% glucose solution (1 g/kg bodyweight). About 150 μL blood was collected from the tail vein at each time point. Blood glucose was measured with a handheld glucose monitor (Accutrend, Roche Diagnostics, IN, USA) at baseline and every 30 min to 120 min; heparinised plasma samples were prepared at each time point for measurement of insulin (RIA kit, Linco Research, St Charles, MO, USA). Oral glucose tolerance tests were repeated at weeks 2, 5, 8, and 14 of the dietary intervention.

For the insulin tolerance test at week 16, rats were fasted for 6 h and given an intraperitoneal injection of insulin (0·5 U/kg bodyweight). Blood glucose was measured with a handheld glucose monitor at baseline, 15 min, 30 min, and 60 min.

At week 7, rats were fasted overnight (16 h), and blood was collected for preparation of heparinised plasma to measure leptin (RIA kit, Linco Research) and triglycerides (GPO-Trinder kit, Sigma Diagnostic, St Louis, MO, USA). Lean body mass and body fat were calculated at week 17, from total body water measured after intraperitoneal injection of tritiated water (ICN, Irvine, CA, USA).[11,12] The distribution space of water was calculated as total radioactivity injected ($3·7\times10^7$ Bq) divided by the specific activity of plasma water (water content of plasma was assumed to be 93·7%). Lean body mass was calculated as the distribution space divided by the water content of lean body mass (73·2%). Adiposity was also assessed on autopsy at week 18, when the rats were 28 weeks old. The animals were killed by means of an overdose of pentobarbital (200 mg/kg by intraperitoneal injection), then epididymal and retroperitoneal fat pads were dissected by an investigator unaware of dietary group assignment. Bodyweight throughout the experiment and fat-pad weight were measured to 0·01 g with a Sartorius scale (Edgewood, NY, USA).

For islet-cell studies, the pancreas was rapidly removed under pentobarbital anaesthesia and cleared of fat. The whole pancreas was fixed in aqueous Bouin's solution overnight and embedded in paraffin. Each pancreatic block was sectioned (5 μm) throughout its length to avoid bias due to regional change in islet distribution. Separate sections were stained with Masson's trichrome stain, with aniline blue used for connective tissue. The extent of fibrosis was assessed on adjacent, non-overlapping portions of pancreas at ×20 magnification; the sections were assessed for the number of islets and presence and severity of fibrotic changes in a masked way. The criteria applied were: no fibrosis, round islets with no detectable collagen tissue present inside or outside of islets; moderate fibrosis, thin strands of collagen tissue around or inside islets but the shape well preserved; and severe fibrosis, thick strands of fibrotic tissue and irregular islet shape. To quantify the extent of the fibrosis, the number of islets identified as severely fibrotic was expressed as a percentage of the total number examined. Immunohistochemical localisation of antigens and double-label immunohistochemistry were done as previously described.[13] 5 μm longitudinal sections in paraffin blocks were rehydrated with xylene followed by decreasing concentrations of ethanol, microwaved in 0·01 mol/L sodium citrate (pH 6·0) for 20 min, and permeabilised with 1% Triton X-100 in phosphate-buffered saline before incubation with primary antisera. Islet morphometry was done as previously described.[13] The primary antibodies were guineapig antibody to insulin and rabbit antibody to glucagon (Zymed Laboratories Inc, South San Francisco, CA, USA). Secondary antibodies were labelled with fluorescein isothiocyanate or rhodamine (Jackson ImmunoResearch Laboratories Inc, West Grove, PA, USA). β-cell area was quantified by acquiring adjacent, non-overlapping images (×10 magnification) of the pancreas from two sections per animal stained with anti-insulin and two stained with anti-glucagon, with a Zeiss Axiovert microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA). Images were analysed for stained area with Open Lab Software density slice software (Improvision Scientific Imaging, Lexington, MA, USA). Results are expressed as the percentage of the total surveyed area that contained cells positive for insulin. For each animal the ratio of β-cell area to total pancreas area was calculated.

