Antecedent protein restriction exacerbates development of impaired insulin action after high-fat feeding

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Holness, Mark J., and Mary C. Sugden. Antecedent protein restriction exacerbates development of impaired insulin action after high-fat feeding. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E85–E93, 1999.—The study investigated whether a persistent impairment of insulin secretion resulting from mild protein restriction predisposes to loss of glucoregulatory control and impaired insulin action after the subsequent imposition of the diabetogenic challenge of high-fat feeding. Offspring of dams provided with either control (20% protein) diet (C) or an isocaloric restricted (8%) protein diet (PR) were weaned onto the maintenance diet with which their mothers had been provided. At 20 wk of age, protein restriction enhanced glucose tolerance despite impaired insulin secretion and an augmented and sensitized lipolytic response to norepinephrine in adipocytes. C and PR rats were then transferred to a high-fat diet (HF, 19% protein, 22% lipid, 34% carbohydrate) and sampled after 8 wk. These groups are termed C-HF and PR-HF. Glucose tolerance was impaired in PR-HF, but not C-HF, rats. Insulin-stimulated glucose disposal rates were significantly lower (by 30%; P < 0.01) in the PR-HF group than in the C-HF group, and a specific impairment of antilipolytic response of insulin was unmasked in adipocytes from PR-HF, but not C-HF, rats. The study demonstrates that antecedent protein restriction accelerates and augments the development of impaired glucoregulation and insulin resistance after high-fat feeding.

Insulin resistance; euglycemic-hyperinsulinemic clamps; adipocytes

PROTEIN–CALORIE MALNUTRITION impairs the insulin secretory response of the pancreatic β-cell (1, 4, 16, 19, 20, 26, 27), and when imposed during pregnancy, mild protein restriction, even in the absence of calorie restriction, elicits a profound impairment in the structural and functional development of the fetal endocrine pancreas (3). There has been considerable research interest in the proposition that changes to nutrition during pregnancy may influence the normal metabolic control mechanisms in the offspring. Deprivation of specific macronutrients, in particular protein, has been suggested to have effects on glucose metabolism in later life, particularly on the secretion of insulin. The continued maintenance of offspring of dams subjected to moderate protein restriction during pregnancy on a protein-restricted diet after birth leads to impaired glucose-stimulated insulin secretion in adulthood (3, 9). Paradoxically, the blunted response of insulin secretion to glucose challenge in rats maintained on low-protein diets is frequently associated with normal or enhanced glucose tolerance and insulin action (5, 10, 16, 19, 20, 28). For example, the imposition of protein-calorie restriction (35% restriction, 5% protein) on 4-wk-old female rats for 4 wk leads to greatly impaired glucose-stimulated insulin secretion but in association with enhanced insulin-mediated glucose uptake (20). In male rats, provision of a 5% protein diet from 4 wk to 15 wk of age also results in slightly enhanced tolerance to intravenous glucose, whereas the insulin response to glucose is severely blunted (19). Furthermore, hypoglycemia induced by intravenous insulin is more sustained in protein-malnourished rats compared with rats fed a normal protein diet (19), suggesting increased whole body insulin sensitivity. In a separate study, normal glucose tolerance (despite a failure to release insulin after intravenous glucose) has been suggested to indicate increased sensitivity to insulin and/or increased peripheral glucose utilization in rats provided with a 6% protein diet for 14 wk (16). Providing 4-wk-old rats with an isocaloric 5% protein diet for 4 wk, while not affecting energy intake, enhances tolerance to an intravenous glucose challenge and improves the actions of insulin to stimulate peripheral glucose uptake and suppress hepatic glucose output, whereas glucose-stimulated insulin secretion remains normal (5). Thus, in general, the evidence to date does not support the hypothesis that protein restriction necessarily elicits impaired glucoregulatory control, despite an impaired insulin secretory capacity, and an enhanced action of insulin action may help to limit the deterioration of glucose tolerance in the face of impaired insulin secretion (see Ref. 20).

