Adaptations in Glucose Homeostasis During Chronic Nutritional Deprivation in Rats: Hepatic Resistance to Both Insulin and Glucagon

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The role of glucagon in glucose homeostasis during chronic malnutrition was studied in weanling-littermate rats either fed ad libitum or restricted to 60% of ad libitum intake for 8 weeks. Fasting glucose and insulin levels were lower in malnourished rats, and their response to glucagon (0.02 mg/kg intravenous [IV]) after a 16-hour fast was significantly less than in control littermates for both glucose ($P = .039$) and insulin ($P = .008$). During euglycemic glucose clamp studies at identical plasma glucose (PG) levels, insulin suppression of hepatic glucose production (HGP) was impaired in malnourished rats, indicating insulin resistance (mean ± SE HGP: 48 ± 5 *v* 32 ± 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for controls, $P = .028$). Glucose disposal was not significantly different in the two groups. However, after IV glucagon, the increase in HGP was markedly impaired in malnourished rats ($P = .0004$), with the total amount of glucose produced by the liver over 15 minutes being 1,397 ± 114 μmol/kg as compared with 2,031 ± 118 in controls ($P = .0047$). The impaired response was not due to defective glycogenolysis, because the release of glucose from prelabeled glycogen in response to glucagon injection contributed only 6% to 8% of the overall increase in glucose output from the liver, and was not different in the two groups. Furthermore, liver glycogen stores were virtually exhausted after the 16-hour fast, without glucagon injection. Glucagon receptor affinity and number were not affected by malnutrition. It is concluded that (1) chronic malnutrition is associated with hepatic resistance to both insulin and glucagon, (2) the glucagon resistance is not due to impaired glycogenolysis, and (3) it is mediated by a postreceptor defect. It is proposed that resistance to glucagon stimulation of gluconeogenesis is an adaptive response that helps conserve protein at the expense of a lower PG level in chronic malnutrition. The risk of hypoglycemia is partially offset by the insulin deficiency and resistance, which are other elements of this adaptive response.

MALNUTRITION is characterized by decreased fasting blood glucose levels despite below-normal insulin levels.[1] The cause of the fasting hypoglycemia has not been identified, but certain peculiar metabolic features of malnutrition suggest that the hormonal regulation of glucose homeostasis may be altered. These features include (1) a diminished ability to mobilize glucose in response to glucagon,[2,3] (2) an absence of ketosis during fasting,[4] despite (3) a normal free fatty acid response to glucagon,[3] and (4) insulin resistance.[5-7] In experimental animals, impaired insulin receptor binding[8,9] and delayed recovery from insulin-induced hypoglycemia[8] have also been described, so there is little doubt that modulation of insulin sensitivity is an important part of the adaptation to chronic nutritional deprivation.[1,10,11] However, little is known regarding the effect of chronic malnutrition on glucagon secretion and action. A similar modulation of glucagon sensitivity at or beyond the glucagon receptor could be invoked to explain the blunted hyperglycemic response to glucagon, lack of ketosis, and fasting hypoglycemia seen in chronic malnutrition.

The role of glucagon in the metabolic adaptation to fasting and starvation has been studied extensively.[12] However, chronic malnutrition needs to be clearly distinguished from states of acute nutritional deprivation. Fasting and starvation are characterized by ketosis, a process attributable to the actions of glucagon.[13] Ketosis is striking in its absence in moderate to severe chronic malnutrition, as noted earlier. A more fundamental difference is that starvation is a severely catabolic state of acute weight loss, whereas chronic malnutrition is a state characterized by long-term weight maintenance at a less than ideal body weight in adults or a below normal rate of weight gain in children.[14,15] Thus, studies in acute nutritional deprivation are not relevant to states of chronic malnutrition, the most widespread form of nutritional deficiency in the world.[14] Similarly, studies of glucagon secretion and action in rat fetuses subjected to severe protein deficiency during intrauterine life[16] cannot be extrapolated to more moderate forms of chronic malnutrition later in life, because of the uniquely crucial role of glucagon resistance in the metabolism of the developing rat[17] and the critical effects of protein deficiency in arresting growth in the fetus.[18]

In the study reported here, a model of malnutrition was selected, based on balanced food restriction in the growing rat, that has been shown in earlier studies[8,19,20] to be characterized by continued growth. This pattern of anabolic rather than catabolic activity mimics the situation in states of chronic human malnutrition. The overall purpose of the study was to examine the role of glucagon in the homeostatic adaptation to chronic malnutrition in rats. This overall objective was approached through five specific objectives, as follows: (1) to study the hyperglycemic response to glucagon in the fasting state in chronically malnourished rats; (2) to study glucose turnover in malnutrition under controlled conditions of steady-state euglycemic hyperinsulinemia using the glucose clamp technique to correct for the fasting hypoglycemia and hypoinsulinemia of malnourished animals; (3) to determine the hepatic response to glucagon in malnutrition under these conditions of equivalent hyperinsulinemia; (4) to establish whether impaired glycogenolysis is responsible for the tendency to fasting hypoglycemia in chronic malnutrition;

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and (5) to identify any abnormalities in the hepatic glucagon receptor that might be responsible for glucagon resistance.

