Changes in Insulin Action and Insulin Secretion in the Rat After Dietary Restriction Early in Life: Influence of Food Restriction Versus Low-Protein Food Restriction

Françoise Picarel-Blanchot, Carmen Alvarez, Danielle Bailbe, Ana-Maria Pascual-Leone, and Bernard Portha

The effect of a limited period of undernutrition in young rats on insulin secretion and insulin action during adulthood has been studied. Four-week-old female rats were either food-restricted (35% restriction, 15% protein diet) or protein-calorie–restricted (35% restriction, 5% protein diet) for 4 weeks. Food-restricted rats gained weight at a lower rate than control rats. In the protein-calorie–restricted group, the alteration of weight gain was more severe. Basal plasma insulin was reduced only in protein-calorie–restricted rats. Glucose-stimulated insulin secretion (ΔI) obtained in vivo after an intravenous glucose load was only moderately decreased in food-restricted group, whereas it was severely blunted in the protein-calorie–restricted group. In this last group, impairment of the insulin secretory response to glucose was related to an intrinsic impairment of β-cell secretory capacity, since the insulin secretory response to glucose or arginine was decreased when tested in vitro (perfused pancreas). In food-restricted rats, basal plasma glucose level was kept normal, while a mild deterioration of glucose tolerance was detectable. This was related, of course, to the decrease of ΔI as identified in vivo. However, data obtained under basal or euglycemic-hyperinsulinemic conditions provided direct evidence that insulin-mediated total glucose uptake (weight-related expression) was paradoxically enhanced. A similar conclusion was reached in protein-calorie–restricted rats; the increase of overall insulin-mediated glucose uptake was even more important. Such an adaptation, which was operating in both types of restriction, may help limit the deterioration of glucose tolerance in the face of impaired insulin release. In the basal postabsorptive state, the higher glucose utilization rate in both models originated from increased hepatic glucose production rates. During hyperinsulinemia, endogenous glucose production in food-restricted rats was normally blunted, but not in protein-calorie–restricted rats, thus indicating resistance of the hepatic glucose production pathway to insulin action in this group.

A HIGH PREVALENCE of malnutrition-related diabetic syndromes has been recognized in the developing world[1,2] and is hypothesized in the elderly in the developed world.[3] Glucose intolerance is a characteristic feature of protein-calorie malnutrition, especially in children suffering from acute severe protein deficiency.[4-7] The possibility that the impaired glucose tolerance could result in that case from insulin deficiency has been raised.[7] However, some confusion exists in the literature concerning this point, since high fasting plasma insulin levels or sustained insulin secretion have also been reported in some malnourished children.[8-10] Concerning insulin action, it has been reported that infants with kwashiorkor are insulin-resistant, whereas those with marasmus are not.[4] Thus, the question of the changes in insulin action during protein-energy malnutrition in humans remains unresolved.

Experimental models of protein-calorie malnutrition are necessary because they provide the opportunity for rigorously controlled nutritional experimentation. Rats have mainly been used[11-19] to identify the impact of protein-energy deficiency on glucose homeostasis. In rats fed long-term with a protein level in the diet comparable to that of humans in developing countries, glucose homeostasis has been evaluated through measurements of basal levels of blood glucose or pancreatic hormones,[15-17] glucose tolerance tests,[14,16,17] or insulin tolerance tests.[17] Most of these studies have provided evidence for a failure of insulin secretion together with an increased insulin sensitivity of peripheral tissues in malnourished rats.[16-19]

However, an important limitation for the interpretation of results obtained in these animal models of malnutrition is that they do not allow a separate analysis of the effect of protein deficiency per se from that of energy deficiency, since young rats given the low-protein diet reduced their food intake spontaneously by 30% to 40%.[16-18]

The experiments reported here are an attempt to dissociate a calorie-restriction effect from a protein-restriction effect on glucose metabolism. For this purpose, normal weaned Wistar 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-week period.

MATERIALS AND METHODS

Diets

The powdered semisynthetic standard diet contained by weight (g/100 g) 68% starch, 4% cellulose, 5% lipid (corn oil), and 15% protein (casein), and by calories 72% carbohydrate, 12% lipid, and 15% protein. The powdered semisynthetic low-protein diet con-

From the Laboratoire Physiopathologie Nutrition, Centre National de la Recherche Scientifique (CNRS) Unite de Recherche Associee (URA) 307, Université D. Diderot, Paris, France; and Instituto de Bioquimica, Centro Mixto Universidad Complutense y CSIC Facultad de Farmacia, Universidad Complutense, Madrid, Spain.

