Dietary restriction and glucose regulation in aging rhesus monkeys: a follow-up report at 8.5 yr

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Gresl, Theresa A., Ricki J. Colman, Ellen B. Roecker, Thomas C. Havighurst, Ze Huang, David B. Allison, Richard N. Bergman, and Joseph W. Kemnitz. Dietary restriction and glucose regulation in aging rhesus monkeys: a follow-up report at 8.5 yr. Am J Physiol Endocrinol Metab 281: E757–E765, 2001.—In a longitudinal study of the effects of moderate (70%) dietary restriction (DR) on aging, plasma glucose and insulin concentrations were measured from semiannual, frequently sampled intravenous glucose tolerance tests (FSIGTT) in 30 adult male rhesus monkeys. FSIGTT data were analyzed with Bergman’s minimal model, and analysis of covariance revealed that restricted (R) monkeys exhibited increased insulin sensitivity (SI, \( P < 0.001 \)) and plasma glucose disappearance rate (\( \text{KG} \), \( P = 0.015 \)), and reduced fasting plasma insulin (\( \text{I}_b \), \( P < 0.001 \)) and insulin response to glucose (AIRG, \( P = 0.023 \)) compared with control (C; ad libitum-fed) monkeys. DR reduced the baseline fasting hyperinsulinemia of two R monkeys, whereas four C monkeys have maintained from baseline, or subsequently developed, fasting hyperinsulinemia; one has progressed to diabetes. Compared with only the normoinsulinemic C monkeys, R monkeys exhibited similarly improved FSIGTT and minimal-model parameters. Thus chronic DR not only has protected against the development of insulin resistance in aging rhesus monkeys, but has also improved glucoregulatory parameters compared with those of otherwise normoinsulinemic monkeys.

Dietary restriction (DR) has been shown to slow the rate of physiological decline and the development of age-associated diseases in rodents and to extend life span in a variety of species (42). We are studying 30 male rhesus monkeys longitudinally to characterize changes in indexes of glucose regulation with age and long-term moderate DR. Because the rhesus monkey is prone to the spontaneous development of obesity and diabetes in midlife (25, 26, 37), it is an excellent animal model for studying the progression of metabolic changes that occur before the onset of type 2 diabetes. This progression is similar to that observed in humans (18). Although it remains unclear whether it is age itself or the changes in body composition that occur with aging that underlie the frequently reported impairments in glucose tolerance in aging subjects, it is likely that the latter, particularly the increase in visceral fat, may play an important role in age-related disease (2).

We have previously reported that, after 2.5 yr of DR, these monkeys had increased insulin sensitivity, whereas fasting plasma insulin and glucose levels were reduced compared with controls’ values. (27). Other studies of DR in both rodents and nonhuman primates (rhesus and cynomolgus monkeys) have produced similar findings. In general, energy restriction markedly increases insulin sensitivity (6, 8), reduces fasting or mean 24-h insulin concentration (6, 20, 29, 32), and, in some (29, 32) but not all cases (6, 8, 20), significantly reduces fasting or mean 24-h glucose concentration compared with control animals. Taken together, these data suggest that DR acts to enhance glucoregulatory health in aging animals, thus retarding the development of insulin resistance and type 2 diabetes. Whether the aging process itself is slowed in conjunction with altered glucose and insulin concentrations is not yet known. It has been suggested that the reduced exposure to insulin over time (34) or the reduction of oxidative damage (40) may be related to the life-span-extending effects of DR. In this report, we describe the glucoregulatory-related effects of DR and ad libitum feeding in adult male rhesus monkeys after 8.5 yr of regular, standardized assessments.

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RESEARCH DESIGN AND METHODS

Experimental design and subjects. The experimental design and methodology have been described in detail previously (12, 27, 28). Briefly, 30 adult male rhesus monkeys (Macaca mulatta), born and raised at the Wisconsin Regional Primate Research Center, have been used in this ongoing, multidimensional study of the effects of moderate DR on aging. The monkeys were 8–14 yr of age at the beginning of the study, and after 8.5 yr, the mean age was 17.8 yr. The median life expectancy of the rhesus monkey in captivity is ~26 yr, with some of the monkeys in this colony living into their late 30s (11).

Animals were individually housed to control access to food and to allow accurate food intake measurements. Animals had continuous access to tap water. Room temperature was maintained at ~21°C, and the animals were maintained on a 12:12-h light-dark cycle with lights on between 6 AM and 6 PM. The animals underwent a 2-mo prebaseline period for adaptation to the environment before the start of the experiment. Semiannual assessment periods included measurements of energy expenditure, body composition, and glucose metabolism, as described below.