MATERIALS AND METHODS

Experimental Animals

Four-week-old male Sprague-Dawley rats were assigned in littermate pairs to either a control group fed ad libitum or an undernourished group that each day received 60% of the food intake of ad libitum–fed littermates for 8 weeks based on daily weighing of food. Details of the diet and pair-feeding design have been previously described.[8,19,20] On the day before study, food was provided to undernourished animals at 4 PM based on the overnight intake of each control littermate over the previous 24 hours, and food bins of the latter were emptied as soon as undernourished rats had consumed their portions (usually in an hour). Thus, the duration of fasting at the time all studies were performed the next morning (9 AM) was approximately 16 hours in both groups. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh Medical Center.

Experimental Protocols

Hyperglycemic effect of glucagon. Under pentobarbital anesthesia, the left femoral artery and vein were cannulated (n = 6 per group). Glucagon was then injected intravenously ([IV] 0.02 mg/kg body weight), and arterial plasma samples (1 mL/kg body weight) were taken at 0, 1, 2, 4, 8, 15, and 30 minutes for glucose and insulin assay. Blood samples were immediately centrifuged in a microcentrifuge, and red blood cells were returned to the animal after each withdrawal, suspended in normal saline.

Euglycemic clamp studies of glucose turnover. Under pentobarbital anesthesia, the left femoral artery and both femoral veins were cannulated (n = 5 per group). A primed-continuous infusion of 3-^{3}H-glucose was then started (20 μCi/kg bolus, 500 nCi/kg/min infusion) for 30 minutes. At the end of this equilibration period, a euglycemic glucose clamp study was commenced with constant insulin (4 mU/kg/min) and varying glucose infusion to maintain plasma glucose (PG) at approximately 5 mmol/L for the next 30 minutes. After a steady state was established, two samples were taken 5 minutes apart and glucagon was injected IV (0.02 mg/kg). Further samples (0.5 mL/kg body weight) were drawn at 0, 1, 2, 4, 8, and 15 minutes while the glucose infusion rate was held constant. The total volume of blood withdrawn did not exceed 7 mL/kg body weight in the entire study, and was accompanied by an equivalent volume of fluid replacement in the form of IV infusions of glucose and insulin. A total withdrawal volume of 7 mL/kg has been shown previously to be the upper limit for blood withdrawal without stress in repetitive sampling experiments.[21]

Glycogen mobilization by glucagon. Rats were fasted for 16 hours, and livers were removed (n = 5 per group) for determination of basal glycogen concentration. In a second experiment (n = 5 per group), glucagon was injected (0.02 mg/kg IV) and livers were removed after 60 minutes. Livers were snap-frozen in liquid nitrogen and stored at −70°C for subsequent glycogen analysis.

Release of 6-[^{3}H]glucose from prelabeled glycogen (n = 6 per group). Rats were fasted for 24 hours to deplete glycogen stores totally, and then injected intraperitoneally with 1 g glucose/kg body weight, which contained 6-[^{3}H]glucose (50 μCi/kg body weight) to label liver glycogen stores. Two basal samples were drawn 16 hours later and glucagon was injected IV (0.02 mg/kg body weight), and further samples were drawn at 1, 2, 4, 8, and 15 minutes to study the appearance of prelabeled glucose from the liver.

Hepatocyte glucagon receptors (n = 6 rats per group). On the day of study, rats were anesthetized with pentobarbital (50 mg/kg body weight intraperitoneally) and hepatocytes were isolated by in situ hepatic perfusion with collagenase.[22] Briefly, the abdomen was opened and ligatures were placed (but not tied) around the portal vein (excluding the bile duct), the celiac and superior mesenteric arteries, and the inferior vena cava (IVC) above the renal veins. A cannula was then introduced into the portal vein, the flow of buffer (Ca^{2+}-free Hanks buffer, pH 8.0, at 37°C and gassed with O_2:CO_2 95:5 vol/vol), was started, the IVC was transected below the renal veins, and arterial ligatures were tied. The chest was then quickly opened, and a second cannula was introduced through the right atrium and advanced into the IVC to be above the hepatic veins, and then tied in place. The IVC was then ligated to isolate the hepatic circuit. After a 10-minute perfusion at 20 mL/min to drain all blood from the circuit, the liver was perfused with collagenase (type IV, 50 mg in 100 mL Hanks at 10 mL/min). The liver was excised, minced with fine-point scissors in ice-cold Hanks, and filtered through several layers of gauze, and the crude preparation was centrifuged at 300 ×*g* for 4 minutes at 4°C. The supernatant was decanted, and the cells were washed three more times in ice-cold Hanks before a final wash in Krebs-Ringer bicarbonate buffer ([KRB] pH 7.5, gassed with O_2:CO_2 95:5 vol/vol). Cell viability (in all cases > 98%) was checked by trypan blue exclusion, and the cells were counted in a hemocytometer.