Submitted June 4, 1994; accepted April 18, 1995.

Supported in part by the France/Spain Programme for Science (Actions Intégrées 149 and 216 and Coopération Franco-Espagnole CNRS/CSIC project 1986). C.A. was the recipient of a fellowship from Université D. Diderot / Paris 7 (Maitre de Conférence invité, emploi no. 234MA0202).

Address reprint requests to Bernard Portha, PhD, Lab. Physiopathology of Nutrition, CNRS URA 307, Université D. Diderot/Paris 7, 2 place Jussieu, Tour 33, 75251 Paris Cedex 05, France.

0026-0495/95/4412-0002$03.00/0

tained by weight (g/100 g) 78% starch, 4% cellulose, 5% lipid (corn oil), and 5% protein (casein), and by calories 83% carbohydrate, 12% lipid, and 5% protein. Energy content per 100 g diet was the same (375 cal) in both diets.

Both diets contained 2 g/100 g yeast, a salt mixture (3.5 g/100 g), and a vitamin mixture (2.2 g/100 g). The vitamin mixture contained the following: folic acid 0.094 mg/g, biotin 0.047 mg/g, vitamin A 469 UI/g, cholecalciferol 94 UI/g, menadione 0.469 mg/g, thiamine 0.703 mg/g, riboflavin 0.703 mg/g, pyridoxine 0.469 mg/g, calcium pantothenate 2.344 mg/g, niacin 2.344 mg/g, *p*-aminobenzoic acid 14.062 mg/g, inositol 23.437 mg/g, tocopherol 14.1 UI/g, choline 93.747 mg/g, vitamin B_{12} 0.0023 mg/g, and cellulose to make 1 g. The following were present in the salt mixture (g/kg): $CaHPO_4$ 500, NaCl 74, $K_3C_6H_5O_7H_2O$ 220, K_2SO_4 52, MgO 24, $MnCO_3$ 3.5, $FeC_6H_5O_7H_2O$ 0.6, ZnO 1.6, $CuCO_3$ + $Cu(OH_2)$ 0.3, KIO_3 0.01, $NaSeO_35H_2O$ 0.01, $CrK(SO_4)_212H_2O$ 0.55, and sucrose to make 1 kg.

Animals

Female Wistar rats bred in our colony were weaned 28 days after birth and then maintained on a standard diet. Food intake and body weight were recorded every 2 days. One member of each pair of littermates was fed ad libitum with daily food intake being measured, and the intake of the other member of the pair was restricted for the next 4 weeks 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). The choice of a moderate level (35%) of food restriction was suggested by the studies of Swenne et al,[16] and Okitolonda et al,[17] who reported that rats offered a low-protein diet after weaning spontaneously reduced their daily food intake to 65% of the food intake of animals receiving a normal isocaloric diet ad libitum. Normal 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 group, since we have verified that rats had an excess of food available most of the time during the nocturnal feeding period and that the restricted rats never consumed their daily food ration in one short meal (in contrast to the findings usually reported in more severe food-restriction protocols). Since in glucose tolerance and clamp experiments performed at 2 PM food was withdrawn in the three groups of rats on the morning of study shortly after light-cycle onset, one may therefore consider that the duration of subsequent fasting was comparable in the three groups.

After feeding the diet for 3 weeks, all animals underwent a glucose tolerance test. After feeding the diet for 4 weeks, 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 sodium pentobarbital (4 mg/100 g body weight intraperitoneally). Isolation and perfusion of the pancreas were performed as previously described.[20] 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 $CaCl_2$, 1.2 mmol/ L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 1.25 g/L fatty acid–free bovine serum albumin (Sigma, St Louis, MO), and 40 g/L dextran T70 (Pharmacia, Uppsala, Sweden). When needed, D-glucose or L-arginine (Sigma) were 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-minute intervals in chilled tubes and frozen for storage at −20°C until assay.