Diet. Monkeys were fed a defined, pelleted diet (Teklad, Madison, WI) comprised of 15% protein as lactalbumin, 10% fat as corn oil, and ~65% carbohydrate as sucrose, starch, and dextrin, by weight. All monkeys were fed in the morning, and 6–8 h later, the remaining or spilled food was removed and weighed, and the animals were given a piece of fresh fruit. Food intake was calculated as previously described (28).

DR was 70% of individual baseline intake. This reduction in energy intake was achieved by random assignment of animals to a treatment group after a 3- to 5-mo period of baseline assessment, during which food intake of the experimental diet was determined for individual monkeys. Food intakes of the monkeys assigned to DR (R; n = 15) were reduced from their baseline period averages by 10%/mo for 3 mo and then maintained at this level. The control animals (C; n = 15) continued to have free access to food during the 6- to 8-h daily feeding period, and all food provided was consumed in that time, subsequent food allotments were increased as necessary.

Body size and composition. Semiannual body weight measurements were made while the monkeys were anesthetized with ketamine HCl (10 mg/kg body wt im). Body mass index (BMI) was calculated as body weight (kg) divided by the square of crown rump length (m²), measured with the animal in lateral recumbency by use of a calibrated rule with a fixed head rest. Abdominal skinfold measurements were taken as previously described (12). Beginning with 1 yr after initiation of DR, body composition was measured annually through 5 yr with dual-energy X-ray absorptiometry (DEXA; model DPX-lunar, Madison, WI) while the monkeys were sedated with ketamine HCl (10 mg/kg body wt im), followed by ketamine HCl-xylazine (7 mg/kg body wt ketamine HCl, 0.6 mg/kg body wt xylazine im) for additional muscular relaxation and anesthesia.

Glucose regulation. Frequently sampled intravenous glucose tolerance tests (FSGITT) were performed semiannually according to the tolbutamide-modified minimal-model protocol (4). After an overnight (~16 h) fast, monkeys were anesthetized with ketamine HCl (15 mg/kg body wt im) and diazepam (1.25 mg/kg body wt im). Sedation was maintained with additional ketamine administration (5–10 mg/kg body wt im) as needed. A central venous catheter was positioned for blood sampling and administration of glucose (300 mg/kg body wt at 20 min) and tolbutamide (5 mg/kg body wt (Orinase Diagnostic, provided courtesy of Pharmacia & Upjohn, Kalamazoo, MI) during the procedure.

Plasma glucose concentration was measured by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was measured by a double-antibody RIA. From baseline through the 5.0-yr assessment period, this assay measured a combination of both insulin and proinsulin (and cross-reactivity with proinsulin was ~30%) (Binax, Portland, ME; inter- and intra-assay coefficients of variation (CVs): 5.56% and 4.50%, respectively). From 5.5 through 8.5 yr, the assay reagents used were more specific for insulin (Linco Research, St. Charles, MO; inter- and intra-assay CVs: 6.39% and 3.29%, respectively). Triglycerides were measured in serum by a spectrophotometric assay. Total glycated hemoglobin was measured using a Glyco-Teck affinity column method (Helena Labs, Beaumount, TX).

Plasma glucose and insulin concentrations were analyzed using the minimal-model method, which describes the dynamics of insulin and glucose after a 3-h FSGITT. The minimal model (version 3.0, R. N. Bergman) provides estimates of insulin sensitivity (SI) and glucose effectiveness (SG), as well as an integrated measure of suprabasal insulin secretion assessed as acute (0–10 min) plasma insulin response to glucose (AIRG) (3). SI reflects the effect of insulin to promote glucose uptake and to inhibit hepatic glucose production. SG reflects the ability of glucose to enhance its own uptake and to suppress hepatic glucose production independently of an increase in insulin above a basal (IB) level. Furthermore, S0 includes an insulin-independent component: glucose effectiveness at zero insulin (GEZI), calculated as GEZI = S0 – (S1 – IB) (23). Fasting plasma glucose (G0) and IB were calculated as the average of four prechallenge plasma values (~15, ~10, ~5, and ~1 min). Glucose disappearance rate (Kd) was calculated as the slope of the log-linear regression of glucose concentration above G0 between 10 and 19 min.