The present study examined whether the persistent impairment in the insulin secretory response of the pancreas introduced as a consequence of protein restriction predisposes to altered insulin action, either in vivo or in vitro, and/or influences the development of peripheral insulin resistance through compromising the response to the potentially diabetogenic challenge of a high-fat diet that in rats leads to the development of whole body insulin resistance (23).

METHODS

Kits for determination of plasma insulin concentrations were from Phadeseph Pharmacia, Uppsala, Sweden. Collagenase was from Lorne Laboratories, Twyford, Berkshire, UK. Human Actrapid insulin was from Novo Nordisk, Bagsvaerd, Denmark. Kits for determination of plasma nonesterified fatty acid (NEFA) concentrations were supplied by Alpha Laboratories, Eastleigh, Hants, UK. Glycerol kinase and
glycerol-3-phosphate dehydrogenase were from Boehringer Mannheim, Lewes, East Sussex, UK. Other biochemicals and chemicals, including glucose assay kits, were from Boehringer Mannheim or from Sigma, Poole, Dorset, U.K. Female Wistar rats were from Charles River, Margate, Kent, U.K.

The composition of the diets is presented in Table 1. Isocaloric control and protein-restricted diets were prepared (pellet form) by Hope Farms BV, Woerden, Netherlands. The control diet contained 20% protein [casein (22 g/100 g diet; 0.88 g protein/g) supplemented with DL-methionine (0.2 g/100 g diet)], 63.15% carbohydrate [cereal (55.15 g/100 g diet) and corn starch (8 g/100 g diet)], 4.3% lipid (soybean oil) by weight, and other dietary components as specified in Table 1 (see also Ref. 12). The isocaloric protein-restricted diet contained 8% protein [casein (9 g/100 g diet; 0.88 g protein/g) supplemented with DL-methionine (0.08 g/100 g diet)], 76.17% carbohydrate [cereal (66.17 g/100 g diet) and corn starch (8 g/100 g diet)], and 4.3% lipid (soybean oil) by weight. Isocaloricity was maintained by increasing the carbohydrate content of the protein-restricted diet to 76% (Table 1; see also Refs. 12 and 24). The high-energy, high saturated fat diet, henceforth referred to as high-fat diet, contained 19% protein [casein (20.6 g/100 g diet; 0.88 g protein/g) supplemented with DL-methionine (0.40 g/100 g diet)] but 34% carbohydrate (maize starch) and 22% lipid [lard as the major source of lipid (20.1 g/100 g diet), together with corn oil (1.9 g/100 g diet) to prevent essential fatty acid deficiency] by weight (Table 1; see also Ref. 7). The lipid component of the high-fat diet comprised 16% saturated fatty acids (mainly stearic), 16% monounsaturated fatty acids (mainly oleic), and 7% polyunsaturated fatty acids (mainly linoleic) by energy. The high-fat diet was prepared at 3-day intervals with components supplied by Special Diet Services (Witham, Essex, UK), with the exception of the saturated fat component (lard), which was purchased locally.

Female Wistar rats were housed in a temperature-controlled room (21 ± 2°C) on a standard 12:12-h light-dark cycle (light from 8:00 AM). Rats were time mated by the appearance of sperm plugs (day 0 of pregnancy) (15), immediately randomly assigned to either the control or protein-restricted diets, and maintained on these diets throughout pregnancy and lactation. The provision of the protein-restricted diet did not influence maternal food intake or body weight gain during pregnancy (24), and litters of normal numerical size were produced. Mean litter sizes were 12 ± 1 (n = 22) and 12 ± 1 (n = 23) in control and protein-restricted groups, respectively. Any litters containing <10 pups or >15 pups were excluded from the study. Preliminary studies indicated that small litters (<10 pups) were associated with accelerated neonatal growth. It is unclear whether pups in the larger litters grew slowly during suckling because of greater competition for nursing or whether pups in litters of <10 pups gain weight during suckling more rapidly because of less competition for nursing.