Glucagon binding studies were performed in KRB (pH 7.4, with aprotinin 1,000 kIU/mL) gassed with O_2:CO_2 (95:5 vol/vol). Hepatocytes (10^6 cells per tube) were incubated with glucagon (10^{-10} to 10^{-5}mol/L) and approximately 20 fmol ^{125}I-glucagon (receptor grade, New England Nuclear, Boston, MA) at 30°C for 8 minutes in a final volume of 0.5 mL.[23] The reaction was stopped by adding 1.5 mL ice-cold KRB, and the cells were separated in a microcentrifuge, washed again with ice-cold KRB, centrifuged again, and counted. Degradation of glucagon, measured after precipitation with 5% ice-cold trichloroacetic acid, averaged 10% (range, 7% to 15%). Nonspecific binding in 100 μmol/L glucagon averaged 8%.

Analytical Methods

PG, plasma insulin, and glucose specific activity (SA_g) were measured as described previously.[21] Glycogen in liver samples was measured after perchlorate digestion, using an enzymatic method of hydrolysis with amyloglucosidase.[24] A 5-g piece of liver was homogenized in 25 mL ice-cold perchloric acid (0.6N), and 0.2 mL homogenate was incubated with 0.1 mL 1-mol/L $KHCO_3$ and 2 mL amyloglucosidase (10 mg/mL in 0.2 mol/L acetate buffer, pH 4.8) for 2 hours at 40°C. The incubation was stopped with 1 mL ice-cold perchloric acid, tubes were centrifuged at 1,500 ×*g* for 15 minutes, and the supernatant was taken for determination of glucose after neutralization with $KHCO_3$. A sample blank was also included (supernatant from homogenate not subjected to enzymatic hydrolysis), and the glucose concentration was subtracted from the final result to determine glucose produced from enzymatic hydrolysis of glycogen. Glycogen content was calculated in micromoles of glucosyl units per gram weight of liver, using a molecular weight of 162 for anhydroglucose present in glycogen. A control sample of oyster glycogen (type II) was included in each run to monitor assay performance. The recovery of added glycogen to liver homogenate was 80% to 117% in standardization experiments.

Calculations

Steady-state (before glucagon). Rates of appearance (R_a) and disappearance (R_d) of glucose ($R_a = R_d$) were calculated from the mean SA_g in two samples taken 5 minutes apart, after maintaining

PG for 30 minutes at approximately 6 mmol/L during the clamp studies, from the following formula: R_a ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) = tracer infusion rate ($nCi \cdot kg^{-1} \cdot min^{-1}$)/$SA_g$ ($nCi/\mu mol$), and hepatic glucose production (HGP) = R_a − glucose infusion rate (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Non–steady-state. Polynomial regression was used to fit curves to the raw data of PG and SA_g in each rat after glucagon injection. The smoothed data were then used in the formulae reported by Steele et al to calculate R_a and R_d of glucose.[25] HGP was calculated as shown earlier. In all 10 rats, the polynomial regression (up to the third degree) resulted in excellent curve fits for PG and SA_g data both by multiple R^2 (>.95 in all cases) and by tests of goodness of fit for the reduction in sum of squares due to the last term in the regression equation. Studies with single bolus injections of 3-^{3}H-glucose (unpublished observations) have shown that the volume of distribution for glucose in malnourished and ad libitum–fed rats is similar (243 ± 19 and 246 ± 24 mL/kg, respectively).[25a] A volume of distribution of 245 mL/kg body weight was therefore used in the calculations. This is in agreement with the observations reported by Katz et al,[26] who found a volume of distribution of 20% to 30% (200 to 300 mL/kg) in their studies in rats.

Statistical Analysis

All analyses were performed on a VAX 6410 computer (Digital Equipment Corp, Maynard, MA) using the BMDP statistical analysis program.[27] Data involving serial measurements over time in the two groups (eg, PG, plasma insulin, HGP, and R_d after glucagon) were subjected to a repeated-measures ANOVA, designed to examine the effect of nutrition on the response parameter, using an omnibus test (Fisher) of the null hypothesis. When the sphericity assumption for repeated-measures design was not met, probabilities were corrected by the Greenhouse-Geisser method. Post-hoc comparisons between individual means at each time point in the two groups were made with the studentized range statistic. Means involving single estimations, as opposed to repeated measurements (eg, glycogen concentration and maximal increase in HGP over baseline), were compared between littermates in the two groups using the paired *t* test. Hepatocyte glucagon receptor binding data (mean of six experiments) were subjected to Scatchard analysis using the LIGAND program of Munson and Rodbard.[27a]

RESULTS

Growth Rate and Weight Gain

Initial body weights in the two groups were virtually identical (95 ± 4 and 96 ± 6 g, mean ± SE). However, thereafter weight gain was much lower in the malnourished group, so that they attained a mean body weight of 184 ± 12 g at the end of 4 weeks, as compared with control rats, who weighed 334 ± 16 g at the same point. On the day of study at 8 weeks, respective body weights were 288 ± 14 and 455 ± 23 g, respectively. Thus, all malnourished animals were in a state of chronic malnutrition with stable growth at a greatly reduced rate but without any growth arrest (Fig 1).