For all data collection, investigators were unaware of dietary group when the acquisition of a sample or analysis of an outcome had a subjective component, as in the grading of islet-cell fibrosis, estimation of β-cell mass, dissection of fat pads, measurement of pancreatic remnants, and assessment of body length. Data for remaining assessments were obtained by the investigator in forms not readily subject to bias, such as scintillation counter print-out of radioactivity in the determination of body composition.

### Cross-over experiment in rats

2 weeks after 60% pancreatectomy (week 0, rats 7 weeks old), rats were randomly assigned high-GI or low-GI diets. At week 7, the animals were crossed over to the other diet and fed for a further 3 weeks, allowing for examination of within-individual change in glucose homoeostasis as a result of dietary change.

Male Sprague-Dawley rats (n=14), aged 6 weeks, were housed, pancreatectomised, randomised, and fed high-GI (n=7) or low-GI (n=7) diets ad libitum, as described above. Food intake and bodyweights were recorded daily. Oral glucose tolerance tests were done at weeks 7

and 10 (immediately before and after the 3-week cross-over period), as described above. At week 7, blood samples were taken after animals had fasted for 16 h; heparinised plasma was prepared for measurement of **adiponectin** (RIA kit, Linco Research).

### Parallel experiment in mice

To find out whether the effects of GI on adiposity in the rat model were apparent in a contrasting animal model (an obesity-prone mouse strain, without partial pancreatectomy), fed throughout the experiment ad libitum, we used the C57BL/6J mouse model, commonly used in studies of obesity and diabetes. Body composition was measured at week 9 of the diets. Male C57BL/6J mice (n=24) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and randomly assigned the high-GI (n=12, mean bodyweight 26·98 g [SE 0·37]) or the low-GI diet (n=12, bodyweight 26·70 g [0·33]) at 11 weeks of age. Mice were housed individually and allowed to consume the diets ad libitum from spill-proof feeders (Lab Products Inc, Seaford, DE, USA). Bodyweight was recorded twice each week. Body fat and lean body mass were measured at week 9 from total body water after an intraperitoneal injection of tritiated water. Blood (50 μL) for the body composition measurement was collected from the tail vein. Plasma from two mice in each group showed evidence of gross haemolysis, and analysis of body composition for each of these animals yielded non-physiological results (body fat below zero). These animals were excluded from calculations of mean body composition by group; however, a missing data analysis that used highly conservative assumptions was also run.