Table 1. Composition of diets

<table>
<thead>
<tr>
<th>Dietary Composition, g/100 g</th>
<th>Control Diet</th>
<th>Protein-Restricted Diet</th>
<th>High-Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>63.15</td>
<td>76.17</td>
<td>34.00</td>
</tr>
<tr>
<td>Casein, 88% protein</td>
<td>22.00</td>
<td>9.00</td>
<td>20.60</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.20</td>
<td>0.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Fat</td>
<td>4.30</td>
<td>4.30</td>
<td>22.00</td>
</tr>
<tr>
<td>Vitamin and mineral mix</td>
<td>5.05</td>
<td>5.45</td>
<td>5.01</td>
</tr>
<tr>
<td>Nondigestible residue</td>
<td>5.00</td>
<td>5.00</td>
<td>17.90</td>
</tr>
<tr>
<td>Energy content, kcal/100 g diet</td>
<td>367.4</td>
<td>365.3</td>
<td>419.0</td>
</tr>
</tbody>
</table>

At 26 days after birth, sexes were separated. The female offspring were then weaned onto the maintenance diet (control or protein restricted) with which their mothers had been provided and maintained on this diet until ~20 wk of age. These are termed the C and PR groups, respectively. Rats from each of the groups were then transferred to a high-fat diet (HF) and studied after a further 8 wk, except for the adipocyte studies that were undertaken after 4 wk. These rats are termed the C-HF and PR-HF groups.

For intravenous glucose tolerance tests, each rat was fitted with a chronic indwelling jugular cannula under Hypnorm [fentanyl citrate (0.315 mg/ml)-fluanisone (10 mg/ml); 1 ml/kg ip] and diazepam (5 mg/ml; 1 ml/kg ip) anesthesia at 5–7 days before study (see Refs. 10, 12). Food was removed at the end of the dark (feeding) phase at 8:00 AM, and intravenous glucose tolerance tests (0.5 g glucose/kg body wt; 150 µl/100 g body wt) were performed in awake, unstressed rats at 6 h after food withdrawal (see Refs. 10, 12). Glucose was injected and blood samples (100 µl) were withdrawn at intervals from the indwelling cannula, which was flushed with saline (2 × 250 µl) after the injection of glucose to remove residual glucose. Samples of whole blood (150 µl) were deproteinized with ZnSO4-Ba(OH)2 and centrifuged (10,000 g) at 4°C, and the supernatant was retained for subsequent assay of blood glucose. The remaining sample was immediately centrifuged (10,000 g) at 4°C, and plasma was stored at −20°C until assayed for insulin. The insulin and glucose responses during the intravenous glucose tolerance tests were calculated as the incremental areas under the plasma insulin and blood glucose curves, respectively, from data obtained during the 30-min period after the glucose injection. The rate of glucose disappearance (K) was calculated from the slope of the regression line obtained with the log-transformed blood
glucose values between 2 and 15 min after glucose administration and expressed as percent per minute.

For euglycemic-hyperinsulinemic clamp studies, each rat was fitted with two chronic indwelling cannulas. One cannula was placed in the right jugular vein, and the other cannula was placed in the left jugular vein (for infusion and sampling, respectively) under Hypnorm [fentanyl citrate (0.315 mg/ml)-fluanisone (10 mg/ml); 1 ml/kg ip] and diazepam (5 mg/ml; 1 ml/kg ip) anesthesia. The infusion studies were conducted at 5–7 days after cannulation. Food was withdrawn at the end of the dark (feeding) phase at 8:00 AM, and rats were studied 6 h after food withdrawal. Whole body glucose kinetics were estimated in awake, unstressed, freely moving rats in the basal (postabsorptive) state and during euglycemic hyperinsulinemia by use of primed (0.5 µCi) continuous (0.2 µCi·min⁻¹·rat⁻¹) intravenous infusion of [3-³H]glucose as described in Refs. 12 and 24. A steady state of glucose specific activity in the basal state was achieved by 60 min.