Glucose and Insulin Response to IV Glucagon

After 16 hours of fasting, PG was significantly lower in malnourished rats (5.6 ± 0.4 mmol/L) as compared with control rats (7.5 ± 0.7, $P < .01$), although significant hypoglycemia (PG < 3 mmol/L) was not seen in any of the animals (Fig 2). Fasting insulin levels were almost half those seen in the ad libitum–fed controls ($P < .01$). PG

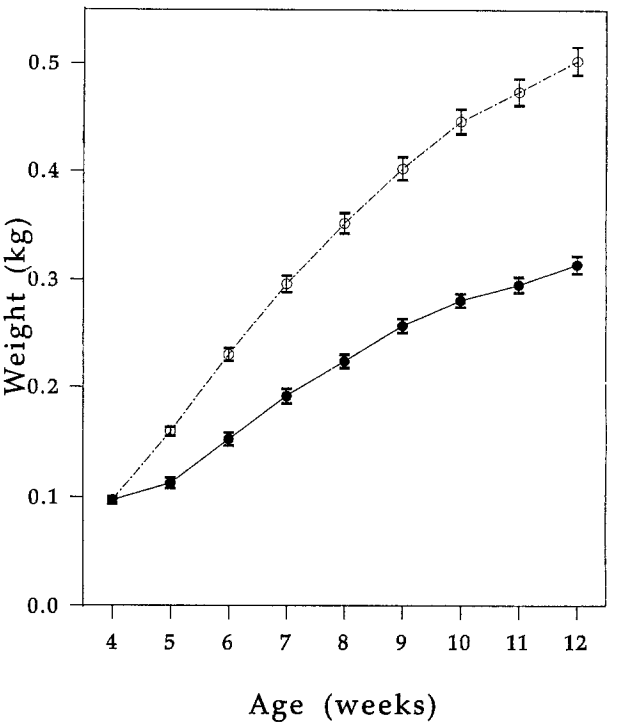

Fig 1. Rate of weight gain in paired littermate rats either allowed ad libitum access to food (○) or pair-fed at 60% of the food intake of ad libitum–fed control littermates (●).

increased in both groups immediately after glucagon administration. However, responses in the two groups were different ($P = .039$ by repeated-measures ANOVA), with PG levels remaining substantially lower in malnourished rats as compared with ad libitum–fed controls ($P < .05$ for each individual time point from 1 to 15 minutes). Insulin responses to glucagon were even more strikingly impaired in malnourished rats ($P = .0079$ by repeated-measures ANOVA). All values from 1 to 30 minutes were significantly decreased in malnourished rats ($P < .01$), indicating that the effect of glucagon on β-cell secretion was substantially impaired.

Glucose Clamp Studies

Since the baseline from which the effect of glucagon was assessed in the above study was significantly different for both glucose and insulin, clamp studies were performed to attain similar plasma steady-state glucose and insulin levels in both groups (Fig 3A and B). Before glucagon injection, there was a striking difference between the two groups in the suppression of HGP by insulin, which was nearly 50% higher in the malnourished group (Fig 3C) than in ad libitum–fed controls ($P = .028$) at the same PG level, indicating a marked degree of insulin resistance. However, the rate of glucose disposal (R_d, Fig 3D) was not significantly different in the two groups ($P = .173$).

After glucagon injection, PG increased immediately in both groups, since the glucose infusion rate was not altered during this phase of the clamp study (Fig 4). Even though the difference in baseline PG had been corrected, the

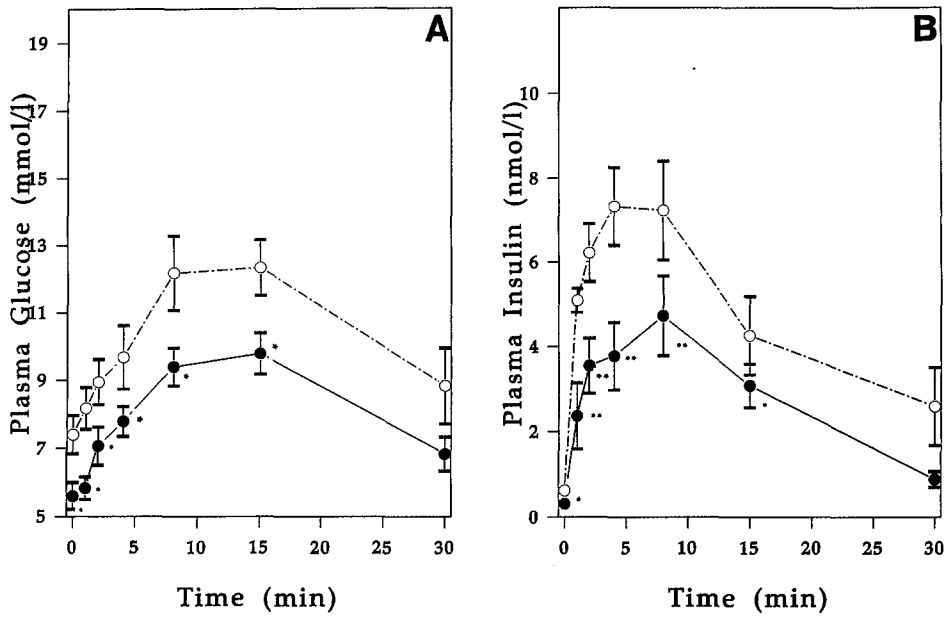

Fig 2. Effect of IV glucagon (0.02 mg/kg body weight) on PG (A) and plasma insulin (B) after a 16-hour fast in ad libitum–fed controls (○) and malnourished littermates (●) (n = 6 per group). *P < .05, **P < .01: v controls after repeated-measures ANOVA.

hyperglycemic response to glucagon was blunted in malnourished rats. The increase in HGP was markedly impaired in the malnourished group as compared with ad libitum–fed controls (P = .0004 by repeated-measures ANOVA). Absolute levels of HGP were lower in malnourished rats throughout the 15-minute period of observation. The effect of glucagon also appeared to be less sustained in the malnourished group. The overall increase in production of glucose over 15 minutes in the malnourished group was only 1,397 ± 114 μmol/kg, as compared with 2,031 ± 118 in ad libitum–fed littermates (P = .0047).

