Effect of early dietary restriction on insulin action and secretion in the GK rat, a spontaneous model of NIDDM

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Alvarez, Carmen, Danielle Bailbe, Francoise Picarel-Blanchot, Eric Bertin, Ana-Maria Pascual-Leone, and Bernard Portha. Effect of early diet restriction on insulin action and secretion in the GK rat, a spontaneous model of NIDDM. Am J Physiol Endocrinol Metab 278: E1097–E1103, 2000.—The availability of the Goto-Kakisaki (GK) rat model of non-insulin-dependent diabetes mellitus prompted us to test the effect of a limited period of undernutrition in previously diabetic young rats on their insulin secretion and insulin action during adult age. Four-week-old female GK rats were either food restricted (35% restriction, 15% protein diet) or protein and energy restricted (35% restriction, 5% protein diet) for 4 wk. Food restriction in the young GK rat lowered weight gain but did not aggravate basal hyperglycemia or glucose intolerance, despite a decrease in basal plasma insulin level. Furthermore, the insulin-mediated glucose uptake by peripheral tissues in the GK rat was clearly improved. We also found that food restriction, when it is coupled to overt protein deficiency in the young GK rat, altered weight gain more severely and slightly decreased basal hyperglycemia but conversely aggravated glucose tolerance. Improvement of basal hyperglycemia was related to repression of basal hepatic glucose hyperproduction, despite profound attenuation of basal plasma insulin level. Deterioration of tolerance to glucose was related to severe blunting of the residual glucose-induced insulin secretion. It is, however, likely that the important enhancement of the insulin-mediated glucose uptake helped to limit the deterioration of glucose tolerance.

Goto-Kakisaki rat; non-insulin-dependent diabetes mellitus; malnutrition; energy restriction; protein-energy restriction; insulin secretion

Based on epidemiological evidence, malnutrition has been envisaged as an etiological factor and/or a precipitating factor for diabetes in malnourished individuals from developing countries (4, 20, 26) as well as in elderly patients who are often undernourished (1). Numerous studies in humans and laboratory animals have also clearly stated that malnutrition exerts a significant deleterious impact on a previously normal pancreatic ß-cell function (reviewed in Ref. 20).

However, some confusion still exists in the literature concerning the relationship between malnutrition and insulin action; a high tissue sensitivity to insulin has been suggested (7, 17, 18), because malnutrition in general and dietary protein deprivation in particular are characterized by low fasting blood glucose levels despite low insulin levels in both humans and rodents (3, 7, 10, 17, 18). As a matter of fact, we have reported that the protein- and energy-restricted rats present an increased utilization of glucose in the basal postabsorptive state and in euglycemic-hyperinsulinemic conditions (18). A similar conclusion was reported in rats subjected to food restriction from the fetal stage (11). On the contrary, insulin resistance has also been reported in undernourished rats (22), and it was found to be aggravated in undernourished rats with mild streptozotocin diabetes (22). Moreover, it has been shown that the deleterious effect of chronic malnutrition on ß-cell function amplifies the overall ß-cell deficit in mild streptozotocin diabetes, and refeeding reverses this effect (21).

Against this background, we propose to reevaluate the interaction between chronic malnutrition and diabetes using the Goto-Kakisaki (GK) rat, a model of hereditary non-insulin-dependent diabetes (NIDDM) that is not overweight (19). The overall objective of the present study was to identify the way chronic malnutrition influences the severity of the ß-cell secretory deficit and the glucose production and glucose utilization defects in the diabetic GK rat. Our experiments were also designed to attempt to dissociate an energy restriction effect from a protein restriction effect on glucose metabolism. For this purpose, weaned GK rats were restricted to 65% of their normal ad libitum daily food intake; one group received a standard diet (15% protein) and the other received an isocaloric but low-protein (5%) diet. Glucose tolerance, in vivo and in vitro glucose-induced insulin release, and basal and insulin-stimulated glucose production and glucose utilization in vivo were evaluated over a 4-wk period.