### Table 2. General characteristics of C and PR rats before and after transfer to high-fat diet for 8 wk

<table>
<thead>
<tr>
<th></th>
<th>C-HF</th>
<th>PR-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma insulin, µU/ml</td>
<td>12 ± 1 (10)</td>
<td>10 ± 1 (12)</td>
</tr>
<tr>
<td>Blood glucose, mM</td>
<td>4.2 ± 0.1 (11)</td>
<td>4.5 ± 0.2 (12)</td>
</tr>
<tr>
<td>Plasma insulin-to-blood glucose ratio, µU/µmol</td>
<td>2.9 ± 0.3 (11)</td>
<td>2.3 ± 0.3 (12)</td>
</tr>
<tr>
<td>Rd, mg glucose·min⁻¹·rat⁻¹</td>
<td>3.1 ± 0.5 (5)</td>
<td>4.1 ± 0.3 (6)</td>
</tr>
<tr>
<td>GMR, ml·min⁻¹·kg⁻¹</td>
<td>19.3 ± 1.9 (6)</td>
<td>16.4 ± 1.1 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of rats is given in parentheses. Rats were sampled in postabsorptive state. C, control; PR, protein restricted. Rd, whole body glucose disposal rate; GMR, glucose metabolic clearance. There were no statistically significant effects of protein restriction, nor were there any statistically significant effects of high-fat (HF) feeding.

![Fig. 2. Blood glucose (A, B) and plasma insulin (C, D) during an intravenous glucose tolerance test in C rats (○) and PR rats (●) before (A, C) and after (B, D) high-fat feeding. Further details are given in METHODS. Values are means ± SE for 5–9 rats in each group. *Statistically significant effects of protein restriction, P < 0.05.](http://ajpendo.physiology.org/DownloadedFrom)
samples were obtained at 60, 75, and 90 min after the commencement of the tracer infusion for determination of basal glucose specific activity. From 90 min, animals were infused with insulin (human Actrapid) at a fixed dose of 4 mU·kg⁻¹·min⁻¹ while blood glucose was maintained at euglycemia for a further 120 min with a variable rate of 30% glucose infusion, which was initiated at 1 min after the start of insulin infusion. Blood was sampled from the right jugular vein at 5- to 10-min intervals, and blood glucose concentrations were determined. Adjustments in the exogenous glucose infusion rate (GIR) were made to maintain glucose concentrations constant. The [3-H]glucose infusion was continued for a further 120 min. Steady-state conditions were achieved after 90 min, after which three blood samples (100 µl) were taken at 15-min intervals (90, 105, and 120 min) for measurement of glucose specific activity. Whole body glucose disposal rates (Rd) were calculated as described previously (12). Glucose metabolic clearance rate (GMR) was calculated as R_d divided by the blood glucose concentration.

For measurements of lipolytic activity, samples of parametrial adipose tissue were removed from rats anesthetized with pentobarbitone (60 mg/kg body wt), and adipocytes were prepared by collagenase digestion as described in Ref. 6. There was no evidence for adipocyte cell breakage during preparation. Adipocyte cell diameter was measured with an Olympus microscope with an OSM-1 eyepiece micrometer. Cell diameters before high-fat feeding for C and PR groups were 29.5 ± 2.1 µm (n = 8) and 26.8 ± 1.9 µm (n = 10), respectively. Cell diameters after high-fat feeding for C and PR groups were 24.9 ± 0.3 µm (n = 9) and 23.3 ± 0.3 µm (n = 8), respectively. Adipocytes were resuspended to 20% lipocrit, and aliquots (100 µl) of adipocyte suspension were added to round-bottomed polypropylene tubes containing 0.7 ml of HEPES-buffered Krebs-Henseleit saline (in mM: 10 HEPES, 1.25 MgSO₄, 2.5 CaCl₂, 140 NaCl, 2 K₂HPO₄, 0.5 KH₂PO₄, pH 7.4) containing 2% (wt/vol) BSA and 2 mg/ml glucose. All subsequent procedures were performed at 37°C. Tubes were preincubated for 10 min (200 oscillations/min) before the addition of norepinephrine and insulin, at the concentrations indicated, to a final volume of 1 ml. Samples were incubated for 30 min (200 oscillations/min), at the end of which period the cell suspension was centrifuged (1,200 g for 1 min). The infranatant was heated at 100°C for 20 min and then centrifuged at 10,000 g for 10 min. The supernatant was assayed spectrophotometrically for glycerol.