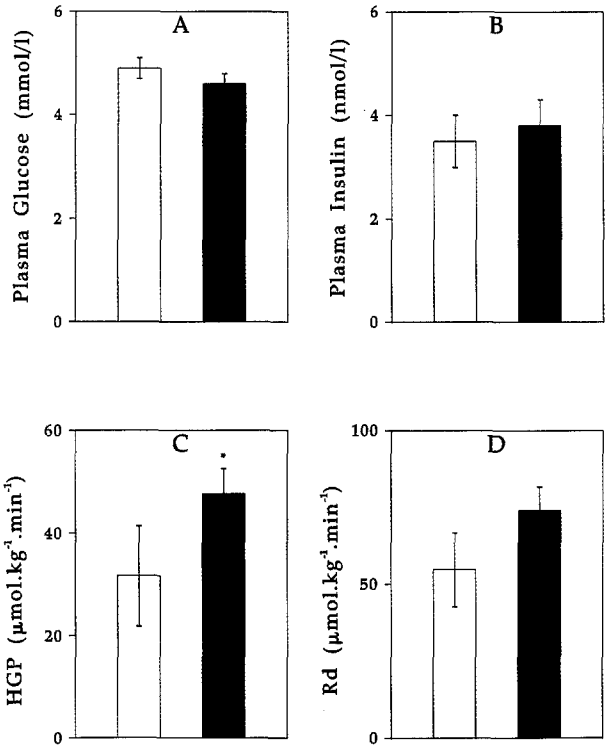

Fig 3. Results of glucose clamp studies in ad libitum–fed controls (□) and malnourished littermates (■) showing that (A) steady-state glucose and (B) insulin levels were comparable (n = 5 each). (C) HGP was significantly greater in malnourished rats (*P = .028), but (D) peripheral glucose R_d was not (P = .173).

Hepatic Glycogen Mobilization

Liver glycogen content after a 16-hour fast was significantly lower in control rats (33.1 ± 6.9 μmol glucosyl units/g wet weight, n = 5) as compared with malnourished rats (56.2 ± 4.8, n = 4, P = .0287). The discrepancy in numbers was caused by the death of one malnourished rat in this experiment. In the livers of rats killed after injection of glucagon, even this small amount of glycogen was virtually completely mobilized (Fig 5).

Release of Glucose From Prelabeled Glycogen

The basal plasma SA of 6-[^{3}H]glucose in the two groups before injection of glucagon was not different in control and malnourished groups (241 ± 39 v 338 ± 66 nCi/mmol, respectively, P > .1). This indicates that the labeling of glycogen in the two groups was similar because basal plasma 6-[^{3}H]glucose SA would be a direct reflection of the SA of hepatic 6-[^{3}H]glycogen, with prelabeled hepatic glycogen being the only source of 6-[^{3}H]glucose in plasma, 16 hours after intraperitoneal injection of labeled glucose. After glucagon administration, similar amounts of radioactivity were released from glycogen prelabeled with 6-[^{3}H]glucose: tritium radioactivity in plasma increased by 215 ± 82 dpm/mL in controls and by 211 ± 64 in malnourished rats (P > .5). This represented an increase of 6.6% ± 1.8% (controls) and 8.7% ± 1.9% (malnourished, P > .5) over the basal tritiated glucose concentration before glucagon administration. On the other hand, the overall 6-[^{3}H]glucose SA in plasma declined by approximately 45% in controls and by 40% in malnourished rats. This indicates that the major contribution to the post-glucagon increase in glucose in plasma came from nonlabeled sources, and that glycogen breakdown contributed little to the hyperglycemic