Separation of data and statistical analysis: criterion of prediabetic status. Guidelines for impaired glucose tolerance in humans are not appropriate to use for rhesus monkeys, because monkeys tend to have higher insulin and lower glucose plasma concentrations compared with humans. However, the progression through prediabetic and diabetic stages in rhesus monkeys has been documented cross-sectionally (18) and longitudinally (19). The first identifiable changes included an increase in insulin response to glucose administration and a rise in IB. In the present study, the 90th percentile of the IB distribution at baseline was taken as the point above which the monkeys were considered relatively hyperinsulinemic and insulin resistant and were possibly “at risk” for subsequent worsening of glucose tolerance. This baseline value was also used through the 5-yr assessment period and then recalculated for the change to a more specific insulin RIA at 5.5 yr; these values were used as a guideline to identify other potentially at-risk animals. At baseline, two monkeys in each treatment group exhibited fasting hyperinsulinemia on the basis of this criterion; these values also fell within the IB range of the early prediabetic hyperinsulinemic stages described by Hansen and Bodkin (18). SI values of these monkeys were also relatively low (mean ± SE: 1.85 ± 0.37 × 10⁻⁶ min⁻¹·pM⁻¹ compared with group mean ± SE of 5.91 ± 0.80 at baseline). Because of the variability of SI, IB may be a more useful indicator of insulin sensitivity than the SI index itself in less insulin-sensitive individuals (36). Because the two variables have very different curves over time, however, this may apply when individuals are in the very

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early stages of the progression toward diabetes, before the reduced insulin secretion observed in the latter stages. Individuals in the highest quartile of fasting insulin concentration are more likely to develop impaired glucose tolerance and diabetes within several years than individuals in the lowest quartile (17). Both at-risk C monkeys exhibited either continual or episodic hyperinsulinemia from baseline, and two additional C monkeys were identified as hyperinsulinemic and relatively insulin resistant for prolonged periods. Data of these four C monkeys (referred to as “hyperinsuline- mic” for simplicity), were removed from one round of the analyses were also performed when data of the four hyperinsuline- mic and relatively insulin resistant C monkeys (referred to as “hyperinsuline- mic C monkeys”) were removed due to the effect on the analyses. The Wilcoxon 2-sample test (for unpaired data) was used to compare within-time treatment group differences (‡P < 0.05), indicating that the treatment group differences varied significantly across time, treatment group comparisons at each time were tested by Fisher’s protected least significant difference procedure (33). No longitudinal statistical analyses were performed with the hyperinsulinemic C animals as a group due to their small number. Within-treatment group differences at baseline and 8.5 yr, shown in Table 1, were tested using the Wilcoxon signed-rank test (for paired data), whereas differences between R and C at each time point were tested using the Wilcoxon signed-rank test (for paired data).

Statistical analyses. Statistical comparisons of treatment groups across time points after baseline were made by repeated-measures analysis of covariance (ANCOVA) using SAS Proc Mixed (SAS Institute, 1989, release 6.09). These analyses were also performed when data of the four hyperinsulinemic C monkeys were removed due to the effect on the distributions. All longitudinal analyses reported contrast in differences (‡P < 0.05) from the means reported above. The Wilcoxon 2-sample test (for unpaired data) was used to compare within-time treatment group differences (‡P < 0.05), indicating that the treatment group differences varied significantly across time, treatment group comparisons at each time were tested by Fisher’s protected least significant difference procedure (33). No longitudinal statistical analyses were performed with the hyperinsulinemic C animals as a group due to their small number. Within-treatment group differences at baseline and 8.5 yr, shown in Table 1, were tested using the Wilcoxon signed-rank test (for paired data), whereas differences between R and C at each time point were tested using the Wilcoxon signed-rank test (for paired data).