| | Mean (SE) in group | | p |
|---|---|---|---|
| | High GI | Low GI | |
| **Rat parallel study** | | | |
| Body composition, week 17 | | | |
| Bodyweight, g | 547·9 (13·4) | 549·2 (15·2) | 0·9499 |
| Body fat, g | 97·8 (13·6) | 57·3 (7·2) | 0·0152 |
| Lean body mass, g | 450·1 (9·6) | 491·9 (11·7) | 0·0120 |
| Adiposity, % | 17·5 (2·1) | 10·3 (1·1) | 0·0062 |
| Fat mass at autopsy, week 18 | | | |
| Epididymal fat, g | 11·6 (1·1) | 8·7 (1·1) | 0·0777 |
| Retroperitoneal fat, g | 16·9 (2·2) | 11·0 (1·1) | 0·0340 |
| Combined, g | 28·5 (3·2) | 19·7 (2·1) | 0·0351 |
| Body length at autopsy, mm, week 18 | 264 (2) | 271 (2) | 0·0261 |
| Fasting plasma studies | | | |
| Leptin, μg/L, week 7 | 8·07 (2·54) | 2·48 (0·24) | 0·0513 |
| Triglyceride, mmol/L, week 7 | 0·31 (0·05) | 0·11 (0·01) | 0·0241 |
| Change in blood-glucose AUC from baseline mmol/L in 120 min | | | |
| OGTT (week 2) | 71·2 (36·9) | 56·9 (39·3) | 0·7844 |
| OGTT (week 5) | 166·9 (35·6) | 16·3 (23·0) | 0·0027 |
| OGTT (week 8) | 55·5 (47·0) | −65·2 (35·8) | 0·0182 |
| OGTT (week 14) | 65·4 (37·0) | −85·8 (24·4) | 0·0154 |
| Mean | 89·9 (26·3) | −19·5 (13·4) | 0·0016 |
| Change in plasma insulin AUC from baseline, pmol/L in 120 min | | | |
| OGTT (week 2) | 29·4 (46·3) | 88·7 (46·1) | 0·6703 |
| OGTT (week 5) | 262·4 (52·0) | 57·7 (49·1) | 0·0091 |
| OGTT (week 8) | .. | .. | .. |
| OGTT (week 14) | 592·0 (50·4) | 261·4 (39·8) | 0·0018 |
| Mean | 294·6 (50·4) | 135·9 (34·6) | 0·0034 |
| Insulin tolerance test, week 16, mmol/L in 60 min* | 143·1 (13·0) | 160·4 (14·1) | 0·4532 |
| Islet morphometry, week 18 | | | |
| Pancreatic mass, g | 1·04 (0·10) | 1·07 (0·07) | 0·8093 |
| Fibrosis, % of total islets | 32 (6) | 8 (3) | 0·0094 |
| β-cell area, % of total area | 0·47 (0·05) | 0·65 (0·09) | 0·0796 |
| **Rat cross-over study** | | | |
| Bodyweight, g, week 7 (before cross-over) | 468·7 (14·9) | 458·5 (17·5) | 0·8245 |
| Bodyweight, g, week 10 (after cross-over) | 515·7 (20·6) | 500·6 (18·9) | 0·6011 |
| Change in blood glucose AUC, mmol/L in 120 min | 239·9 (47·1)† | −85·9 (51·1)† | 0·0005 |
| Change in plasma insulin AUC, pmol/L in 120 min | 522·0 (58·2)† | −38·5 (58·6)† | <0·0001 |
| Plasma adiponectin, mg/L, week 7 | 2·8 (0·2) | 4·7 (0·2) | 0·0020 |
| **Mouse parallel study‡** | | | |
| Bodyweight, g, week 9 | 31·79 (0·83) | 30·21 (0·49) | 0·1202 |
| Body fat, g | 8·13 (0·81) | 4·21 (0·67) | 0·0011 |
| Lean body mass, g | 23·66 (0·64) | 26·00 (0·58) | 0·0108 |
| Adiposity, % | 25·3 (2·1) | 13·8 (2·1) | 0·0009 |

OGTT=oral glucose tolerance test. *Decremental area under baseline glucose concentration. †Group assignment after crossover. ‡n=20.

***Table:*** **Summary of the main outcome variables**

### Statistical analysis

We used independent *t* tests to compare the low-GI and high-GI groups for mean bodyweight, body composition, adiposity, change in blood-glucose AUC, change in plasma-insulin AUC, insulin tolerance test, islet morphometry, and plasma concentrations of adiponectin, leptin, and triglycerides. Simple linear regression was used to assess the relations between plasma insulin 30 min after glucose administration at week 0 (independent variable) and bodyweight or body fat at week 18 (dependent variables); and between islet-cell fibrosis (independent variable) and blood-glucose AUC at week 14 (dependent variable). To analyse the blood-glucose AUC and plasma-insulin AUC in the cross-over experiment, we calculated the difference between the high-GI and the low-GI diet for each animal and estimated the mean difference in the full sample, adjusting for period effects and testing for carry-over as detailed by Senn.[14] In the mouse parallel experiment, a conservative missing-data model was used to analyse bodyweight and body composition. The mean from the opposite treatment was used as a surrogate measurement for the samples that were missing owing to haemolysis. Nevertheless, we have no reason to believe that diet would affect occurrence of haemolysis (and the number of haemolysed samples was the same in both diet groups).

### Role of the funding source

The study sponsors had no role in study design; collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

**Adiponectin**
This protein, otherwise known as ACRP30, GBP28, Adipo-Q, and APM, is secreted from adipose tissue. The plasma concentration is low in people with obesity, insulin resistance, type 2 diabetes, and coronary-artery disease.