Plasma insulin concentrations were measured by radioimmunoassay with a kit from Phadeseph Pharmacia. Blood glucose concentrations during the clamp were determined with a glucose analyzer (YSI, Yellow Springs, OH). In other studies, blood glucose concentrations were measured in deproteinized samples by the glucose oxidase method with kits supplied by Boehringer Mannheim. Plasma NEFA concentrations were measured with a spectrophotometric method with a WAKO c-test kit (supplied by Alpha Laboratories) according to the instructions of the manufacturer.

Results are expressed as means ± SE. Statistical comparisons were made with StatView (Abacus Concepts, Berkeley, CA). Multiple comparisons were made by analysis of variance (ANOVA) and individual comparisons by Fisher’s post hoc tests.

RESULTS

Exposure to the protein-restricted diet during fetal life and suckling resulted in a 33% reduction (P < 0.001) in body weights of the female offspring at weaning (Fig. 1; see also Ref. 9). Body weights of the PR rats were consistently lower than C rats from weaning to adulthood and, at 20 wk of age, were ~14% lower than control. Weight differences between the C and PR groups were maintained during high-fat feeding (Fig. 1), and body weight gains after transfer to the high-fat diet, expressed as a percentage of initial body weight, were unaffected by antecedent protein restriction (C, 7.2 ± 1.1% (n = 11); PR, 10.1 ± 1.0% (n = 16)).

The general metabolic characteristics of C and PR rats in the postabsorptive state before and after transfer to the high-fat diet for 8 wk are shown in Table 2. Plasma insulin and blood glucose concentrations did not differ significantly between the groups (C, PR, C-HF, and PR-HF). The insulin-to-glucose ratio, an index of the relative response of insulin to fasting glycemia, was also not significantly affected by protein restriction, either before or after high-fat feeding.
Glucoregulation after dietary interventions

Nuclear studies, in which rats were infused with [3-3H]glucose, were undertaken to evaluate the effects of mild protein restriction on whole body glucose turnover before and after challenge by the provision of the high-fat diet. Rd values (expressed per rat) in the postabsorptive state did not differ significantly between PR rats and C rats before transfer to the high-fat diet. Basal Rd values also did not differ significantly between PR-HF and C-HF groups. GMR values (which take into account differences in basal blood glucose concentrations) also did not differ significantly between C and PR rats before or after transfer to the high-fat diet.

To evaluate glucoregulatory control, blood glucose (Fig. 2, A and B) and plasma insulin concentrations (Fig. 2, C and D) were measured at intervals after the intravenous administration of a glucose bolus (500 mg/kg body wt). Protein restriction enhanced glucose tolerance (Fig. 2A) with a significantly higher (1.5-fold, P < 0.01) K value for glucose disappearance (Table 3), despite impaired insulin secretion as demonstrated by significantly lower insulin concentrations at 2 min (41%; P < 0.05) after intravenous glucose administration (Fig. 2C; see also Ref. 9). Blood glucose concentrations in PR-HF rats were significantly higher than in C-HF rats at 15 min (by 36%, P < 0.05) and 30 min (69%; P < 0.05) after intravenous glucose administration (Fig. 2B). Plasma insulin concentrations were significantly lower at 5 min (by 41%; P < 0.01) and 10 min (29%; P < 0.05) after intravenous glucose administration in PR-HF, compared with C-HF, rats (Fig. 2D).