MATERIALS AND METHODS

Diets. The powdered semisynthetic standard diet contained by weight (g/100 g) 68% starch, 4% cellulose, 5% lipid (maize oil), and 15% protein (casein) (remaining 8% moisture) and by calories 72% carbohydrate, 12% lipid, and 15% protein. The powdered semisynthetic low-protein diet contained by weight (g/100 g) 78% starch, 4% cellulose, 5% lipid (maize oil), and 5% protein (casein) and by calories 83% carbohydrate, 12% lipid, and 5% protein. The two diets were isoenergetic, and the energy content per 100-g diet was 375 calories. Both diets contained 2 g/100 g yeast, a salt mixture...
Isolation and perfusion of the pancreas were performed as described previously (18). Animals. Female GK rats issued from our GK colony (19) were weaned 28 days after birth and from this age were fed either a standard or a protein-restricted diet for 4 wk onward. One member of each pair of littermates was fed ad libitum (standard diet), with daily food intake being measured, and the intake of the other member of the pair was restricted for the next 4 wk to 65% of the ad libitum intake (standard diet or low-protein diet), with the food being placed in the cage each evening (1 hour before dark cycle onset). GK rats fed the standard diet ad libitum were used as controls.

It is important to mention that no major alteration of the feeding pattern took place in the restricted groups, because we have verified that rats had an excess of food available most of the time during the nocturnal feeding period, and the restricted rats never consumed in one short meal their daily food ration (in contrast to the findings usually reported in more severe food restriction protocols). Because in the glucose tolerance and clamp experiments performed at 1400, food was withdrawn in the three groups of rats in the morning of the study shortly after the light cycle onset, one may therefore consider that the duration of subsequent fasting was comparable in the three groups.

After following the diet for 3 wk, animals from each group underwent a glucose tolerance test. After following the diet for 4 wk, randomly selected animals from each group were used for in vitro perfusion of the pancreas. The remaining animals in each group underwent measurement of in vivo insulin action with the glucose-insulin clamp technique.

Isolated pancreas perfusion technique. Rats were anesthetized with pentobarbital sodium (4 mg/100 g body wt ip). Isolation and perfusion of the pancreas were performed as previously described (12). The perfusate was a Krebs-Ringer bicarbonate buffer with the following components: 2.8 mmol/l D-glucose (Merck, Darmstadt, Germany), 118 mmol/l NaCl, 4 mmol/l KCl, 2.5 mmol/l CaCl2, 1.2 mmol/l MgSO4, 1.2 mmol/l KH2PO4, 25 mmol/l NaHCO3, 1.25 g/l fatty acid-free BSA (Sigma, St. Louis, MO), and 40 g/l dextran T70 (Pharmacia, Uppsala, Sweden). When needed, D-glucose or L-arginine (Sigma) was administered through a side-arm syringe. In all protocols, the complete effluent (3 ml/min) was collected from the cannula in the portal vein at 1-min intervals in chilled tubes and was frozen for storage at −20°C until assay.

Glucose tolerance tests. Intravenous glucose tolerance tests (0.5 g glucose/kg body wt) were performed under pentobarbital sodium anesthesia (4 mg/100 g body wt ip) at 1400 in rats fasted from 0900. Blood was withdrawn from the tail vein, and samples (250 µl) were immediately centrifuged at 4°C; plasma was stored at −20°C until assayed.

Euglycemic-hyperinsulinemic clamp studies. Studies were performed at 1400 in rats fasted from 0900 according to a previously detailed procedure (6, 13). The rats were consid- ered to be in the postabsorptive period, and the rate of glucose production was a measure of endogenous glucose production. Rats were anesthetized with pentobarbital sodium. Body temperature was maintained at 37–38°C with heating lamps. One carotid artery was catheterized for blood sampling, and a tracheotomy was systematically performed to avoid respiratory problems during anesthesia.