Glucose Tolerance Tests

Intravenous glucose tolerance tests (0.5 g glucose/kg body weight) were performed under pentobarbital anesthesia (4 mg/100 g body weight intraperitoneally) at 2 PM in rats fasted from 9 AM. 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 2 PM in rats fasted from 9 AM according to a previously detailed procedure.[21,22] Rats were considered to be in the postabsorptive period, and the rate of glucose production was a measure of endogenous glucose production. Rats were anesthetized with pentobarbital. Body temperature was maintained at 37° to 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 minutes after the end of the surgery for determination of basal blood glucose and plasma insulin concentrations. Then insulin was infused at a constant rate of 20 μL/min (3.6 mmol · h^{-1}. kg^{-1}) in a saphenous vein, and 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 (Infors, Basel, Switzerland). Insulin (porcine monocomponent insulin Actrapid; Novo, Copenhagen, Denmark) was dissolved in 0.9% NaCl containing 0.2% bovine serum albumin (Sigma). Infusion of exogenous glucose (7.5% solution) was started 5 minutes after insulin infusion. Then 25 μL blood was sampled from the carotid artery every 5 minutes, and plasma glucose concentrations were determined within 60 seconds with a glucose analyzer (Beckman, Palo Alto, CA).

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

Endogenous Glucose Production

Endogenous glucose production in the basal state and during hyperinsulinemic clamp studies was assessed by a primed-continuous infusion of (3-^{3}H)glucose (New England Nuclear, Dreiech, Germany). 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 minutes both in the basal state and in the clamp studies. The rate of glucose appearance (R_a) was then equal to the rate of glucose disappearance (R_d)and these two parameters were calculated by dividing the (3-^{3}H) glucose infusion rate (dpm/min) by the steady-state value of glucose specific activity (dpm/g). In the basal state, the rate of endogenous glucose production is equal to R_a. In clamp studies, the rate of endogenous glucose production was calculated by subtracting the exogenous steady-state glucose infusion rate (SSGIR) from R_a. The rate of glucose utilization by the whole-body mass (GUR) was calculated as GUR = R_d, and the glucose production rate (GPR) in the liver was calculated as GPR = R_a-SSGIR.

Samples, Analytical Techniques, and Calculations

Plasma glucose was determined using a glucose analyzer (Beckman). Blood samples for measuring glucose specific activity were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and immediately centrifuged. An aliquot of the supernatant was used for 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 counted with 3 mL ReadySolv-MP scintillation solution (Beckman). Plasma immunoreactive insulin was estimated using purified rat (studies in the basal state) or porcine (clamp studies) insulin as standards (Novo), with the antibody to mixed (porcine + bovine) insulin cross-reacting similarly with pork and rat insulin standards and porcine monoiodinated ^{125}I-insulin.[21,22] Charcoal was used to separate free from bound hormone. The method allows determination of 2 μU/mL (0.08 ng/mL or 14 pmol/L) with a coefficient of variation within and between assays of 10%.

Insulin and glucose responses during the glucose tolerance test were calculated as incremental plasma insulin values integrated over the 30-minute period following the glucose injection ([ΔI] nmol/L · min) and corresponding incremental integrated plasma glucose values ([ΔG] mmol/L · min).

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 planimetry of individual perfusion profiles and expressed as the difference in hormonal secretion rate (nanomoles of insulin per minute) relative to mean hormonal output recorded at the end of the prestimulation period.

Statistical Analysis

Results are presented as the mean ± SE. Statistical analysis was performed using ANOVA (Scheffe F test) or Student's *t* test for unpaired data.

RESULTS

Characteristics of the Rats

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

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

Basal characteristics of the rats killed at age 8 weeks are given in Table 1. In food-restricted rats, basal plasma glucose and insulin levels measured in the postabsorptive state were not significantly different as compared with respective levels in unrestricted rats. In protein-calorie–restricted rats, basal plasma glucose and insulin levels were both significantly decreased ($P < .001$) as compared with levels in either control group or food-restricted group (Table 1).

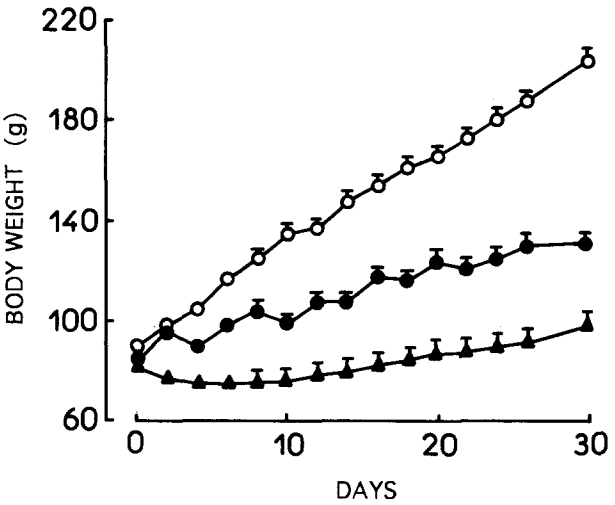

Fig 1. Evolution of body weight of food-restricted (●), protein-calorie–restricted (▲), and control unrestricted (○) female rats. The x axis refers to the number of days after initiation of food restriction. Values are the mean ± SEM for 16 to 21 rats per group.