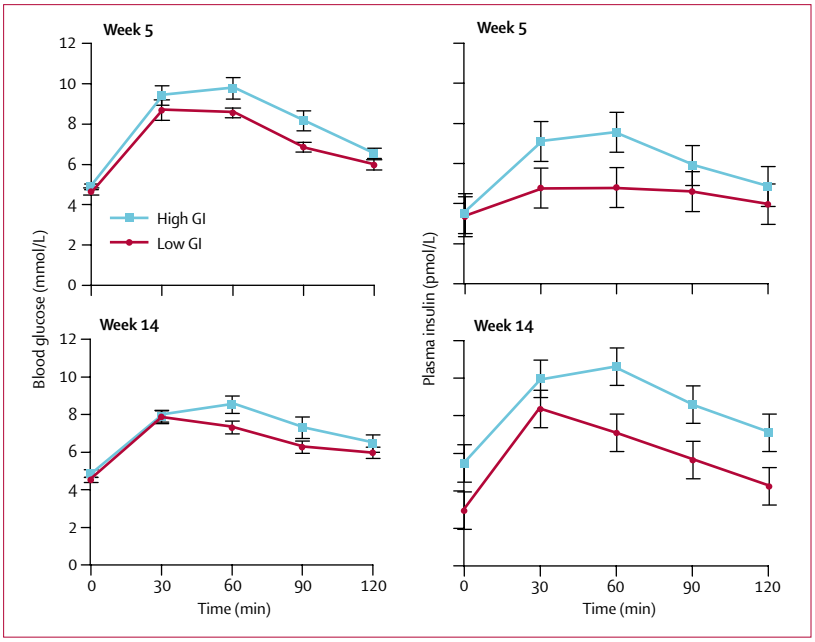


*Figure 2*: **Blood glucose and plasma insulin during oral glucose tolerance tests at weeks 5 and 14 of the high-GI and low-GI diets**
Error bars=SE.

## Results

The table summarises the main results for the three experiments by dietary group assignment.

Figure 1 shows changes in mean bodyweight by group throughout the parallel experiment in rats. The animals were allowed to feed ad libitum for the first 7 weeks of the experiment, and a modest energy restriction was initiated at week 8 in the high-GI group to prevent excessive weight gain, as described in the methods section (figure 1). The difference in cumulative food intake between groups was 13%.

Body composition was measured at week 17 by the tritiated water method (table). Both groups had very similar mean bodyweights (p=0·9499) but the high-GI group had 71% more body fat (p=0·0152) and 8% less lean body mass (p=0·0120) than the low-GI group. In post-hoc analysis, the power to detect the observed difference in body fat was 71%. Adiposity was significantly greater in the high-GI group than in the low-GI group (p=0·0062). At autopsy, at week 18, the high-GI animals had more combined epididymal and retroperitoneal fat and were significantly shorter than the low-GI animals. At week 7, the mean plasma triglyceride concentration was almost three times higher in high-GI than in low-GI animals; mean plasma leptin concentration was higher but not significantly so (table).

We examined change over time in blood-glucose AUC and plasma-insulin AUC by the oral glucose tolerance test (table, figure 2). Blood-glucose AUC had increased significantly more in the high-GI than in the low-GI group at week 5, and the difference persisted for the duration of the experiment. Plasma-insulin AUC also increased significantly more in the high-GI group by week 5, and the difference persisted at week 14. Insulin sensitivity, assessed by insulin tolerance test at week 16, did not differ between the groups (table).

We had previously hypothesised that susceptibility to the obesity-promoting effect of a high-GI diet might be mediated partly by individual differences in the insulin response to ingested glucose.[15] We therefore examined the associations between plasma insulin at baseline and final bodyweight. As shown in figure 3, insulin concentration 30 min after oral glucose at week 0 strongly predicted bodyweight at week 18 among the high-GI group ($R^2$=0·84, p=0·0001) but not among the low-GI group ($R^2$=0·0027, p=0·9430). A similar association was observed between insulin concentration at 30 min and body fat for the high-GI group ($R^2$= 0·51, p=0·0131) but not for the low-GI group ($R^2$=0·27, p=0·1300).