Blood samples of 150 µl were collected 20 min after the end of the surgery for the determination of basal blood glucose and plasma insulin concentrations. Next, insulin was infused at a constant rate of 20 µU/min (3.0 mmol·h⁻¹·kg⁻¹ in control group and 3.6 mmol·h⁻¹·kg⁻¹ in diet-restricted group) in a saphenous vein, and the blood glucose level was clamped at the level measured in the basal state by a variable infusion of glucose through the other saphenous vein with a Precidor pump (Inforis, Basel, Switzerland). Insulin (porcine monocomponent insulin Actrapid; Novo, Copenhagen, Denmark) was dissolved in 0.9% NaCl containing 0.2% BSA (Sigma). The variable infusion of exogenous glucose (7.5% solution) was started 5 min after the insulin infusion. Next, 25 µl blood were sampled from the carotid artery every 5 min, and plasma glucose concentrations were determined within 60 s with a glucose analyzer (Beckman, Palo Alto, CA).

Steady-state plasma insulin levels were reached 30 min after starting the insulin infusion, and steady-state blood glucose levels were reached after 45–50 min. Blood samples (200 µl) were collected at 55, 60, and 65 min to determine blood glucose specific activity and plasma insulin concentrations. The coefficients of variation in plasma glucose and insulin concentrations during the clamp were 5 and 15%, respectively.

Endogenous glucose production and whole body glucose utilization. Endogenous glucose production in the basal state and during the hyperinsulinemic clamp studies was assessed by a primed continuous infusion of [3-³H]glucose (New England Nuclear, Dreieich, Germany). The labeled glucose was administered as an initial intravenous priming dose (4 µCi) followed immediately by a continuous intravenous infusion at a rate of 0.2 µCi/min. Steady-state glucose specific activity was established by 40 min both in the basal state and the clamp studies. The rate of glucose appearance (Ra) was then equal to the rate of glucose disappearance (Rd), and these two parameters were calculated by dividing the [3-³H]glucose infusion rate [disintegrations·min⁻¹·dpm·min⁻¹] by the steady-state value of glucose specific activity (dpm/g). In the basal state, the rate of endogenous glucose production is equal to Rd. In the clamp studies, the rate of endogenous glucose production was calculated by subtracting the exogenous steady-state glucose infusion rate (SSGIR) from Rd. The rate of glucose utilization by the whole body mass (GUR) was calculated as GUR = Rd − SSGIR and the glucose production rate (GPR) in the liver was calculated as GPR = Rd·SSGIR.

Samples, analytical techniques, and calculations. Plasma glucose was determined with a glucose analyzer (Beckman, Palo Alto, CA). Blood samples for measuring glucose specific activity were deproteinized with Ba(OH)2-ZnSO4 and immediately centrifuged. An aliquot of the supernatant was used for the determination of glucose using a glucose oxidase method. Another aliquot of the supernatant was evaporated to dryness at 60°C to remove tritiated water. The dry residue was dissolved in 0.1 ml distilled water and was counted with 3 ml of ReadySolv-MP scintillation solution (Beckman). Plasma immunoactive insulin was estimated using purified rat insulin (Novo), antibody to mixed (porcine bovine) insulin cross-reacting similarly with porcine and rat insulin standards, and porcine monoidinated 125I-labeled insulin (6, 18). Charcoal was used to separate free from bound hormone. The method allows the determination of 2 µlU/ml (0.08 ng/ml or 14 pmol/l) with a coefficient of variation within and between assays of 10%.

The insulin and glucose responses during the glucose tolerance test were calculated as the incremental plasma insulin values integrated over the 30-min period after the glucose injection (ΔI, mmol·l⁻¹·min⁻¹) and the corresponding incremental integrated plasma glucose values (ΔG, mmol·l⁻¹·min⁻¹).

The insulin secretion rate per total pancreas was calculated by multiplying the insulin concentration in the samples by the flow rate and was expressed as nanomoles per minute. Total insulin response to a given stimulus was obtained by
RESULTS

Characteristics of the GK rats. After weaning (4 wk), female GK rats fed ad libitum gained weight and continued to grow throughout the observation period (Fig. 1). Food-restricted GK rats gained weight at a considerably lower rate during the same observation period (Fig. 1). The deficiency state in this group could be regarded as one of combined protein-energy malnutrition. Calculation of the daily protein intake per gram body weight at the end of the 4-wk period of restriction indicated that it was not significantly different in the restricted rats from that in the unrestricted rats (1.7 ± 0.2 g protein/100 g body wt, n = 9, and 1.4 ± 0.2, n = 6, respectively), thus suggesting that the protein deficiency remains mild under these experimental conditions.

