Maternal protein restriction leads to early life alterations in the expression of key molecules involved in the aging process in rat offspring

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Maternal protein restriction leads to early life alterations in the expression of key molecules involved in the aging process in rat offspring. Am J Physiol Regul Integr Comp Physiol 294: R494–R500, 2008. First published December 19, 2007; doi:10.1152/ajpregu.00530.2007.—Recent findings demonstrate that nutrition during the fetal and neonatal periods can affect the life span of an organism. Our previous studies in rodents using a maternal low protein diet have shown that limiting protein and growth during lactation [postnatal low protein (PLP) group] increases longevity, while in utero growth restriction (IUGR) followed by “catch up growth” (recuperated group) shortens life span. The aim of this study was to investigate mechanisms in early postnatal life that could underlie these substantial differences in longevity. At weaning, PLP animals had improved insulin sensitivity as suggested by lower concentrations of insulin required to maintain concentrations of glucose similar to those of the control group and significant upregulation of insulin receptor-β, IGF-1 receptor, Akt1, Akt2, and Akt phosphorylated at Ser 473 in the kidney. These animals also had significantly increased SIRT1 (mammalian sirtuin) expression. Expression of the antioxidant enzymes catalase, CuZnSOD, and glutathione peroxidase-1 was elevated in these animals. In contrast, recuperated animals had a significantly increased fasting glucose concentration, while insulin levels remained comparable to those of the control group suggesting relative insulin resistance. MnSOD expression was increased in these animals. These data suggest that early nutrition can lead to alterations in insulin sensitivity and antioxidant capacity very early in life, which may influence life span.

insulin signaling; antioxidant enzymes; longevity; SIRT1

IT IS WIDELY ACCEPTED THAT permanent calorie restriction (CR) can affect the rate of aging and longevity of a number of organisms including mammals (14). In recent years, however, it has emerged that nutrition during the fetal and neonatal periods can also affect life span. Studies in rodents using maternal low protein diets, without CR, have shown that limiting growth during lactation [postnatal low protein (PLP group)] increases longevity, while in utero growth restriction (IUGR) followed by accelerated postnatal growth (recuperated group) shortens life span (22, 28, 37, 44). These data indicate that both nutrition during fetal life and nutrition during lactation can affect the rate and phenotype of aging; however, the molecular mechanisms involved are largely unknown.

The kidney is one of the most metabolically active tissues (53) and is an important regulator of glucose homeostasis in humans (10, 17). It can both utilize and release glucose in the postabsorptive state, accounting for ~40% of all gluconeogenesis (17, 47). Renal glucose release can compensate to a certain extent for impaired hepatic glucose production and contributes to hyperglycemia in both type 1 and type 2 diabetes (10, 34). Renal failure is the most common cause of death amongst laboratory rats (20) and the prevalence of chronic renal disease in humans is rising by 8% annually (1), suggesting this is an important organ to consider in aging studies.

The IGF-1/insulin signaling pathway not only has a critical role in glucose homeostasis but also has been implicated in the aging process and determining longevity (6). Numerous studies in diverse species, including yeast, fruit flies, worms, and rodents have shown that reduced levels of IGF-1 and insulin signaling are associated with significantly extended longevity and that long-lived animals share several important phenotypic characteristics including reduced insulin signaling, increased insulin sensitivity, and increased resistance to oxidative stress (3, 30). It has been postulated that the beneficial effects of reduced IGF-1/insulin signaling on life span are exerted via FOXO transcription factors regulated by IGF-1/insulin signals via the PKB/Akt pathway (46) and by oxidative stress via SIRT deacetylases (8).

The loss of telomeric DNA has been linked to numerous age-related diseases including renal failure (2), and telomere length has been suggested as a predictor of biological aging (5, 13, 31). The rate of telomere shortening has been shown to be accelerated by oxidative damage (55) caused by a progressive imbalance between intracellular concentrations of reactive oxygen species (ROS) and oxidative defenses (53). Long-lived animals have reduced oxidative damage of macromolecules and increased stress resistance (15). We have recently shown that slower growth during the suckling period is associated with increased renal protein expression of the antioxidant enzymes: glutathione peroxidase-1 (GPX-1) and glutathione reductase (GR) at 3 mo of age, MnSOD at 12 mo, and with significantly longer renal telomeres at 12 mo (48). Alterations in glutathione metabolism have also been reported in liver of rats exposed to low protein diet in utero (27). In addition, administration of antioxidants during gestation in in utero protein-restricted rats has been shown to prevent detrimental alterations associated with aging (9). In particular, taurine supplementation during gestation normalized fetal β-cell proliferation and insulin secretion in low-protein offspring (24).

Most of our studies to date have focused on adult animals. However, to identify primary factors mediating the effect of changes in maternal nutrition, it is important to study early
times. Therefore, in this current study, we focused on the weaning time point. The hypothesis tested was that maternal protein restriction leads to early life alterations in the expression of key molecules involved in the aging process. Our main focus has been on the components of the insulin/IGF-1 signaling pathway and SIRT1. Additionally, we assessed the antioxidant capacity by measuring major antioxidant enzymes, including MnSOD, CuZnSOD, GPX-1, GR, and catalase.