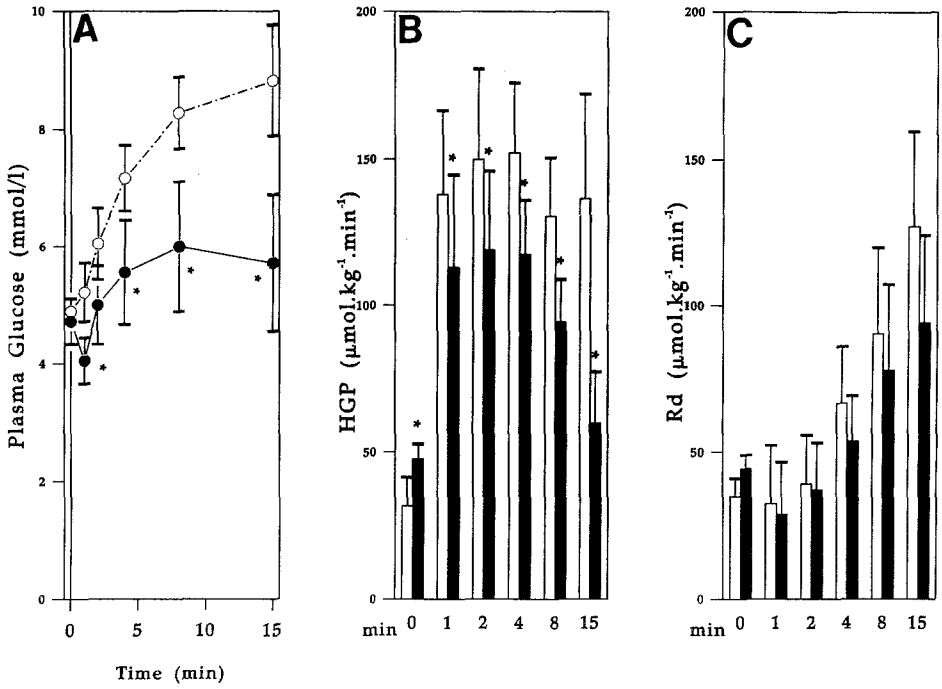

Fig 4. Effect of glucagon (0.02 mg/kg IV) on glucose levels (A), HGP (B), and R_d (C) during glucose clamp studies in ad libitum–fed controls (○ and □) and malnourished littermates (● and ■). *$P < .05$ *v* controls after repeated-measures ANOVA (n = 5 each).

response in either group after the 16-hour fast to which the rats had been subjected after labeling the glycogen.

Hepatocyte Glucagon Receptors

Binding displacement curves of radiolabeled glucagon on isolated hepatocytes were virtually superimposable in control and malnourished littermate rats (Fig 6). Scatchard analysis of the data showed that binding affinity and receptor concentrations were identical in both groups (Table 1).

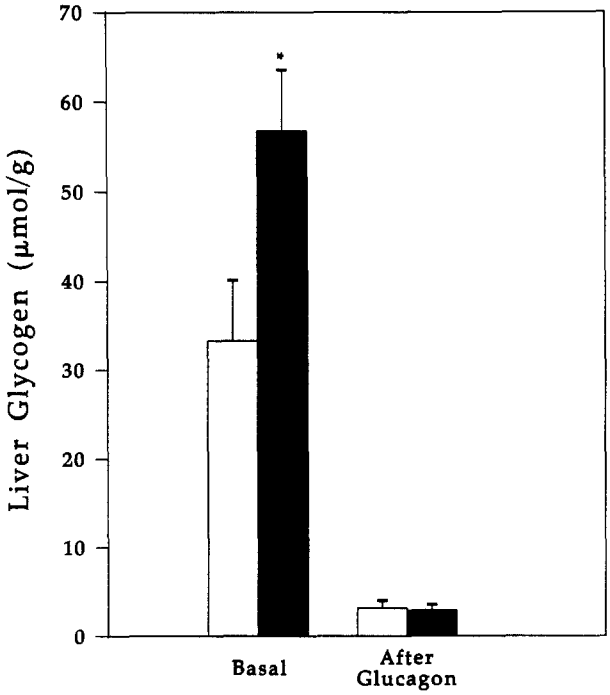

Fig 5. Hepatic glycogen levels after a 16-hour fast in ad libitum–fed controls (□) and malnourished littermates (■). *$P = .0287$ by *t* test.

DISCUSSION

The results of this study show that the adaptive response to chronic nutritional deprivation is characterized by two important alterations in the hormonal regulation of fasting glucose homeostasis: resistance to the suppressive effect of insulin on glucose production, and resistance to the glucose-mobilizing action of glucagon on the liver. The first of these, insulin resistance, is in accordance with an earlier study showing that chronic malnutrition is accompanied by an alteration of the insulin receptor in hepatocytes, resulting in reduced affinity of binding to insulin.[8] This has also been shown to occur in red blood cells of malnourished animals.[9] The present study establishes the physiologic significance of these receptor changes, since insulin resistance has been shown in this study to be present in chronically malnourished rats using the glucose clamp technique: HGP was 50% greater in malnourished rats at the same level of insulinemia. In previous studies in both experimental and human malnutrition, the existence of insulin resistance has been inferred from responses during insulin tolerance tests.[5-8] Unfortunately, such comparisons are not reliable because of the differences in baseline caused by the relative fasting hypoglycemia and hypoinsulinemia of malnutrition. The present study thus represents the first confirmation that chronic malnutrition (as differentiated from catabolic states of acute nutritional deprivation, such as fasting, and starvation) is an insulin-resistant state.