Table 1. Baseline and 8.5-yr characteristics of body composition and FSIGTT/minimal-model variables for control and restricted monkeys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>8.5 Yr</th>
<th>C</th>
<th>R</th>
<th>C</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>9.6 ± 0.4</td>
<td>9.5 ± 0.5</td>
<td>17.9 ± 0.4</td>
<td>17.7 ± 0.5</td>
<td>16.4–22.2</td>
<td>16.3–22.4</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>989 ± 67</td>
<td>1009 ± 47</td>
<td>717 ± 26</td>
<td>494 ± 18</td>
<td>547–900</td>
<td>394–605</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>11.3 ± 0.5</td>
<td>11.3 ± 0.4</td>
<td>15.1 ± 0.6*</td>
<td>9.7 ± 0.4§</td>
<td>(11.2–19.7)</td>
<td>(7.5–12.5)</td>
</tr>
<tr>
<td>BMI</td>
<td>39.3 ± 1.2</td>
<td>40.9 ± 1.4</td>
<td>49.8 ± 2.1*</td>
<td>35.0 ± 1.5§</td>
<td>(36.2–62.9)</td>
<td>(29.2–47.1)</td>
</tr>
<tr>
<td>Abd circ, cm</td>
<td>46 ± 2</td>
<td>47 ± 1</td>
<td>66 ± 2*</td>
<td>44 ± 1§</td>
<td>(51–77)</td>
<td>(36–53)</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9.6 ± 0.4</td>
<td>9.5 ± 0.5</td>
<td>17.9 ± 0.4</td>
<td>17.7 ± 0.5</td>
<td>16.4–22.2</td>
<td>16.3–22.4</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>279 ± 61</td>
<td>336 ± 37</td>
<td>303 ± 43</td>
<td>101 ± 20§</td>
<td>(132–642)</td>
<td>(48–276)</td>
</tr>
<tr>
<td>Go, mM</td>
<td>3.2 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>4.2 ± 0.7</td>
<td>3.2 ± 0.1‡</td>
<td>(12–9.6)</td>
<td>(7.4–11.7)</td>
</tr>
<tr>
<td>Is, pM</td>
<td>65.1 ± 0.71</td>
<td>8.38 ± 1.20</td>
<td>4.36 ± 0.41‡</td>
<td>8.50 ± 1.27§</td>
<td>(8.8–13.2)</td>
<td>(7.4–11.7)</td>
</tr>
<tr>
<td>Sg, ×10^−2·min^−1</td>
<td>3.60 ± 0.36</td>
<td>4.15 ± 0.97</td>
<td>2.66 ± 0.14*</td>
<td>3.56 ± 0.63</td>
<td>(3.63–7.20)</td>
<td>(2.57–8.41)</td>
</tr>
<tr>
<td>AIRg, ×10^−3·pM^−1·min^−1</td>
<td>8.550 ± 1.391</td>
<td>8.840 ± 1.738</td>
<td>8.349 ± 1.563</td>
<td>4.070 ± 0.529§</td>
<td>(3.876–23.520)</td>
<td>(1.860–29.880)</td>
</tr>
<tr>
<td>S1, ×10^−5·min^−1·pM^−1</td>
<td>6.85 ± 1.38</td>
<td>4.99 ± 0.78</td>
<td>2.78 ± 0.57*</td>
<td>14.19 ± 2.14§</td>
<td>(0.78–17.23)</td>
<td>(1.05–11.43)</td>
</tr>
<tr>
<td>Sg, ×10^−2·min^−1</td>
<td>2.45 ± 0.20</td>
<td>2.72 ± 0.34</td>
<td>1.93 ± 0.22*</td>
<td>2.38 ± 0.54</td>
<td>(0.5–5.35)</td>
<td>(0.99–6.55)</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>223 ± 41</td>
<td>59 ± 9§</td>
<td>9.8 ± 0.8</td>
<td>8.0 ± 0.2‡</td>
<td>(36–507)</td>
<td>(9–112)</td>
</tr>
<tr>
<td>Glycated Hb, %</td>
<td>9.6 ± 0.4</td>
<td>9.5 ± 0.5</td>
<td>17.9 ± 0.4</td>
<td>17.7 ± 0.5</td>
<td>16.4–22.2</td>
<td>16.3–22.4</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE (range). FSIGTT, frequently sampled intravenous glucose tolerance test; C, control (n = 15 at baseline; n = 13 at 8.5 yr); R, restricted (n = 15 at baseline; n = 12 at 8.5 yr); BMI, body mass index; Abd circ, abdominal circumference; Go, fasting plasma glucose; Is, fasting plasma insulin; Kg, glucose disappearance rate; Sg, glucose effectiveness; AIRg, integrated acute insulin response to glucose from 0 to 10 minutes; S1, insulin sensitivity index; GEZI, glucose effectiveness at zero insulin. No differences in these variables existed between groups at baseline, and differences between groups were not tested for age and energy intake. The Wilcoxon signed-rank test was used to compare within-treatment group differences from 0 to 8.5 yr (*P < 0.01; ‡P < 0.05). Because this test is used for paired data, animals missing data at either time point are not included in the analysis, and group means reflecting the true number of animals may differ slightly from the means reported above. The Wilcoxon 2-sample test (for unpaired data) was used to compare within-time treatment group differences (‡P < 0.01; §P < 0.05). There were no measurements taken at baseline for triglycerides and total glycated hemoglobin, and no dual-energy X-ray absorptiometry estimates of %body fat and lean body mass.
tested using the Wilcoxon two-sample test (for unpaired data) with significance at $P < 0.05$. Finally, Spearman’s rank correlation analysis was used to examine the associations among estimates of adiposity (i.e., percent body fat, abdominal circumference, abdominal skinfold thicknesses, BMI), serum triglyceride content, $K_g$, and $S_i$. The Wilcoxon and Spearman’s analyses were performed using JMP statistical software (SAS Institute, 1994, version 3.2).