Glucose Tolerance and In Vivo Insulin Secretory Response to Glucose

In response to an intravenous glucose load, the mean incremental glucose area in the food-restricted group was significantly increased ($P < .001$) as compared with the unrestricted group. The same tendency ($P < .02$) was observed in the protein-calorie–restricted group.

Mean incremental insulin areas were significantly decreased in the food-restricted group (39% decrease, $P < .02$) and in the protein-calorie–restricted group (88% decrease, $P < .001$) (Fig 2 and Table 1). This indicates that the in vivo glucose-induced insulin secretion was moderately deficient in rats submitted to food restriction, whereas it was more severely impaired ($P < .001$ food restricted group) in rats submitted to protein-calorie restriction.

In Vitro Insulin Secretory Response

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 food-restricted rats as compared with controls (Table 2). Exposure for 20 minutes to a 16-mmol/L glucose concentration, which induces a typical biphasic pattern of insulin release in control pancreases, elicited a similar increase of insulin output in food-restricted pancreases. Also, the incremental insulin response to 19 mmol/L arginine remained unchanged in the food-restricted group versus the control group. The 16-mmol/L glucose concentration elicited a biphasic insulin response in protein-calorie–restricted rats also (Fig 3). However, it was severely decreased ($P < .001$), since the incremental insulin re-

Table 1. Characteristics of Wistar Female Rats Submitted to Food Restriction (35% restriction) or to Protein-Calorie Restriction (35% food restriction + low-protein diet) for 4 Weeks

Group	Body Weight (g)	Plasma Glucose (mmol/L)	Plasma Insulin (nmol/L)	ΔG (mmol/L · min)	ΔI (nmol/L · min)
Controls (n = 18)	195 ± 6	7.5 ± 0.2	0.91 ± 0.10	10.8 ± 1.0	6.5 ± 0.9
Food-restricted (n = 18)	137 ± 6†	7.3 ± 0.2	0.83 ± 0.19	16.7 ± 0.6†	4.0 ± 0.3*
Protein-calorie–restricted (n = 16)	89 ± 2†‖	5.1 ± 0.4†‖	0.30 ± 0.07†§	11.9 ± 0.7‡	0.8 ± 0.1†‖

NOTE. Values are the mean ± SEM. Mean incremental insulin areas (ΔI) and mean incremental plasma glucose areas (ΔG) were calculated from data obtained during 30-minute glucose tolerance tests (0.5 g/kg intravenously) performed in the postabsorptive state.

*$P < .02$, †$P < .001$: *v* control group.

‡$P < .02$, §$P < .01$, ‖$P < .001$: *v* food-restricted group.

sponse was only 30% of the control response (Table 2). A similar pattern ($P < .001$) was observed during 19-mmol/L arginine stimulation (Table 2).

In Vivo Insulin Action

In both food-restricted rats and protein-calorie–restricted rats, the basal rate of glucose production, which reflects hepatic glucose production (postabsorptive state), was similar to that in control rats when expressed on a per-animal basis. However, when expressed per kilogram body weight, it was significantly increased ($P < .001$). Following submaximal hyperinsulinemia (3 nmol/L as a mean), endogenous glucose production in food-restricted rats was suppressed to a residual value similar to that in the control group, whether the results are expressed per rat or per kilogram body weight (Table 3). Under similar hyperinsulinemia, endogenous glucose production was only modestly decreased in protein-calorie–restricted rats, and accordingly, its value remained significantly higher ($P < .01$) than that of the control group and that of the food-restricted group.