**METHODS**

Animal maintenance and breeding. All procedures involving animals were conducted under the British Home Office Animals Act (1986). Adult female Wistar rats were housed individually and were maintained at 22°C on a 12:12-h light-dark cycle. When they reached weight of between 235 and 250 g, they were mated. The day on which vaginal plugs were expelled was taken as day 0 of gestation. Dams were fed ad libitum either a control diet (containing 20% protein) or an isocaloric low protein (8%) diet (both diets were purchased from Arie Blok, Woerden, the Netherlands) during gestation and lactation. Detailed diet composition is provided in Table 1. Cross-fostering techniques were used at birth to establish these study groups: 1) controls (offspring of control dams, culled to 8 (4 males and 4 females) and suckled by control dams); 2) recuperated (offspring of control dams fed a low-protein diet during pregnancy, but nursed by control dams); and 3) postnatal low-protein (offspring of control dams nursed by low-protein-fed dams, unculled to minimize the plane of nutrition). Litter size standardization was carried out randomly. Body weights of animals were recorded at birth and at days 3, 7, 14, and 21 of age. At day 21 pups were removed from dams and starved overnight. One female was selected at random from each litter in the current study. Fasting blood was collected by decapitation, allowed to clot for 30 min, and then centrifuged at 7,200 g for 3 min to obtain serum. Both kidneys were removed. Kidneys and serum were snap frozen and stored at -80°C until use.

**Glucose and insulin measurements.** Blood glucose was measured using a blood glucose analyzer (Hemocue, Angelholm, Sweden). Serum insulin concentrations were measured using a rat insulin ELISA kit (Merckodia Ultra-sensitive Rat Insulin ELISA, Merckodia, Uppsala, Sweden). All samples were assayed in duplicate, and an intra-assay coefficient of variation of up to 5% was accepted.

**Protein expression: Western blot analysis.** Total protein was extracted from each kidney by homogenization in ice-cold lysis buffer (50 mmol/l HEPES (pH 8), 150 mmol/l sodium chloride, 1% Triton X100, 1 mmol/l sodium orthovanadate, 30 mmol/l sodium fluoride, 10 mmol/l sodium pyrophosphate, 10 mmol/l EDTA, and a protease inhibitor cocktail III (Calbiochem Novabiochem Biociences, Nottingham, UK)). The total protein concentration in the lysates was determined using a Sigma copper/bicinchoninic assay. Protein content of kidney tissue did not differ between any of the groups. Samples were diluted to concentration of 1 mg/ml in Laemmli’s buffer. Twenty micrograms of total protein were subjected to overnight SDS-PAGE, and the proteins were then transferred to PVDF Immobilon-P (Millipore, Billerica, MA) membrane, blocked for 1 h (5% nonfat dehydrated milk, 1× TBS, and 0.1% Tween 20) and incubated overnight with antibody against: Akt1, Akt2 and phospho-Akt (Ser 473) from Cell Signaling (Beverly, MA); IGF-1 receptor (IGF-1R), insulin receptor-β (IR-β), PKCγ, phosphatidylinositol 3-kinase (PI3-kinase) p110β from Santa Cruz Biotechnology, (Santa Cruz, CA); PI3-kinase p85α, SIRT1, MnSOD from Upstate Biotechnology (Millipore, Billerica, MA); GPX-1 and GR (Ab-cam), and CuZnSOD (R&D). Autoradiographed images were captured and were quantified densitometrically using AlphaEase software (AlphaImager).

Twenty-four samples were run on a single gel alongside molecular weight markers and a positive control. Additionally, 20 and 10 μg of one sample were loaded onto each gel to ensure the linearity of the signal. Before analysis, control blots were performed for each antibody with varying amounts of protein (5, 10, and 20 μg) loaded onto the gel to ensure that the chemiluminescent signal changed in a linear manner. Primary and secondary antibody concentrations were also optimized.

**Telomere length detection.** Nonsheared, high molecular size DNA (average size 97 kb) was isolated from kidney samples using a commercial method of DNA extraction (Qiagen). DNA quality and integrity was determined using a spectrophotometer (GeneQuant; Pharmacia Biotech). DNA (1.2 μg) was digested with Hinf1 and Rsa1 restriction enzymes (16.6 U/μg DNA; Roche Diagnostics, Mannheim, Germany) for 2 h at 37°C. The digested DNA was then separated using Pulsed Field Gel Electrophoresis (Chef-DR III; Bio-Rad, Hercules, CA; Ref. 11). Controls used in each gel were a mid range Pulsed Field Gel maker (New England Biolabs) and a dioxogenin (DIG; low range) molecular weight marker.

After electrophoresis, the gel was washed for complete digestion of the digested DNA by staining with ethidium bromide (50 μl/l). The gels were visualized using an Alpha Imager UV light source (Alpha Innotech) and photographed with P/N Polaroid film.