In the protein- and energy-restricted GK group, body weight gain was almost obtunded (Fig. 1). This indicates that the overt protein deficiency that is superimposed to the same energy restriction as in the food-restricted group exerts a proper aggravating influence upon growth.

The basal characteristics of the GK rats killed at the age of 8 wk are given in Table 1. In the food-restricted GK rats, although the basal plasma glucose level measured in the postabsorptive state was not significantly different compared with unrestricted GK rats, the basal plasma insulin level was significantly lower (P < 0.02). In the protein- and energy-restricted GK rats, the basal plasma glucose and insulin levels were both significantly decreased (P < 0.001) compared with the control group (Table 1), and the plasma insulin level was lower (P < 0.001) than that in the food-restricted group.

Glucose tolerance and in vivo insulin secretory response to glucose. The mean incremental glucose area (ΔG) in the food-restricted group tended to increase in response to an intravenous glucose load, whereas in the protein- and energy-restricted group it was clearly increased (P < 0.001) compared with the unrestricted group.

Values of the mean incremental insulin area (ΔI) were significantly decreased in the protein- and energy-restricted group (P < 0.05 compared with the control group and P < 0.01 compared with the food-restricted group; Table 1). This indicates that in vivo glucose-induced insulin secretion was severely impaired in the GK rats submitted to protein-energy restriction, whereas no major degradation was detectable in the food-restricted GK rats.

In vitro insulin secretory response. The in vitro insulin release in response to glucose and arginine was studied with the isolated perfused pancreas preparation. Basal insulin secretion in the presence of 2.8 mmol/l glucose in the perfusion medium was not significantly different in the food-restricted rats compared with the controls but was decreased (P < 0.05) in the protein- and energy-restricted GK rats (Table 2). Exposure for 20 min to a 16 mmol/l glucose concentration that induces the pattern of impaired insulin release typical of the control GK pancreases elicited a similar increase of insulin output in the food-restricted GK pancreases. Also, the incremental insulin response to 19 mmol/l arginine remained unchanged in the food-restricted group compared with that in the control.
female GK rats. Glucose concentration in the perfusate in the basal state was severely blunted (P < 0.001) in the protein- and energy-restricted GK rats by 16 mmol/l glucose or 19 mmol/l arginine above basal release in food-restricted, protein- and energy-restricted, or unrestricted (control) female GK rats.

### Table 2. Basal insulin release (2.8 mmol/l) and ΔI to 16 mmol/l glucose or 19 mmol/l arginine above basal release in food-restricted, protein- and energy-restricted, or unrestricted (control) female GK rats

<table>
<thead>
<tr>
<th>GK Rats</th>
<th>Basal Release, pmol/min</th>
<th>ΔI, pmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>6 70 ± 20</td>
<td>2,890 ± 700</td>
</tr>
<tr>
<td>Food restricted</td>
<td>6 70 ± 20</td>
<td>2,790 ± 500</td>
</tr>
<tr>
<td>Protein-energy restricted</td>
<td>8 22 ± 4</td>
<td>290 ± 80</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Animals were 8 wk old when killed. They had received either the control diet or the restricted diets from the age of 4 wk (weaning) and were tested in the postabsorptive state. aP < 0.05, bP < 0.01, and cP < 0.001 vs. related control group. dP < 0.001 and eP < 0.05 vs. related food-restricted group.

By contrast, the insulin response elicited in the protein- and energy-restricted GK rats by 16 mmol/l glucose was severely blunted (P < 0.01; Fig. 2), because the incremental insulin response was only 30% of the control response (Table 2). A similar blunted pattern (P < 0.001) was observed during 19 mmol/l arginine stimulation (Table 2).