RESULTS

Energy intake. Figure 1 shows energy intake over 8.5 yr among all C, normoinsulinemic C, and R groups. Although the intended level of restriction was 30%, 6-mo averages have ranged from 23 to 37% of C intake (mean ± SE: 29 ± 1) from 2 through 8.5 yr. Due to a voluntary reduction in food intake by controls in the initial months of the study, food allotment was reduced for all R monkeys at 1.5 and 4.5 yr to reestablish the ~30% difference in group intake (27). Between 6 and 8.5 yr, however, food allotments for seven restricted monkeys were increased incrementally to maintain a minimal level of 5% body fat. In addition, if a C monkey consistently consumed all food provided on average over a 3-mo period, the food allotment was increased by 20 g.

Eight and one-half years after initiation of DR, the animals ranged in age from 16 to 22 yr (mean ± SE: 17.8 ± 0.3). Both C and R animals appeared healthy, although two deaths in each group occurred since our last report on this topic (27). One death in each group was anesthesia related; the other C group death was due to herniation of the colon, and the R group death was due to asymptomatic cardiomyopathy.

Table 1 shows group characteristics and indexes of body composition and glucose metabolism at baseline and 8.5 yr. Univariate analyses revealed that $G_h$, $I_b$, AIRG, glycated hemoglobin, and serum triglycerides were significantly lower, and $K_g$ and $S_i$ were higher in R vs. C monkeys at 8.5 yr. The treatment group difference in glycated hemoglobin remained marginally significant ($P = 0.073$) after the data from one monkey with diabetes were removed from the analysis. $I_b$ and triglyceride levels were also reduced, and $S_i$ was significantly elevated from baseline (or 5.0 yr for triglycerides) in R monkeys. Among C monkeys, $K_g$, $S_g$, GEZI, and $S_i$ were significantly reduced from baseline values. R monkeys were leaner than C monkeys, and this observation is confirmed by the significant cross-sectional differences observed at 8.5 yr in body weight, BMI, percent body fat, and abdominal circumference found between groups (all $P < 0.001$). Longitudinal changes through 8.5 yr in body weight, body fat, abdominal circumference, and lean body mass were also apparent between R and C groups (Fig. 2, A-D, all $P < 0.001$). These changes in body composition were reported from 1.0 through 7.5 yr (12). Furthermore, at 8.5 yr and despite the loss of body weight and fat with this diet, R monkeys exhibited a significant increase from 1.0 yr in lean tissue mass ($P = 0.044$).

Figure 3, A and B, shows $G_h$ and $I_b$ over time. When all C were compared with R monkeys, there was a significant time-by-treatment interaction for $I_b$ ($C > R$, $P < 0.001$) but not $G_h$ ($P = 0.139$). However, when the four hyperinsulinemic monkeys were removed from the analysis (i.e., R vs. normoinsulinemic C), the time-by-treatment interactions for both were significant ($I_b$, normoinsulinemic C $> R$, $P < 0.001$; $G_h$, normoinsulinemic C $> R$, not shown, $P = 0.028$). Likewise, regardless of whether the comparison was between R and all C monkeys or R and normoinsulinemic C monkeys, there were significant time-by-treatment interactions for AIRG (Fig. 4, R $< C$, $P = 0.023$; $R < $ normoinsulinemic C, $P < 0.001$) and for $S_i$ (Fig. 5, C $< R$, and $R < $ normoinsulinemic C, both $P < 0.001$).

The time-by-treatment interaction for $K_g$ (Fig. 6) reached statistical significance when all C were compared with R ($P = 0.015$), but this was only marginally significant when R were compared with normoinsulinemic C monkeys ($P = 0.064$). Treatment groups did not differ over time with respect to either $S_g$ (R vs. normoinsulinemic C, $P = 0.314$; R vs. all C, $P = 0.319$) or GEZI (R vs. normoinsulinemic C, $P = 0.572$; R vs. all C, $P = 0.478$).