The basal rate of glucose utilization in food-restricted rats and protein-calorie–restricted rats was similar to that in control rats when expressed on a per-animal basis. When expressed per kilogram body weight, it was significantly increased ($P < .001$). During clamp experiments performed at similar blood glucose levels in the three groups of rats, the rate of exogenous glucose infusion (SSGIR, Table 3) required to maintain blood glucose at euglycemia and at steady-state plasma insulin was taken as a measure of the effect of insulin on total-body glucose metabolism. Total-body glucose metabolism in food-restricted rats and protein-calorie–restricted rats was significantly higher ($P < .001$) than in control rats when values were expressed per kilogram body weight. This suggests that total-body glucose metabolism is most responsive to insulin in both restricted groups as compared with the control group. Following submaximal hyperinsulinemia, overall glucose utilization was similar in food-restricted rats, protein-calorie–re-

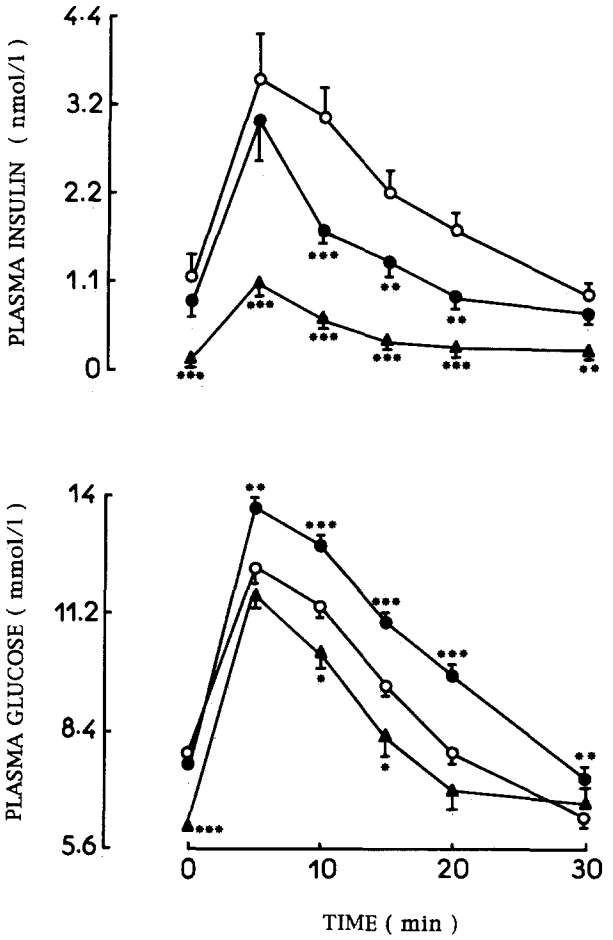

Fig 2. Glucose tolerance and plasma insulin response to glucose (0.5 g/kg intravenously) in food-restricted (●), protein-calorie–restricted (▲), and control unrestricted (○) female rats. Rats were tested after 3 weeks on their respective diet. Values are the mean ± SEM for 16 to 18 rats per group. *$V < .05$, **$V < .01$, *$V < .001$: respective control value.**

Table 2. Basal Insulin Release (2.8 mmol/L) and Incremental Insulin Response (ΔI) to 16 mmol/L Glucose or to 19 mmol/L Arginine Above Basal Release in Food-Restricted, Protein-Calorie–Restricted, or Unrestricted (control) Female Wistar Rats

Group	Basal Release (nmol/min)	ΔI (nmol/min) Glucose	ΔI (nmol/min) Arginine
Controls (n = 10)	0.10 ± 0.03	4.07 ± 0.25	3.13 ± 0.37
Food-restricted (n = 11)	0.06 ± 0.02	3.96 ± 0.35	3.22 ± 0.39
Protein-calorie–restricted (n = 7)	0.02 ± 0.01*	1.25 ± 0.28†‡	0.72 ± 0.12†‡

NOTE. Values are the mean ± SEM.

*$P < .05$, †$P < .001$: *v* control group.

‡$P < .001$ *v* food-restricted group.