In vivo glucose metabolism and insulin action. The basal rate of glucose production (reflective of hepatic glucose production in the postabsorptive state) was depressed (P < 0.001) in the food-restricted and protein- and energy-restricted GK rats relative to controls when expressed per animal. However, when expressed per kilogram body weight, the rate of exogenous glucose infusion was significantly higher (P < 0.05) than in the control rats when the values were expressed per kilogram body weight. This suggests that the total body glucose metabolism is more responsive to insulin in both restricted groups compared with the control group. After submaximal hyperinsulinemia, overall glucose utilization was significantly lower (P < 0.001) in the food-restricted GK rats and the protein- and energy-restricted GK rats compared with the control GK rats (when the results are expressed per animal). However, it increased (P < 0.001) in the food-restricted and the protein- and energy-restricted GK rats when it was expressed per kilogram body weight (Table 3). Furthermore, glucose utilization during hyperinsulinemia was elevated (P < 0.001) in the food-restricted group relative to the protein- and energy-restricted GK rats (Table 3).

### DISCUSSION

This study extends to young diabetic GK rats previous observations in young normal rats (7, 9, 11, 17, 24) that food restriction (30–50% restriction) does not support normal growth. The moderate growth retardation in rats exposed to food restriction contrasts with the drastic growth arrest observed in the protein- and energy-restricted group. Of course, at the beginning of the restriction protocol, the rats have to cope not only with energy restriction but also with an insufficient protein intake. However, the preservation of a daily protein intake (when expressed per unit body weight) that we found in the food-restricted GK rats at the end of the 4-wk protocol suggests that these rats did not experience irreversible and major protein restriction. We previously came to the same conclusion in normal Wistar rats using the same dietary manipulation (18). Note that, using diet compositions close to the present...
Table 3. Levels of glucose and insulin and glucose kinetics during hyperinsulinemic-euglycemic clamp in female GK rats submitted to food restriction (35% restriction) or to protein-energy restriction (35% food-restriction plus low protein diet) for 4 wk

<table>
<thead>
<tr>
<th>GK Rats</th>
<th>n</th>
<th>IIR, mmol·h⁻¹·kg⁻¹</th>
<th>SSPI, mmol/l</th>
<th>BBG, mmol/l</th>
<th>SSBG, mmol/l</th>
<th>SSGIR, µmol/min</th>
<th>SSIGIR, µmol·min⁻¹·kg⁻¹</th>
<th>GPR, µmol/min</th>
<th>GPR, µmol·min⁻¹·kg⁻¹</th>
<th>GUR, µmol/min</th>
<th>GUR, µmol·min⁻¹·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>12</td>
<td>1.2 ± 0.1</td>
<td>9.9 ± 0.2</td>
<td>10.0 ± 0.3</td>
<td>13.9 ± 0.1</td>
<td>65 ± 4</td>
<td>13.9 ± 0.1</td>
<td>65 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food restricted</td>
<td>12</td>
<td>2.4 ± 0.2</td>
<td>9.6 ± 0.3</td>
<td>6.0 ± 0.2</td>
<td>10.3 ± 0.5</td>
<td>50 ± 3</td>
<td>5.3 ± 0.5</td>
<td>26 ± 2</td>
<td>15.6 ± 1.0</td>
<td>76 ± 5</td>
<td></td>
</tr>
<tr>
<td>Protein energy restricted</td>
<td>8</td>
<td>3.6 ± 0.3</td>
<td>6.9 ± 0.2</td>
<td>14.1 ± 0.5</td>
<td>152 ± 5</td>
<td>0.4 ± 0.2</td>
<td>12.9 ± 0.5</td>
<td>136 ± 2</td>
<td></td>
<td></td>
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</tr>
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</table>

Values are means ± SE; n, no. of rats. IIR, insulin infusion rate; SSPI, steady-state plasma insulin; BBG, basal blood glucose; SSBG, steady-state blood glucose; SSGIR, steady-state glucose infusion rate; GPR, glucose production rate; GUR, glucose utilization rate. Animals were 8 wk old when killed. They had received either the control diet or the restricted diets from the age of 4 wk (weaning) and were tested in the postabsorptive state. Comparison was made with rats raised in parallel and fed a standard diet ad libitum (controls). a P < 0.05, b P < 0.01, and c P < 0.001 compared with related control group. d P < 0.01, P < 0.001, and P < 0.05 compared with related food-restricted group.
value found in normal nondiabetic Wistar rats). By contrast, their tolerance to intravenous glucose was lower than in control rats, as shown by a significantly increased incremental plasma glucose area (ΔG value).