Switching to the high-fat diet for 8 wk resulted in a significant decrease in the K value (45%; P < 0.001) and a significant increase in the incremental area under the curve (IAUC) for glucose (61%; P < 0.01) in rats previously subjected to protein restriction, but it did not alter glucose tolerance, the K value, or the IAUC for glucose in control rats (Table 3). As a consequence, whereas K values before high-fat feeding were higher in PR compared with C rats, K values of PR-HF rats were significantly lower (by 29%; P < 0.05) than those of C-HF rats (Table 3).

Because it was not clear whether impaired glucose tolerance in PR-HF rats was a consequence of impaired insulin secretion or insulin resistance, whole body glucose kinetics were measured during hyperinsulinemia. Insulin was infused into conscious, unrestrained rats at a fixed rate to raise steady-state insulin concentrations approximately sixfold (Table 4). Clamp blood glucose concentrations in the four groups were similar (Table 4). The coefficients of variance for blood glucose concentrations were <15% for all groups (results not shown). The steady-state GIRs required to maintain euglycemia between 90 and 120 min of hyperinsulinemia were similar in the C and PR rats. There were also no statistical differences between GIR values for C-HF and PR-HF rats (Table 4), but high-fat feeding resulted

### Table 5. Basal and norepinephrine-stimulated rates of adipose tissue lipolysis (assessed from glycerol release) for C and PR rats before and after HF feeding

<table>
<thead>
<tr>
<th>Glycerol release, pg·h⁻¹·cell⁻¹</th>
<th>C</th>
<th>PR</th>
<th>C-HF</th>
<th>PR-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>8.2 ± 2.3 (4)</td>
<td>6.7 ± 3.3 (5)</td>
<td>9.2 ± 2.1 (5)</td>
<td>6.8 ± 2.9 (4)</td>
</tr>
<tr>
<td>Norepinephrine, 0.1 µM</td>
<td>29.6 ± 5.3 (4)</td>
<td>114.4 ± 14.9 (5)*</td>
<td>20.5 ± 2.6 (5)</td>
<td>102.5 ± 27.5 (4)*</td>
</tr>
<tr>
<td>Norepinephrine, 1 µM</td>
<td>69.6 ± 3.2 (4)</td>
<td>144.2 ± 13.3 (5)*</td>
<td>63.5 ± 6.1 (5)</td>
<td>133.8 ± 22.9 (4)*</td>
</tr>
<tr>
<td>Norepinephrine, 1 µM + insulin, 75 µU/ml</td>
<td>16.1 ± 2.3 (4)</td>
<td>12.5 ± 3.4 (5)*</td>
<td>12.2 ± 1.8 (5)</td>
<td>73.0 ± 23.9 (4)*</td>
</tr>
</tbody>
</table>

Values are means ± SE for no. of adipocyte preparations in parentheses. Individual incubations were undertaken in triplicate. Statistically significant effects of protein restriction are indicated by *P < 0.01; †P < 0.001. Statistically significant effects of HF feeding are indicated by ‡P < 0.001.
in a significant decline in GIR in both groups [43% (P < 0.01) in C-HF rats and 36% (P < 0.05) in PR-HF rats].

R_d values, estimated at steady-state hyperinsulinemia with [3-3H]glucose, expressed either relative to body weight (Fig. 3) or per rat [C, 7.4 ± 0.7 mg·min^{-1}·rat^{-1} (n = 5); PR 6.3 ± 0.3 mg·min^{-1}·rat^{-1} (n = 6)], did not differ significantly between C and PR rats before high-fat feeding. Plasma insulin-glucose disposal relationships before (A) and after (B) high-fat feeding are shown in Fig. 3. Before transfer to a high-fat diet, the gradients of the lines were similar in the C and PR groups, indicating that the response of whole body glucose disposal to this degree of hyperinsulinemia is unaffected by mild protein restriction. R_d values after high-fat feeding were significantly (30%; P < 0.01) lower in PR-HF rats than in C-HF rats. This arises as a consequence of an almost twofold greater decline in insulin-stimulated R_d (47%; P < 0.001) in response to high-fat feeding in previously PR rats than in the C group (27%; P < 0.01). In contrast, there was greater suppression of endogenous glucose production in the PR-HF group compared with the C-HF group (results not shown).