Pancreatic islets were studied by histochemistry and morphometry at week 18. 494 islets from rats in the high-GI group (n=9) and 512 from rats in the low-GI group (n=9) were examined. A much higher proportion of islets in the high-GI group than the low-GI group were distinctly abnormal, with severely disorganised architecture and extensive fibrosis (table). The proportion of fibrotic islets in each individual was strongly associated with blood-glucose AUC at week 14 ($R^2$=0·36, p=0·0096) among both groups. Figure 4 shows a representative islet from a high-GI animal demonstrating this abnormality and a normal islet from a weight-matched low-GI animal. Mean β-cell area, calculated by point-counting morphometry of insulin-stained pancreatic tissue sections, did not differ significantly between groups (table).

In the rat cross-over experiment, mean bodyweights were similar in the two dietary groups before (week 7) and after (week 10) cross-over to the alternative diets (table). Animals that were changed from the low-GI to the high-GI diet showed greater increases in blood-glucose AUC (p=0·0005) and plasma-insulin AUC (p<0·0001) than those that were changed from the high-GI to the low-GI diet. The estimated difference between the high-GI and the low-GI groups, after adjustment for period effects, was 163 mmol/L in 120 min (95% CI 87–239) for blood-glucose AUC and 280 pmol/L in 120 min (194–366) for plasma-insulin AUC. No significant carry-over effect (diet×period interaction) was detected for either blood-glucose AUC (p=0·8375) or plasma-insulin AUC (p=0·7674). Before cross-over at week 7, plasma adiponectin concentration was significantly lower in animals consuming the high-GI diet than in those on the low-GI diet (table).

Finally, we examined the effects of GI on adiposity in a second animal model, the obesity-prone C57BL/6J mouse. At week 9, both groups had similar mean bodyweight, but the high-GI group had 93% more body

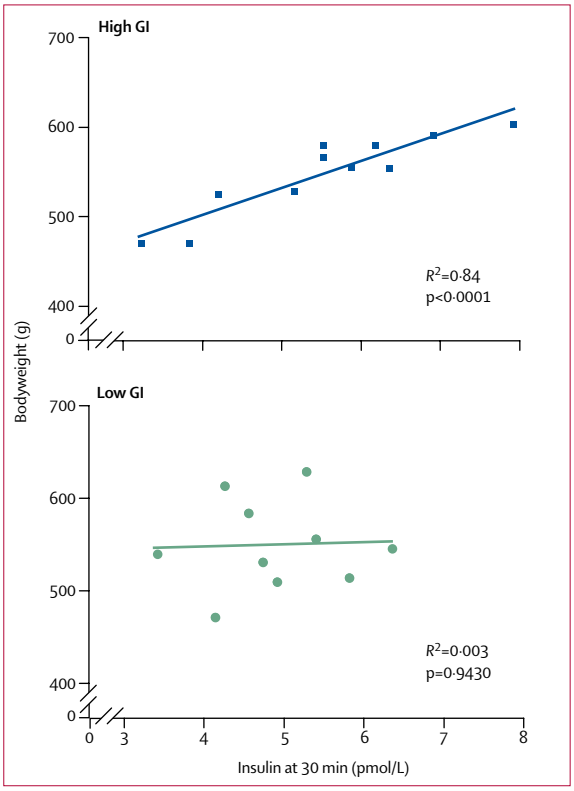


*Figure 3:* **Association between plasma insulin 30 min after oral glucose at week 0 and bodyweight at week 18**