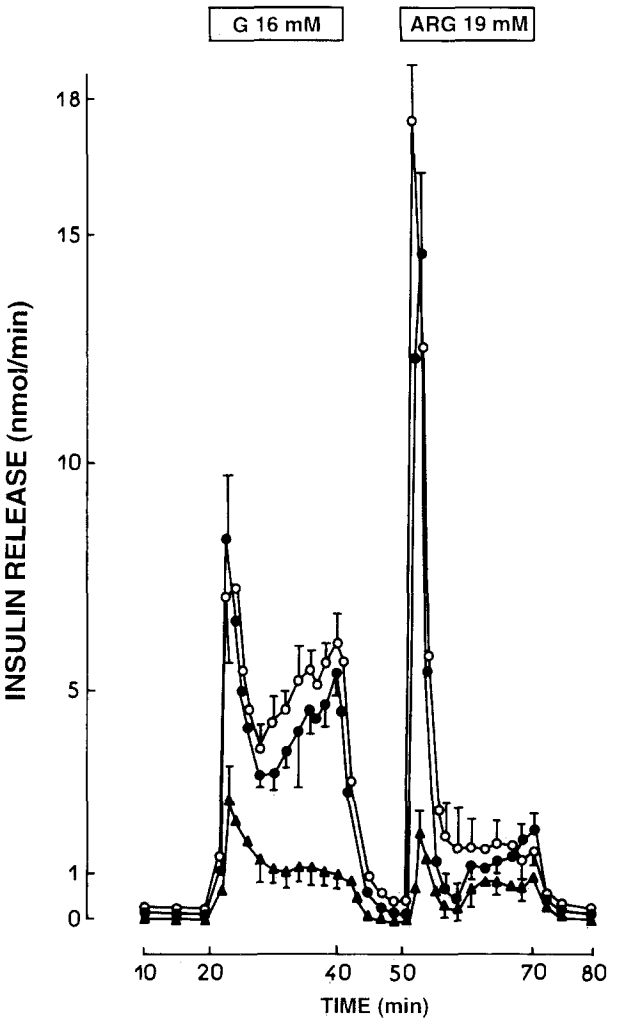

Fig 3. Effect of 16.5 mmol/L glucose or 19 mmol/L arginine on insulin release from perfused pancreas of food-restricted (●), protein-calorie–restricted (▲), and control unrestricted (○) female rats. Glucose concentration in the perfusate in the basal state was 2.8 mmol/L. Rats were tested after 4 weeks on their respective diet. Each point is the mean ± SEM of 7 to 11 observations per group.

stricted rats, and control rats when the results were expressed per animal. However, it was significantly increased ($P < .001$) in food-restricted and protein-calorie–restricted rats when expressed per kilogram body weight. Besides, glucose utilization during hyperinsulinemia in protein-calorie–restricted rats was significantly increased ($P < .01$) as compared with food-restricted rats (Table 3).

DISCUSSION

This study confirms previous observations[15-19] that food restriction (30% to 50% restriction) does not support normal growth in young rats. In these reports, including ours, the common result after food restriction was a moderate growth retardation in rats. This contrasts with the drastic growth arrest observed in protein-calorie malnutrition studies[15,17,19] (and the present study). Of course, at the beginning of the restriction protocol, rats have to cope not only with calorie 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 food-restricted rats at the end of the 4-week protocol suggests that these rats did not experience irreversible and major protein restriction. Moreover, using diet compositions similar to ours, Wade et al[23] reported that serum albumin level was not significantly affected by a reduction in caloric consumption as high as 50% after 6 weeks (18% protein diet), whereas a 50% food restriction combined with 4% protein in the food significantly decreased serum albumin. Given these data, one may retain as important for the significance of our study that the food-restricted group exhibits mainly calorie deficiency with limited associated protein malnutrition, whereas the protein-calorie–restricted group is clearly protein-deficient. This is an issue requiring attention, since it helps to delineate the respective effects of two parameters that are intimately associated in most animal studies of malnutrition.[15-19]

In food-restricted rats, basal plasma insulin levels were maintained. By contrast, a decreased in vivo insulin response to glucose was detected in the same animals. Such an impairment of the insulin secretory response to glucose was not related to an intrinsic impairment of β-cell secretory capacity, since the insulin secretory response to glucose or to arginine was normal when tested in vitro using perfusion of isolated pancreatic glands. These observations indicate that food restriction as used in our present experimental protocol did not exert any major detrimental structural or functional damage on pancreatic β-cells. They also suggest that the attenuation of the insulin response as observed in vivo during a glucose challenge is related

Table 3. Glucose and Insulin Levels and Glucose Kinetics During a Hyperinsulinemic-Euglycemic Clamp in Wistar Female Rats Submitted to Food Restriction (35% restriction) or to Protein-Calorie Restriction (35% food restriction + low-protein diet) for 4 Weeks