The sensitivity and responsiveness of adipose tissue lipolysis (assessed from glycerol release) to stimulation by norepinephrine in C rats and PR rats before and after transfer to the high-fat diet are shown in Table 5. Basal rates of adipose tissue lipolysis before and after high-fat feeding were unaffected by protein restriction. Lipolysis was maximally stimulated by norepinephrine at 1 µM for adipocytes from all four groups (results not shown). With adipocytes from C rats, 0.1 µM norepinephrine led to a 3.6-fold (P < 0.01) increase in lipolysis. In contrast, the response of lipolysis to stimulation by 0.1 µM norepinephrine was increased by 17.2-fold (P < 0.001) in adipocytes prepared from PR
rats. Lipolysis rates at 1 µM norepinephrine were approximately twofold higher (P < 0.001) with adipocytes from PR rats compared with adipocytes from C rats. The increased sensitivity of lipolysis to stimulation by norepinephrine at low concentrations and the increased responsiveness to norepinephrine at the maximal concentration were retained in adipocytes prepared from PR-HF rats compared with C-HF controls.

To assess the antilipolytic action of insulin, adipocytes prepared from rats in each of the groups were incubated with norepinephrine at a fixed concentration of 1 µM in the presence or absence of insulin at 75 µU/ml (a concentration comparable to that attained during the insulin infusion studies in vivo) (Table 5). This concentration of insulin produced 80 and 90% inhibition of norepinephrine-stimulated lipolysis in adipocytes prepared from C and PR rats, respectively. The response to 75 µU/ml insulin was unimpaired in the C-HF group (80% inhibition of lipolysis). In contrast, the response of norepinephrine-stimulated lipolysis to suppression by 75 µU/ml insulin was markedly and selectively blunted in adipocytes prepared from the PR-HF rats, with only ~50% inhibition of norepinephrine-stimulated lipolysis.

Dose-response curves for the antilipolytic action of insulin are shown in Fig. 4. Adipocytes prepared from C and PR rats responded to insulin with suppression of norepinephrine-stimulated lipolysis at all insulin concentrations tested (Fig. 4, A and C). Inulin at concentrations from 5 to 75 µU/ml led to significant (P < 0.05) suppression of norepinephrine-stimulated rates of lipolysis with adipocytes prepared from C-HF rats (Fig. 4, B and D). In contrast, insulin at 5 µU/ml was without significant effect on norepinephrine-stimulated lipolysis in adipocytes from PR-HF rats, and there was little or no enhancement of the antilipolytic action of insulin when the insulin concentration was increased from 10 to 75 µU/ml (Fig. 4B and D). A significant (P < 0.05) effect of insulin to suppress lipolysis was observed only at 75 µU/ml insulin. As a consequence, rates of lipolysis were significantly higher in the PR-HF group compared with the C-HF group at all insulin concentrations studied (Fig. 4B). The percentage suppression of lipolysis was significantly less in the PR-HF group (C-HF, 80%; PR-HF, 47%; P < 0.01) (Fig. 4D); however, by virtue of the higher response of lipolysis to norepinephrine stimulation in the PR-HF group, the absolute decrease in glycerol release at the highest insulin concentration studied (75 µU/ml) was similar in the C-HF and PR-HF groups (Fig. 4B).

Plasma NEFA concentrations in vivo did not differ significantly between the C-HF and PR-HF groups in either the basal (postabsorptive) state [C-HF, 0.69 ± 0.20 mM (n = 6); PR-HF, 0.42 ± 0.07 mM (n = 6)] or after insulin infusion [C-HF, 0.11 ± 0.02 mM (n = 12); PR-HF, 0.10 ± 0.01 mM (n = 6)].