The results of this study must be contrasted with earlier studies in other forms of chronic nutritional deprivation. In rats subjected to severe protein restriction, insulin sensitivity is increased: glucose levels decrease more rapidly in an insulin tolerance test, and recovery from hypoglycemia is

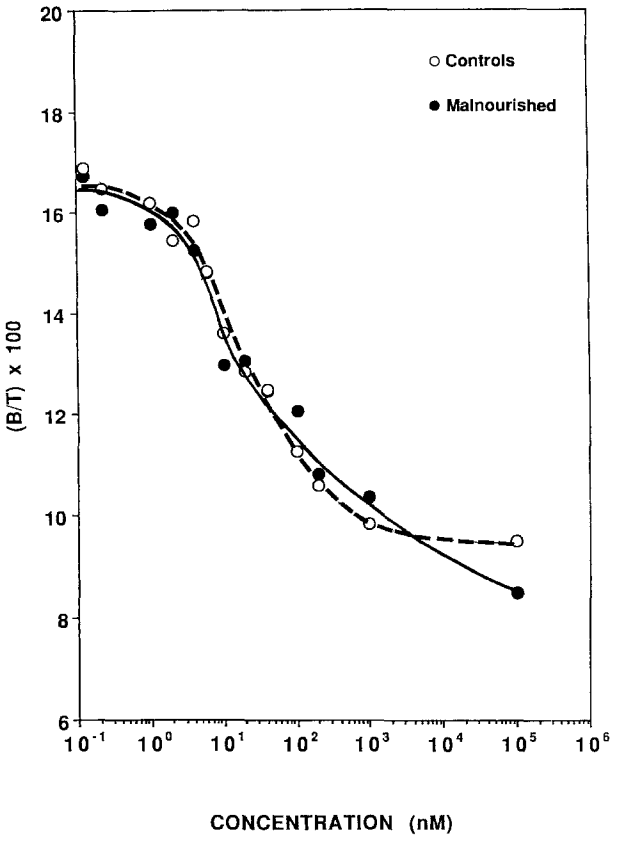

Fig 6. Glucagon binding and displacement in hepatocytes isolated from ad libitum–fed (○) and malnourished (●) littermate rats. Data represent the mean of binding ratios in six rats. Curves were not significantly different in a repeated-measures ANOVA (P = .947).

delayed.[28,29] The severely protein-restricted rat is characterized by complete cessation of growth, in sharp contrast to the continuing weight gain seen in the rats studied here. The difference in metabolic activity in the two circumstances is reason enough to doubt that the insulin responsiveness of the former will also be seen in the latter. In fact, insulin resistance, not increased insulin sensitivity, is a feature of the chronically malnourished model used here, as shown indirectly in an earlier study using insulin tolerance tests[8] and more conclusively in this report using clamp studies. Similarly, the insulin resistance described in cancer-associated cachexia[30] is not a valid basis for extrapolation to the adaptation to chronic malnutrition itself, because it is probably a stress-related phenomenon. Previously, it was shown that corticosterone levels were not elevated in this model,[8] so that the insulin resistance is clearly not due to stress.

Table 1. Scatchard Analysis (one-site model analyzed by LIGAND program) of Binding Displacement Data for Glucagon in Hepatocytes Isolated From Ad Libitum–Fed and Malnourished Littermate Rats (n = 6 each)

	Ad Libitum–Fed		Malnourished	
	Mean	95% CI	Mean	95% CI
Receptor affinity ($10^7 \cdot M^{-1}$)	5.62	1.38-9.87	6.50	4.4-8.6
Receptor concentration (10^{-9} M)	1.58	0.42-2.75	1.39	0.94-1.83

Abbreviation: CI, confidence interval.

The second finding of major interest is that chronically malnourished rats are glucagon-resistant in the liver. The β-cell resistance to glucagon may be either a part of this or a manifestation of the overall insensitivity of the malnourished β cell to stimulation.[1] In contrast, the glucagon resistance of the liver appears to be a primary defect. HGP does not increase to the same extent after glucagon in malnourished animals under controlled conditions of euglycemic hyperinsulinemia during a glucose clamp study, even though it is not fully suppressed by insulin in malnourished animals (ie, in the face of insulin resistance). The impaired hyperglycemic response to glucagon is not due to either defective release or inadequate reserves of glycogen in the liver. First, glycogen levels were extremely low in livers from both groups after 16 hours' fasting, so that glucose release from glycogen could not have contributed to any great extent to HGP. The higher basal glycogen level in malnourished rats may reflect either glucagon resistance to endogenous glucagon or more simply an inability to match exactly the duration of fasting in the two groups because of differences in feeding behavior in the two groups (ie, meal-driven in malnourished rats *v* recurrent nibbling in ad libitum–fed animals). Second, the contribution of glycogen to the response was small, as shown in the experiment on mobilization of prelabeled glucose: it increased by only 6% to 8% even though HGP increased 100%, and glucose SA in plasma declined. Third, the increase in prelabeled glucose after glucagon was not different in the two groups. Fourth, exogenous glucagon administration resulted in virtually complete mobilization of the small fraction of glycogen remaining in the liver at the end of the 16-hour fast: measured values of 33.1 ± 6.9 and 56 ± 4.8 μmol glucosyl units/g in this study versus 338 ± 17 in livers of nonfasted rats.[31] Fifth, the time course of development of hypoglycemia (ie, after approximately 12 hours of fasting) suggests that it occurs when glycogen depletion is complete. For all these reasons, it is logical to conclude that the hepatic glucagon resistance shown here is not due to impaired glycogenolysis, but rather to defective gluconeogenesis. It is possible that glycogenolysis after glucagon may also be impaired in fed rats, but this does not appear to be relevant to the fasting hypoglycemia seen in malnourished animals.