Associations between the estimates of adiposity and serum triglyceride level vs. $K_g$ and $S_i$ within both C and R groups at the 8.5-yr assessment period did not achieve statistical significance, with the following exceptions. In C monkeys only, serum triglyceride level was associated inversely with $S_i$ ($r = -0.62$; $P = 0.042$) and marginally associated with $K_g$ ($r = -0.46$; $P = 0.094$). In addition, among C monkeys there was a marginally significant association between abdominal circumference and $K_g$ ($r = -0.52$; $P = 0.070$).

In addition, serum triglyceride level was not associated with any estimate of adiposity in either group, whereas $K_g$ was significantly associated with $S_i$ in
Fig. 2. Body weight (kg; A), body fat (%; B), abdominal circumference (cm; C), and lean body mass (kg; D) among all C (n = 13–15) and R (n = 11–15) groups through 8.5 yr after initiation of dietary restriction (DR). Dual-energy X-ray absorptiometry data for % body fat were available annually, beginning at 1 through 5 yr and semiannually through 8.5 yr. The time-by-treatment interaction was significant for all 3 parameters (P < 0.001) for R vs. C groups. However, when the hyperinsulinemic controls (including the diabetic monkey) were removed from the analysis, the time-by-treatment interaction was significant between R and NIC (not shown; P = 0.028) with significant cross-sectional treatment group differences using Fisher’s protected least significant difference evident at each time point from 1.0 yr for body weight, body fat, and abdominal circumference, and from 2.0 yr for lean body mass (all P < 0.05). All values shown are means ± SE. Scale of graph may obscure SE bars in some cases.

Fig. 3. Fasting plasma glucose (Gb; A) and fasting plasma insulin (Ib; B) among all C (n = 13–15) and R (n = 11–15) groups through 8.5 yr after initiation of DR. A: neither the time-by-treatment interaction (P = 0.139) nor the main effect of treatment (P = 0.132) for glucose was significant for R vs. C groups. However, when the hyperinsulinemic controls (including the diabetic monkey) were removed from the analysis, the time-by-treatment interaction was significant between R and NIC (P = 0.028) with significant cross-sectional treatment group differences using Fisher’s protected least significant difference evident at 2.0, 2.5, 3.5, 6.0, and 7.0–8.5 yr (P < 0.05). The elevated glucose means among C monkeys is due almost entirely to the monkey that developed diabetes. B: time-by-treatment interaction for insulin was significant when comparing both C vs. R and NIC (n = 9–11) vs. R groups (both P < 0.001), with significant cross-sectional treatment group differences evident at all points from 2.0 yr (P < 0.05). The same longitudinal results were seen for comparisons between C vs. R and NIC vs. R when this analysis was carried out from baseline through 5.0 yr only, before the change in insulin RIA kit. All values shown are means ± SE. Scale of graph may obscure SE bars in some cases.
plasma samples taken from another group of rhesus model parameters (data not shown). From FSIGTT examine its effect, if any, on FSIGTT and minimal-5-yr assessment (Binax), we performed a small study to at 5.5 yr (Linco) for the one we had used through the groups.

Because we substituted a more specific insulin RIA during a frequently sampled intravenous glucose tolerance test (FSIGTT) among all C (n = 13–15), NIC (n = 9–11), and R (n = 11–15) groups through 8.5 yr after initiation of DR. The time-by-treatment interaction was significant for R vs. C groups (P = 0.023) as well as for R vs. NIC groups (P < 0.001). Significant cross-sectional treatment group differences using Fisher’s protected least significant difference for R vs. C groups were evident from 2.0 yr and for R vs. NIC groups at 2.5, 3.0, and 4.5–8.5 yr (P < 0.05). All values shown are means ± SE. Scale of graph may obscure SE bars in some cases.

Both C (r = 0.62; P = 0.040) and R (r = 0.66; P = 0.026) groups.

Because we substituted a more specific insulin RIA at 5.5 yr (Linco) for the one we had used through the 5-yr assessment (Binax), we performed a small study to examine its effect, if any, on FSIGTT and minimal-model parameters (data not shown). From FSIGTT plasma samples taken from another group of rhesus monkeys on the same purified diet, we measured insulin concentration with both Binax and Linco kits, and each data set was analyzed with the minimal model. Although the Linco-measured insulin concentrations were generally lower than the corresponding Binax-measured values throughout the FSIGTTs and fasting insulin concentration differed significantly (P = 0.046), there was no evidence that group means differed for any other FSIGTT and minimal-model estimates (e.g., SI, S_G, AIR_G, and GEZI, all P > 0.05).