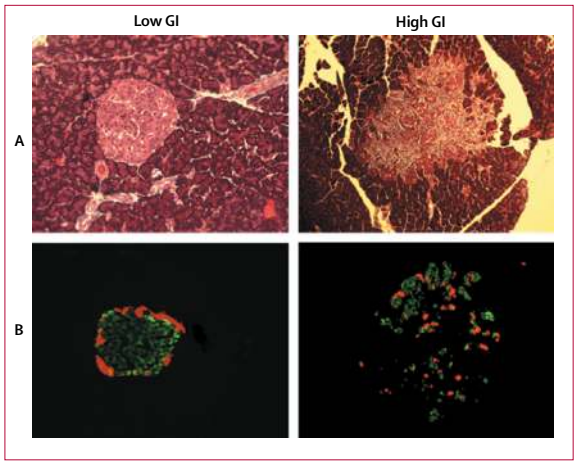


*Figure 4:* **Representative abnormal islet from a high-GI animal compared with a normal islet from a weight-matched low-GI animal**
A: Masson's trichrome staining, collagen shown in blue, magnification ×10. Pronounced islet fibrosis is accompanied by irregular shape and infiltration into exocrine tissue. B: Immunostaining with antibodies against insulin (green) and glucagon (red) visualised with fluorescein isothiocyanate or rhodamine, magnification ×10. The low-GI islet shows the normal peripheral distribution of α cells (staining for glucagon) and central distribution of β cells (staining for insulin); cellular localisation is disrupted in the high-GI islet.

fat (n=20, p=0·0011, table). This result did not differ substantially when a conservative missing-data model was used, including the four mice for which blood samples could not be analysed owing to severe haemolysis (n=24, p=0·0201).

## Discussion

Several dietary modifications, including high-fat feeding and the "cafeteria diet", can cause obesity in susceptible rodents.[16,17] These diets induce excess adiposity partly by increasing the palatability, energy density, or bioavailable energy of food. By contrast, this study shows that GI has an independent effect on body composition. Rats in both groups of the parallel experiment consumed the same amount of food ad libitum and gained the same amount of weight for 7 weeks, by which point the high-GI animals had developed hyperinsulinaemia. This time course indicates that the diets did not differ substantially in any of the potentially confounding factors mentioned above. Furthermore, to control for subtle, possibly cumulative differences in any of these factors, we maintained identical mean bodyweight between groups by use of a modest energy restriction in the high-GI animals after 7 weeks.

Compared with those in the low-GI group, rats in the high-GI group (parallel experiment) required less food to gain the same weight from week 8, which suggests that they had become more metabolically efficient. The nature of this metabolic change warrants further investigation, but it may involve lower resting energy expenditure or activity related to emerging differences in body composition (ie, decreased lean body mass).

We also studied the obesity-prone C57BL/6J mouse fed ad libitum, without doing partial pancreatectomy. After 9 weeks, mice on the high-GI diet had decreased lean body mass and almost twice the body fat of those on the low-GI diet, though mean bodyweight did not differ between groups. The similarity of findings from the two different mammalian models, rat and mouse, provides stronger evidence about the effect of GI on body composition.

We speculate that the striking chronic primary peripheral hyperinsulinaemia induced by the high-GI diet alters nutrient partitioning in favour of fat deposition, shunting metabolic fuels from oxidation in muscle to storage in fat. Support for this idea comes from four lines of evidence. First, long-term insulin administration in normal rats causes increased insulin sensitivity in fat tissue and weight gain.[18] Second, muscle-specific inactivation of the insulin receptor results in increased adiposity in mice.[19] Third, rats given high-GI food for 3–5 weeks show increased Glut4 expression in fat, fatty-acid synthase activity in fat, glucose incorporation into total lipids, and adipocyte size compared with animals given low-GI diets.[20,21] Fourth, in our analyses, insulin concentration 30 min after glucose administration at the beginning of the experiment predicted 84% of the individual variance in bodyweight at the end of the experiment among the high-GI rats, but none of the variance among the low-GI rats.