A deficiency in the glucagon effect in the liver can therefore be implicated as the probable cause of the decreased glucose level seen after 16 to 18 hours of fasting in malnourished rats, rather than increased insulin secretion or increased insulin sensitivity. It is likely that the decreased fasting glucose after a 16-hour fast is due to defective hepatic gluconeogenesis, since this is the predominant means of sustaining HGP in rats after this duration of fasting.[32] However, this must remain speculative at the present time. The exact role of the hepatic gluconeogenetic response to glucagon in the hypoglycemia of malnutrition, as well as the pathogenetic mechanisms underlying it, can

only be determined by direct measurement of the rate of turnover of gluconeogenic substrates in malnourished rats and of intracellular metabolism in hepatocytes.

In broad terms, hepatic control of glucose production appears to be altered in malnutrition to make it less responsive to both insulin and glucagon by different mechanisms. The modulation of insulin responsiveness appears to occur at the level of the receptor,[8,9] although a contribution from postreceptor processes cannot be ruled out.[20] In contrast, hepatocyte glucagon receptors appear to be unaffected by chronic malnutrition. This would suggest that the modulation of glucagon responsiveness occurs through postreceptor events. The exact step at which this might occur is not known. One study in malnourished subjects with diabetes concluded that the adenylyl cyclase system may be affected,[33] based on observations of defective lipolytic responses to adrenergic stimulation. The relevance of this finding is questionable, because lipolytic responses to glucagon are normal in malnutrition.[3]

The demonstration in this study of acquired hepatic glucagon resistance in chronic malnutrition may be particularly relevant in the context of the pathophysiology of chronic nutritional deprivation in humans, for several reasons. First, protein-energy malnutrition (PEM) is characterized by hypoglycemia and insulin resistance, as is the animal model used here.[1,5-7] Second, the glycemic response to glucagon in human malnutrition is also impaired.[2,3] Third, ketosis is notable for its absence during fasting in PEM.[4] It is reasonable to speculate that glucagon resistance may also exist in human chronic malnutrition, since glucagon is an important modulator of both the response to hypoglycemia and the synthesis of ketones. Finally, the conclusion that glucagon resistance is manifest principally as inadequate gluconeogenesis rather than glycogenolysis is in accordance with metabolic studies in PEM. Glycogenolysis accounts for only 7% of total glucose production after 20 hours of fasting in infants regardless of whether they have PEM,[4] whereas the contribution of gluconeogenesis from amino acids is reduced from 21% to only 9% of total energy production in the presence of PEM.[34] This is accompanied by a 50% reduction in utilization of protein for energy (reflected in urinary nitrogen excretion), which is out of proportion to the reduction in lean body mass (estimated from urinary creatinine). Therefore, the predominant source of substrate for gluconeogenesis during fasting in malnourished infants is the recycling of intermediary products of glycolysis.[33] Since glucagon is known to play a major role in diverting these products to gluconeogenesis at several points in the Embden-Meyerhof pathway,[35] glucagon resistance is a logical explanation for the inadequacy in gluconeogenesis and the impaired glycemic response to glucagon that occur in human chronic nutritional deprivation.

The phenomenon of glucagon resistance would also explain another puzzling feature of PEM—the fact that ketosis is absent. Kerr et al[4] found that basal energy requirements in infants with PEM were predominantly met by fat oxidation (>94% of energy utilization). Despite this degree of fat oxidation, excretion rates of β-hydroxybutyrate averaged 0.3 μmol/kg/min, as compared with 2.7 in the same subjects after nutritional rehabilitation. When viewed in the context of the insulin deficiency and insulin resistance of malnutrition, this inability to generate ketones is difficult to explain. Glucagon resistance could well be the explanation, indicating once again that the present study is likely to be relevant to understanding the hormonal and metabolic adaptations to chronic nutritional deprivation in humans.

With regard to the five specific objectives that prompted this study, it can be concluded that the decreased fasting PG and plasma insulin of chronic malnutrition are associated with (1) a reduction in both the hyperglycemic and insulin-secretory responses to IV glucagon, (2) significant hepatic resistance to the actions of insulin, (3) a marked impairment in the stimulatory effect of glucagon on HGP, (4) no change in glucagon receptor number and affinity, and (5) near-complete mobilization of glycogen, confirming that impaired glycogenolysis is not the basis for the glucagon resistance. In summary, chronic malnutrition in rats induces resistance to the action of glucagon on the liver, resulting in defective production of glucose, and the impaired glycemic response to glucagon is most likely due to impaired gluconeogenesis. On the basis of these findings, the metabolic paradox of the coexistence of hypoglycemia with insulinopenia and insulin resistance in chronic malnutrition can be explained and incorporated into the framework of a coherent hypothesis regarding the adaptive homeostatic response to chronic nutritional deprivation. Glucagon resistance leading to impaired gluconeogenesis could be responsible for protein conservation in chronic nutritional deprivation. The fasting hypoglycemia that would inevitably develop under such circumstances when glycogen stores are exhausted is partially offset by the insulin deficiency and resistance that accompany it. This adaptive process may be crucial to survival during periods of chronic nutritional deprivation, because it results in selective sparing of tissue proteins while protecting the organism from profound hypoglycemia.

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