DISCUSSION

We have previously reported that 2.5 yr of adult-onset DR in rhesus monkeys resulted in reduced body weight and lower central adiposity, as well as reduced fasting insulin and glucose concentrations, glucose-stimulated insulin responses, and enhanced insulin sensitivity compared with age-matched, ad libitum-fed controls (27). After 8.5 yr, as the animals were advancing into middle age, these changes were still apparent. Glucose tolerance was maintained similar to baseline levels in R monkeys, whereas among C monkeys it had begun to decline. These results are consistent with the DR-induced changes in glucose regulation seen in other studies of aging nonhuman primates (6, 8, 29, 30), among humans subjected to brief (10, 31) and chronic reduced energy intake (43) and, in general, with the disease-retarding effects of DR observed in other species (42).

The R animals were lean and appeared healthy after 8.5 yr. They had lost ~14% of their adult body weight from the onset of the study and >50% of the fat mass present at 1 yr. Despite the loss of body weight and fat mass, lean mass was ~9% greater at 8.5 yr than at 1 yr, and abdominal circumference was not different from the baseline value. In contrast, and although they also appeared to be in good health, body weight and abdominal circumference of C monkeys had gradually
increased from the beginning of the study, consistent with the observed increase in adiposity of aging rhesus monkeys (22, 37) and humans (39). It is worth noting that, for the R monkeys, this is not simply a weight (or fat) reduction study. Weight and fat reduction were expected outcomes in these animals, but in contrast to many or most human weight reduction studies, our data support maintenance or an increase in fat-free mass (12, 13), suggesting an adaptation to chronic moderately restricted energy intake. The wide range of body fat after 8.5 yr among monkeys subjected to a similar level of DR supports the notion that body composition, particularly total body fat, is not indicative of the level of restriction and suggests that energy efficiency varies greatly in this group. Furthermore, it is not clear that DR exerts its effects through the loss of fat mass.

Development of hyperinsulinemia among four control animals. At baseline, two R and two C monkeys exhibited $I_b$ above the 90th percentile of the $I_b$ baseline distribution ($n = 30$). These animals were considered relatively hyperinsulinemic and insulin resistant and were potentially at risk at that time for worsening of glucose tolerance. $I_b$ levels of the two baseline hyperinsulinemic R monkeys were reduced to levels of other R monkeys by 2.0 yr (R group mean ± SE at 2 yr: 189 ± 27 pM), whereas the $S_I$ levels were increased, in the case of one animal >10-fold to $14.95 \times 10^{-5}\cdot\text{min}^{-1}\cdot\text{pM}^{-1}$. Insulin responses to glucose, in turn, were also reduced. $I_b$ values of the two baseline hyperinsulinemic C monkeys, in contrast, remained elevated most if not all of the time from baseline, whereas their $S_I$ levels remained low (at 8.0 yr: $S_I = 0.54 \times 10^{-5}\cdot\text{min}^{-1}\cdot\text{pM}^{-1}$). By 4 yr, two additional C monkeys had developed hyperinsulinemia for prolonged periods, while $S_I$ remained reduced. One of these latter two C monkeys subsequently developed diabetes mellitus and received daily insulin therapy. Although this animal was not hyperinsulinemic at baseline as two other C monkeys were, it progressed relatively quickly to type 2 diabetes, indicative of the individual variability of the development of impaired glucose tolerance in monkeys (19).

The monkeys in this study ranged in age from <10 to 17 yr, corresponding to young adulthood and early middle age in humans (11), when changes in glucose metabolism began to emerge and diabetes was diagnosed (19).

Longitudinal analyses. Differences in $I_b$ and $G_b$ began to emerge only after an adjustment in food allotment at 1.5 yr, which reestablished the ~30% difference in intake between R and C groups (27). After 8.5 yr, these differences over time continued to be significant between the R monkeys and either all C or normoinsulinemic C monkeys. In agreement with our preliminary findings (28), 1 yr of DR in cynomolgus monkeys did not result in reduced $G_b$ despite a similar (~34%) level of restriction (8). Lane et al. (29) found $G_b$ in rhesus monkeys to be reduced only after 3–4 yr of DR but noted that $G_b$ had increased in young adult R monkeys after 7 yr, resulting in only a small difference between the groups. Although that study lacked an older adult R group with which to compare, the $G_b$ levels of older C monkeys appeared to increase over the same time period as well. It is possible that small but significant DR-induced reductions of $G_b$ in younger adulthood may be attenuated with increasing age in nonhuman primates. It is not clear whether age or differences in feeding protocol explain the findings of Bodkin et al. (6), who reported no reduction in $G_b$ after ~9 yr of weight clamping (i.e., maintaining body weight at a constant level by titrating energy intake).