A high-GI diet has been linked to increased risk of type 2 diabetes independent of bodyweight,[3,22,23] and the results of this study provide support for this possibility. Hyperglycaemia appears to lower β-cell insulin content partly through the effects of chronic overstimulation.[24] The failure to increase β-cell mass in the setting of reduced islet insulin stores could contribute to a deterioration in glucose homoeostasis observed among obese individuals.[25,26] In this study, the high-GI animals showed no significant increase (and a decrease that did not achieve statistical significance) in β-cell area; they also developed severe abnormalities in islet-cell architecture. Islet fibrosis is a feature of several rat models of diabetes, accelerated by sucrose-induced hyperglycaemia and prevented by rosiglitazone treatment.[27,28] Furthermore, the high-GI group had much lower plasma concentrations of adiponectin, a newly identified risk factor for type 2 diabetes, than the low-GI animals.[29,30] Were these functional and structural changes to progress over time, frank type 2 diabetes might develop.

Human studies have identified higher risk of myocardial infarction among individuals consuming high-GI versus low-GI diets.[3] These studies are consistent with our findings of increased triglyceride concentrations and decreased adiponectin concentrations[31] in the high-GI group.

One feature of the diets used in this study merits further consideration. The diets had identical macronutrient and micronutrient composition, differing only in the nature of the starch (100% amylopectin *vs* 60% amylose/40% amylopectin). Depending on many physiochemical factors, amylose contains varying amounts of "resistant starch" that is not fully digested in the small intestine but is instead fermented in the colon or excreted in the faeces.[32] However, we do not think that our primary findings can be attributed in any significant way to differences in resistant starch concentrations for the following reasons. First, previous studies in rats with high amylose starch from maize (used for our low-GI diet) found little to no undigested starch in the distal colon.[33,34] Second, the low-GI and high-GI rats in the parallel experiment gained weight at similar rates while eating a similar amount of food for 7 weeks. Third, the adjustments made in food intake for rats in the parallel experiment after 7 weeks would have compensated for any subtle, potentially cumulative differences in energy availability between the diets.

In conclusion, this study shows that consumption of a high-GI diet per se adversely affects body composition and risk factors for diabetes and cardiovascular disease in animal models. These findings provide a mechanistic basis for interpretation of data from previous epidemiological analyses and intervention studies. Large-scale, long-term trials of low-GI diets should be given high priority.

**Contributors**

Dorota Pawlak helped design the study, carried out the experiments, and took part in data analysis and preparation of the report. Jake Kushner assisted with β-cell staining and data analysis. David Ludwig, the principal investigator, obtained funding, supervised all parts of the work, and took part in preparation of the report.

**Conflict of interest statement**

None declared.

**Acknowledgments**

We thank Jack Leahy, Susan Bonner-Weir, Henry Feldman, and Joseph Majzoub for advice and encouragement, and Erica Garcia-Lago and Lindsey Wong for technical assistance. The study was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (1R01DK059240, T32HD07277), the Charles H Hood Foundation, and the Iacocca Foundation.

**References**

1 Pirozzo S, Summerbell C, Cameron C, Glasziou P. Advice on low-fat diets for obesity (Cochrane Review). In: *The Cochrane Library*, issue 2. Oxford:Update Software, 2002.

2 Willett WC. Dietary fat plays a major role in obesity: no. *Obesity Reviews* 2002; **3:** 59–68.

3 Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes and cardiovascular disease. *JAMA* 2002; **287:** 2414–23.

4 Bouche C, Rizkalla SW, Luo J, et al. Five-week, low-glycemic index diet decreases total fat mass and improves plasma lipid profile in moderately overweight nondiabetic men. *Diabetes Care* 2002; **25:** 822–28.

5 Spieth LE, Harnish JD, Lenders CM, et al. A low-glycemic index diet in the treatment of pediatric obesity. *Arch Pediatr Adolesc Med* 2000; **154:** 947–51.

6 Leahy JL, Bonner-Weir S, Weir GC. Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J Clin Invest* 1988; **81:** 1407–14.

7 Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; **52:** 102–10.

8 Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr* 2000; **71:** 1455–61.

9 Frost G. Dietary advice based on the glycaemic index improves dietary profile and metabolic control in type 2 diabetic patients. *Diabet Med* 1994; **11:** 397–401.

10 Jenkins DJ, Wolever TM, Collier GR, et al. Metabolic effects of a low-glycemic-index diet. *Am J Clin Nutr* 1987; **46:** 968–75.