Group	No.	IIR (mmol/h/kg)	SSPI (nmol/L)	BBG (mmol/L)	SSBG (mmol/L)	SSGIR		GPR		GUR	
						μmol/min	μmol/min/kg	μmol/min	μmol/min/kg	μmol/min	μmol/min/kg
Controls	12	0	1.0 ± 0.1	5.7 ± 0.2	5.3 ± 0.2	—	—	9.2 ± 0.4	44 ± 2	9.2 ± 0.4	44 ± 2
	12	3.6	3.1 ± 0.2	6.4 ± 0.1	5.1 ± 0.1	14.8 ± 0.6	70 ± 2	1.1 ± 0.3	5.0 ± 1.5	15.1 ± 0.7	71 ± 3
Food-restricted	10	0	0.9 ± 0.1	6.3 ± 0.3	5.9 ± 0.2	—	—	10.8 ± 1.0	73 ± 7†	10.8 ± 1.0	73 ± 7†
	8	3.6	2.7 ± 0.2	6.5 ± 0.1	5.4 ± 0.1	15.1 ± 1.3	107 ± 8†	2.3 ± 0.8	17 ± 6	16.8 ± 2.0	120 ± 13†
Protein-calorie–restricted	7	0	0.6 ± 0.1*	4.9 ± 0.1*	5.2 ± 0.3	—	—	10.8 ± 0.8	110 ± 2§	10.8 ± 0.8	110 ± 2
	9	3.6	3.2 ± 0.2	5.0 ± 0.1*	5.4 ± 0.1	12 ± 1	116 ± 7†	7.0 ± 1.1†‡	69 ± 9†§	18.7 ± 1.3	185 ± 10†‡

NOTE. Values are the mean ± SEM.

Abbreviations: 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.

*$P < .01$, †$P < .001$: *v* control group.

‡$P < .01$, §$P < .001$: *v* food-restricted group.

mainly to an impaired environment of pancreatic β-cells. Determinants of such an altered environment for β-cell function in food-restricted rats are presently unknown. However, one may imagine that food restriction impairs the role of extrapancreatic modulators of β-cell function, such as the central nervous system via sympathetic and parasympathetic fibers afferent to the endocrine pancreas. This possibility remains to be investigated, but it represents an issue of interest, since there are several lines of evidence suggesting that early undernutrition exerts a substantial deleterious effect on anatomical, neurological, and neurochemical parameters.[24-26]

In protein-calorie–restricted rats, basal plasma insulin was clearly decreased and the in vivo insulin response to glucose was poor. This is in agreement with previous reports in similar models.[13-15] This impairment of insulin release in vivo is dramatic and, at variance with that found in food-restricted rats, it is related to an intrinsic abnormality(ies) of pancreatic β cells, since low responsiveness of β cells in protein-calorie–deficient rats could be evidenced in vitro in the perfused pancreas experiments. In fact, there is no doubt that protein-calorie malnutrition causes a generalized insensitivity of β cells, since in vitro responses to arginine and to glucose were blunted. It is also notable that protein-energy malnutrition has been reported to cause a diminution of β-cell mass,[27] and that such β-cell atrophy is a feature typical of protein shortage.[27-29]

Under basal conditions, food-restricted rats maintained a normal plasma glucose level. 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). Such glucose intolerance is clearly related to the decreased glucose-induced insulin release in vivo. Alternatively, glucose intolerance could also be related to a decreased sensitivity to insulin in food-restricted rats. Since the relationship between the ΔG value and the effects of insulin on glucose uptake is not a direct one, we have investigated insulin action in food-restricted rats using the insulin-glucose clamp technique in conjunction with isotopic measurements of glucose turnover. Total-body glucose metabolism in food-restricted rats, as measured by the rate of exogenous glucose infusion required to maintain blood glucose at euglycemia and at steady-state plasma insulin, was significantly higher than in control rats at submaximal insulin levels. This indicates that total-body glucose metabolism is more responsive to insulin in food-restricted rats as compared with control rats. The basal glucose utilization rate, as estimated by glucose turnover, was significantly higher in food-restricted rats. During the clamp studies, glucose utilization induced by submaximal insulin levels was significantly greater (when related to body mass) in food-restricted rats than in control rats. These data suggest that insulin-mediated glucose uptake is enhanced in food-restricted rats. Others have also studied insulin sensitivity in dietary-restricted rats. However, contradictory results have been reported. Kalant et al[30] concluded that dietary restriction started at 4 weeks of age reduced the ability of insulin to stimulate glucose uptake in vivo, whereas Ivy et al,[31] using a hindlimb perfusion preparation, reported that dietary restriction increased glucose uptake. Reaven et al[32] and Masoro et al[33] also concluded that dietary-restricted old rats were less insulin-resistant than ad libitum–fed rats.