$I_b$ levels were markedly reduced for R monkeys; in contrast, $I_b$ of the normoinsulinemic C group increased and then appeared to level off for a few years, consistent with findings reported by Lane et al. (29). The decline in $I_b$ in both C and R groups at the 5.5-yr assessment is likely due to the change to a more specific insulin RIA at that time; however, among C monkeys, the mean $I_b$ level subsequently rose to pre-5-yr levels, whereas in the R monkeys, $I_b$ remained at lower levels. When the lower $I_b$ levels are taken into account after the change in insulin assay in both groups, these data suggest an increase in $I_b$ with age among ad libitum-fed normoinsulinemic monkeys with perhaps little, if any, rise observed among the R animals.

As observed in other chronically restricted monkeys (29), the acute plasma insulin response to glucose was also elevated among C vs. R monkeys. This difference was evident both before and after the change in insulin assay. The apparent drop in insulin response values among R monkeys after this change may be responsible, in part, for the elevated $S_I$ levels from 5.5 yr on. However, the apparent reduction in insulin response values among controls at 5.5 yr did not result in a sustained enhancement of $S_I$. Furthermore, because the substantial increase in $S_I$ among R monkeys is evident 6 mo earlier (i.e., at 5 yr), it is more likely that this was due to the small reduction in food allotment following the 4.5-yr assessment period. Likewise, an increase in $S_I$ was seen after the reduction in food allotment in R monkeys after 1.5 yr. It is reasonable to speculate that $S_I$ levels may also reflect an adaptation to chronic DR.

In agreement with our findings that $S_I$ was markedly increased and insulin responses were reduced in R monkeys within 1 yr of reestablishing ~30% difference in energy intake between treatment groups, Cefalu et al. (8) also reported $S_I$ to be significantly improved after only 1 yr of DR in adult cynomolgus monkeys. Likewise, Bodkin et al. (6) reported enhanced maximally insulin-stimulated glucose uptake as measured during a hyperinsulinemic euglycemic clamp procedure. Although this measure is not directly comparable with the minimal-model-derived $S_I$, they are highly correlated (4, 15). Agreement of these findings from studies with such diverse methodologies and among animals of varied ages and species further suggests a protective effect of energy restriction on the development of insulin resistance with increasing age. Moreover, the elevated fasting insulin and insulin response levels among ad libitum-fed C monkeys suggests a potentially greater exposure of these animals to insulin...
over time. Hyperinsulinemia itself, independent of hyperglycemia, has recently been proposed to be a major contributor to oxidative damage with age (14). Taken together, these data are consistent with a proposed theory that DR may exert its disease-retarding and life-extending benefits by reducing exposure to insulin over time (34).

The results from our longitudinal analyses are also consistent with the work of Hansen and Bodkin (20), demonstrating that prevention of obesity by weight clamping prevented the development of insulin resistance and type 2 diabetes in older rhesus monkeys. DR reduced total body fat over time in R monkeys, whereas monkeys in the C group gained a substantial amount of fat (12), including an increase in centrally located fat mass. Clearly, increasing adiposity is associated with hyperinsulinemia and insulin resistance (5, 9, 16, 35) and likely contributed to or resulted from the observed reduction in insulin sensitivity over time among some C monkeys. Insulin resistance, however, frequently occurs in the absence of overweight or obesity (1), and the question of whether the loss of fat mass, particularly visceral fat mass, with DR is requisite for improved insulin sensitivity is not resolved. The loss of body fat or prevention of fat gain does not appear to entirely explain DR-induced improvement of glucose metabolism, because chronic DR has had this effect in younger, lean adult monkeys (29). Short-term DR among older adult monkeys has also improved insulin levels and insulin response to glucose before a detectable change in body composition (30). Furthermore, when obese humans with type 2 diabetes are briefly energy restricted, most individuals exhibit rapid improvement of glucose metabolism, because chronic DR has had this effect in younger, lean adult monkeys (29).

In summary, we performed tolbutamide-modified FSIGTTs semiannually for 8.5 yr in male rhesus monkeys, one-half of which were subjected to chronic, moderate DR, and we analyzed the data with the use of Bergman’s minimal model. Changes in indexes of glucose metabolism were apparent within 0.5–1 yr after successfully reestablishing ~30% difference in treatment group energy intake, and after 8.5 yr, as the animals had entered middle age, DR protected against the development of insulin resistance and type 2 diabetes with no apparent adverse effect.

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