The total body glucose metabolism in the food-restricted GK rats, as measured by the rate of exogenous glucose infusion required to maintain the blood glucose level at euglycemia and at steady-state plasma insulin, was significantly higher than that in the control GK rats at submaximal insulin levels. This indicates that the total body glucose metabolism was more responsive to insulin in the food-restricted GK rats compared with the control GK rats. The basal glucose utilization rate as estimated by the glucose turnover value was significantly higher in the food-restricted GK rats. During the clamp studies, the glucose utilization induced by submaximal insulin levels was significantly greater (4.6-fold increase when related to body mass) in the food-restricted GK rats than in the control GK rats. These data suggest that insulin-mediated glucose uptake is enhanced in the food-restricted GK rats.

Under basal postabsorptive conditions and in the face of drastically lowered plasma insulin levels, the protein- and energy-restricted GK rats experienced a significant decrease in plasma glucose, but it remained in the diabetic range. Tolerance to intravenous glucose was aggravated, as shown by an increased incremental plasma glucose area value. Such a glucose intolerance is obviously related to the decreased glucose-induced insulin release in vivo. Alternatively, it could also be related to a decreased sensitivity to insulin. In fact, the total body glucose metabolism (rate of exogenous glucose infusion at submaximal insulin levels) in the protein- and energy-restricted GK rats was significantly higher than that in the control GK rats. Their basal glucose utilization rate (glucose turnover) was significantly higher (1.6-fold increase) and, during the clamp studies, the glucose utilization induced by submaximal insulin levels was significantly greater (1.8-fold increase) in the protein- and energy-restricted GK rats than in the control GK rats. Therefore, one may conclude that insulin-mediated glucose uptake was indeed enhanced in the protein- and energy-restricted GK rats.

Furthermore, the comparison of insulin action between the two protocols of restriction investigated here suggests that glucose utilization was more efficiently increased by insulin in the food-restricted GK rats than in the protein- and energy-restricted GK rats.

One of the aims of our study was also to evaluate the effect of insulin on endogenous glucose production in the food-restricted GK rats. In the basal state (postabsorptive state), the hepatic glucose production value (when expressed per body mass and when compared with the value in control GK rats) was increased in the protein- and energy-restricted GK rats, whereas it remained unchanged in the food-restricted GK rats. Without knowing at the present time the circulating levels of the counterregulatory hormones, we cannot conclude that insulin action in the liver of both restricted groups was impaired in the basal state. In the presence of submaximal insulin levels (euglycemic clamp studies), the glucose production in the food-restricted GK rats, instead of being suppressed, was paradoxically enhanced. We are presently without any satisfactory explanation for such a pattern. However, it is consistent with our previous proposal that food restriction from weaning (18) or from the fetal stage (11) promotes hepatic insulin resistance. In the protein- and energy-restricted GK rats, the pattern was clearly different; although the hepatic glucose production in control GK rats remained elevated in the presence of submaximal insulin levels, it was almost blocked in the protein- and energy-restricted GK rats.

In conclusion, we have found that a 35% food restriction in the young GK rat does not aggravate basal hyperglycemia or glucose intolerance, despite a decrease of the basal plasma insulin level. Furthermore, our data provide direct evidence that food restriction determines changes in the effect of insulin on some target tissues, because the insulin-mediated glucose uptake by peripheral tissues in the GK rat was indeed improved. We also found that the 35% food restriction, when coupled to overt protein deficiency, slightly decreases basal hyperglycemia but conversely aggravates glucose tolerance. Improvement of basal hyperglycemia was related to repression of basal hepatic glucose hyperproduction, despite profound attenuation of the basal plasma insulin level. Deterioration of tolerance to glucose was related to severe blunting of the residual glucose-induced insulin secretion. It is, however, likely that the important enhancement of the insulin-mediated glucose uptake helps to limit the deterioration of glucose tolerance.

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