11 Rothwell NJ, Stock MJ. In vivo determination of body composition by tritium dilution in the rat. *Br J Nutr* 1979; **41:** 625–28.

12 Tisavipat A, Vibulsreth S, Sheng HP, Huggins RA. Total body water measured by desiccation and by tritiated water in adult rats. *J Appl Physiol* 1974; **37:** 699–701.

13 Kushner JA, Ye J, Schubert M, et al. Pdx1 restores beta cell function in Irs2 knockout mice. *J Clin Invest* 2002; **109:** 1193–201.

14 Senn S. Cross-over trials in clinical research, 2nd edn. Chichester: John Wiley, 2002.

15 Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High glycemic index foods, overeating, and obesity. *Pediatrics* 1999; **103:** E26.

16 Oscai LB. Dietary-induced severe obesity: a rat model. *Am J Physiol* 1982; **242:** R212–15.

17 West DB, York B. Dietary fat, genetic predisposition, and obesity: lessons from animal models. *Am J Clin Nutr* 1998; **67** (suppl): 505S–12S.

18 Cusin I, Rohner-Jeanrenaud F, Terrettaz J, Jeanrenaud B. Hyperinsulinemia and its impact on obesity and insulin resistance. *Int J Obes Relat Metab Disord* 1992; **16** (suppl 4): S1–11.

19 Kim JK, Michael MD, Previs SF, et al. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J Clin Invest* 2000; **105:** 1791–97.

20 Kabir M, Rizkalla SW, Champ M, et al. Dietary amylose-amylopectin starch content affects glucose and lipid metabolism in adipocytes of normal and diabetic rats. *J Nutr* 1998; **128:** 35–43.

21 Lerer-Metzger M, Rizkalla SW, Luo J, et al. Effects of long-term low-glycaemic index starchy food on plasma glucose and lipid concentrations and adipose tissue cellularity in normal and diabetic rats. *Br J Nutr* 1996; **75:** 723–32.

22 Salmeron J, Ascherio A, Rimm EB, et al. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* 1997; **20:** 545–50.

23 Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA* 1997; **277:** 472–77.

24 Song SH, Rhodes CJ, Veldhuis JD, Butler PC. Diazoxide attenuates glucose-induced defects in first-phase insulin release and pulsatile insulin secretion in human islets. *Endocrinology* 2003; **144:** 3399–405.

25 Rastogi GK, Sinha MK, Dash RJ. Insulin and proinsulin content of pancreases from diabetic and nondiabetic subjects. *Diabetes* 1973; **22:** 804–07.

26 Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU. Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res*; **4:** 110–25.

27 Finegood DT, McArthur MD, Kojwang D, et al. Beta-cell mass dynamics in Zucker diabetic fatty rats: rosiglitazone prevents the rise in net cell death. *Diabetes* 2001; **50:** 1021–29.

28 Koyama M, Wada R-i, Sakuraba H, Mizukami H, Yagihashi S. Accelerated loss of islet β cells in sucrose-fed Goto-Kakizaki rats, a genetic model of non-insulin-dependent diabetes mellitus. *Am J Pathol* 1998; **153:** 537–45.

29 Spranger J, Kroke A, Mohlig M, et al. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003; **361:** 226–28.

30 Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7:** 941–46.

31 Zietz B, Herfarth H, Paul G, et al. Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. *FEBS Lett* 2003; **545:** 103–04.

32 Cummings JH, Englyst HN. Gastrointestinal effects of food carbohydrate. *Am J Clin Nutr* 1995; **61** (suppl)**:** 938S–45S.

33 Lajvardi A, Mazarin GI, Gillespie MB, Satchithanandam S, Calvert RJ. Starches of varied digestibilities differentially modify intestinal function in rats. *J Nutr* 1993; **123:** 2059–66.

34 Morita T, Kasaoka S, Hase K, Kiriyama S. Psyllium shifts the fermentation site of high-amylose cornstarch toward the distal colon and increases fecal butyrate concentration in rats. *J Nutr* 1999; **129:** 2081–87.