**DISCUSSION**

The present study was undertaken to evaluate whether antecedent protein restriction predisposes to the later development of glucose intolerance and insulin resistance. The effects of antecedent protein restriction on whole body glucose homeostasis and insulin action were examined after the dietary challenge of the provision of a high-fat diet for 8 wk. A previous study also demonstrated a modest, but not statistically significant, loss of glucose tolerance after intraperitoneal glucose administration after 4 wk of high-fat feeding in the adult offspring of mothers fed a low (5%) protein diet during pregnancy and lactation (28). The present results demonstrate that mild protein restriction from conception to adulthood (20 wk of age) leads to significantly enhanced tolerance to intravenous glucose, despite an impaired insulin response and a greatly augmented and sensitized lipolytic response of the adipocyte to norepinephrine, but does not predispose to either glucose intolerance or impaired insulin action with respect to glucose disposal. However, an effect of high-fat feeding to elicit a deterioration in the action of insulin to stimulate peripheral glucose disposal is exaggerated by antecedent protein restriction; Fig. 5 illustrates the more rapid and exaggerated decline in the incremental insulin-stimulated increase in peripheral glucose disposal elicited by the provision of a high-fat diet in rats previously exposed to protein restriction. This effect is observed in conjunction with a specific impairment of the antilipolytic response of insulin in adipocytes and manifests as impaired glucose tolerance after intravenous glucose administration. Although plasma NEFA concentrations did not differ significantly between the C-HF and PR-HF groups in either the basal (postabsorptive) state or after insulin infusion [C-HF, 0.69 ± 0.20 mM (n = 6); PR-HF, 0.42 ± 0.07 mM (n = 6)] or after insulin infusion [C-HF, 0.11 ± 0.02 mM (n = 12); PR-HF, 0.10 ± 0.01 mM (n = 6)].
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Increased insulin action in the rat after protein restriction alone and the role of subsequent dietary-lifestyle factors in determining the onset of impaired glucose tolerance.

Impaired insulin secretion and reduced insulin action are both thought to contribute to the pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus (2, 14, 22). The degree to which glucoregulatory control deteriorates varies as a function of the magnitude of loss of in vivo insulin action in relation to the capacity of the β-cell to compensate for this defect by insulin hypersecretion (21). Growth retardation evoked by suboptimal protein nutrition during early life leads to decreased basal glucose disposal and glucose utilization by a range of tissues and the fetus during a subsequent pregnancy, but this effect is not associated with any major permanent impairment of glucose-stimulated insulin secretion or whole body insulin action at day 19 of pregnancy (12), even though it is recognized that late pregnancy is an insulin-resistant state (13, 17) (reviewed in Ref. 25). The physiological stimulus of pregnancy is associated with an enhanced insulin secretory response to glucose (18) (reviewed in Ref. 25), and this adaptation appears to be intact in pregnant rats that have experienced early growth retardation (12). The present study involves separate analyses of two dietary interventions known to target either insulin secretion (protein restriction) or insulin action (high-fat feeding). The results indicate that protein restriction alone, while impairing insulin secretion, enhances whole body and peripheral insulin action. As a consequence, there is no adverse effect on glucose tolerance. Conversely, a low-carbohydrate, high-fat diet leads to a modest impairment in peripheral insulin action without a deterioration of glucose tolerance, because insulin secretion is enhanced (see also Ref. 9). However, the sequential imposition of the two dietary interventions results in synergistic interactions, leading to a marked impairment in peripheral insulin action in combination with a blunted insulin secretory response, leading to glucose intolerance. The study has relevance to the interpretation of studies in humans demonstrating an increased incidence of diabetes on change from a nutritionally poor diet to a high-energy diet containing a relatively high percent-age of lipid, characteristic of Western affluent societies, and may also be germane to the hypothesis that diseases that manifest in adult life, such as non-insulin-dependent diabetes mellitus, may originate in infant or early life as a consequence of poor early nutrition (for a recent review see Ref. 8).

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