Under basal postabsorptive conditions and in the face of drastically decreased plasma insulin levels, protein-calorie–restricted rats did not succeed in maintaining normal plasma glucose levels. However, tolerance to intravenous glucose was maintained, as shown by a normal glucose disappearance rate (K value). This last characteristic suggests an increased sensitivity to insulin in rats fed the protein-calorie–restricted diet (since their ΔI value was decreased by 88% during the same test). This conclusion was confirmed by the clamp experiments. Total-body glucose metabolism (rate of exogenous glucose infusion at submaximal insulin levels) in protein-calorie–restricted rats was significantly higher than in control rats. Their basal glucose utilization rate (glucose turnover) was significantly higher (2.5-fold increase). During the clamp studies, glucose utilization induced by submaximal insulin levels was significantly greater (2.6-fold increase) in protein-calorie–restricted rats than in control rats. These data show that insulin-mediated glucose uptake is indeed enhanced in protein-calorie–restricted rats. This conclusion confirms and extends (since it is obtained with a more appropriate methodology) those previously reported in similar protein-calorie–restricted rat models. In such models, it was reported that despite reduced basal plasma insulin levels, glucose clearance from the blood was maintained at a normal rate,[34] exogenous insulin caused a more sustained hypoglycemia,[17] and soleus muscle preparations in vitro were more sensitive to insulin.[35]

Furthermore, comparison of insulin action between the two models of calorie restriction investigated here suggests that glucose utilization is more efficiently increased by insulin in protein-calorie–restricted rats (2.6-fold increase) than in food-restricted rats (1.7-fold increase).

To the extent that our present model of food restriction can be considered a model of calorie deficiency mainly, one may propose that calorie restriction per se is a determinant of the increased insulin action reported during chronic malnutrition. However, it cannot be considered the unique determinant, since we have previously reported that protein restriction per se (with no associated calorie malnutrition) was also followed by increased insulin action in rats.[36]

One of the aims of our study was also to evaluate the effect of insulin on endogenous glucose production in the restricted rats. Since the rats were in the postabsorptive state, it can be assumed that the rate of glucose production represents hepatic glucose production. In the basal state, hepatic glucose production (when expressed per body mass) was increased in food-restricted and protein-calorie–restricted rats. Without knowing at present the circulating levels of counterregulatory hormones, we believe it should not be concluded that insulin action in the liver of both restricted groups was impaired in the basal state. However, in food-restricted rats, suppression of glucose production induced by submaximal insulin levels during euglycemic

clamp studies was indeed similar to that in control rats. Therefore, we propose that in this group insulin action is maintained normal at the level of the liver in the range of hyperinsulinemia. In protein-calorie–restricted rats, the pattern was different. Whereas hepatic glucose production in control rats was almost blocked by a submaximal insulin level, it remained high in protein-calorie–restricted rats. This demonstrates hepatic insulin resistance in these rats in the range of hyperinsulinemia. We have reported a similar conclusion in adult rats submitted to food restriction from the fetal stage.[19]

In conclusion, we have found that a 35% food restriction in the young rat induces a moderate impairment of glucose tolerance, whereas basal plasma glucose levels are still normal. This is probably related to the mild decrease of glucose-induced insulin secretion identified in vivo. Furthermore, these data provide direct evidence that food restriction determines changes in the effect of insulin on some target tissues, since insulin-mediated glucose uptake by peripheral tissues is indeed enhanced. Such a high sensitivity of peripheral tissue to insulin may help to limit the deterioration of glucose tolerance in the face of the attenuated insulin release. We also found that the 35% food restriction, when coupled with long-standing and overt protein deficiency, does not aggravate glucose tolerance. This is a paradoxical situation, in view of the severely blunted glucose-induced insulin secretion. Again, the important enhancement of insulin-mediated glucose uptake helps to maintain glucose tolerance in the normal range. However, it remains possible that long-lasting and/or earlier malnutrition facilitates development of diabetes in particular genetic backgrounds or after toxic or vital aggression, by increasing the vulnerability of pancreatic β cells and/or impairing their growth capacity.[7,37]

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