Southern blotting was used to transfer the resolved DNA fragments onto a positively charged nylon membrane (Roche Diagnostics, Mannheim, Germany), which was then cross-linked onto the nylon membrane using a UV Stratalinker (TM 2400; Stratagene, CA). Telomeric repeat length was determined using a slightly modified commercial method of Chemiluminescent detection (Roche Diagnostics, Mannheim, Germany; Ref. 11). The telomere signals were analyzed using Adobe Photoshop and MacBas computer software.

**Telomere length analysis and quantification.** Telomere length was measured as we have described previously (49) whereby the percentage intensity (telomere length) of the telomeric signal was determined in four molecular size regions, as defined by molecular weight markers. Specific grid squares were placed around the telomeric smear according to the following molecular weights: 145-48.5, 48.5-8.6, 8.6-4.2, and 4.2-1.3 kb. Percent telomere length (expressed as photo-stimulated luminescence) was measured as previously described (11).

**Statistical analysis.** All data was presented as means ± SE, except insulin data, which was log transformed before examination and is shown as geometric mean (confidence intervals). Protein expression and telomere length data were statistically analyzed using a one-way ANOVA with maternal diet as the independent variable factor. When the effect of maternal diet was significant, Duncan’s post hoc testing was used to analyze significance differences between groups. A P value <0.05 was considered statistically significant.

**RESULTS**

**Litter sizes and body weights.** No significant difference in the litter size was seen between the groups (on average, control: 12.9 ± 0.6 pups per litter; PLP: 12.8 ± 0.7; and recuperated: 13.6 ± 0.6). At birth pups of mothers fed the low-protein diet (recuperated group) were significantly smaller...
than controls (6.6 ± 0.2 vs. 7.5 ± 0.2 g, respectively; P < 0.001). By day 7 PLP animals were smaller than controls (11.3 ± 0.5 vs. 17.0 ± 1.2 g; P < 0.001) and remained so until weaning (day 14: 17.4 ± 1.1 vs. 35.4 ± 0.7 g; P < 0.001 and day 21: 25.1 ± 1.6 vs. 54.9 ± 1.1 g; P < 0.001). In contrast, recuperated offspring remained smaller than control animals at day 7 (14.3 ± 0.6 vs. 17.0 ± 1.2 g; P < 0.001) but underwent catch-up growth, so by day 14 the body weights of recuperated and control groups were comparable (33.8 ± 1.2 vs. 35.4 ± 0.7 g, respectively) and remained comparable until weaning (55.0 ± 1.9 vs. 54.9 ± 1.1g).

Glucose and insulin analysis. Fasting blood glucose concentrations were similar in control and PLP groups; however, glucose was significantly elevated in the recuperated group (P < 0.001; Table 2). Fasting serum insulin levels were significantly lower in PLP animals (P < 0.001 compared with the control group; Table 2). There was no statistical significant difference between serum insulin concentrations in control and recuperated animals.

Protein expression of insulin-signaling molecules. There was a significant effect of maternal diet on protein expression of IR-β (P < 0.01) with expression being increased in the PLP group (by 77%) compared with control animals (Fig. 1A). Maternal diet also had a significant effect (P < 0.01) on IGF-1R protein expression, with a 70% increase in the PLP group compared with the control group (Fig. 1B). The effect of maternal diet on the protein expression of PI3-kinase p110β subunit tended to be significant (P = 0.053) with the PLP group showing a reduction of 25% (P < 0.05) compared with control animals (Fig. 1C). There was no significant difference in the protein expression of p110β between control and recuperated offspring. Maternal diet also exerted a significant effect (P < 0.001) upon Akt1 with protein expression being significantly increased (70%) in the PLP group (P < 0.001) compared with controls. There was also a small but statistically significant increase in Akt1 in recuperated animals (27%; P < 0.05) compared with control offspring (Fig. 1D). A similar effect of maternal diet was observed on the protein expression of Akt2 (P < 0.001, which was significantly increased in both the PLP (P < 0.001) and recuperated groups (P < 0.001) compared with controls (Fig. 1E). Maternal diet had a significant effect on phosphorylation of Akt at Ser 473 (P < 0.01) with the PLP group having a higher phosphorylation level of nearly 300% compared with the control group. No significant difference in Akt phosphorylation was observed between the control and recuperated groups (Fig. 1F). There was no effect of maternal diet on either PKCζ protein expression (Fig. 1G) or PI3-kinase p85α subunit expression (Fig. 1H).

Protein expression of SIRT1. There was a significant effect of maternal diet on SIRT1 protein expression (P < 0.001), with SIRT1 levels being increased in the PLP group by nearly 70% compared with the control group (Fig. 2). No significant difference in SIRT1 protein expression was observed between control and recuperated animals.

Telomere length data. No effect of maternal diet was seen on renal telomere lengths at 22 days of age (Fig. 3).

Protein expression of antioxidant enzymes. Maternal diet had a significant effect (P < 0.001) on MnSOD protein expression with MnSOD protein expression being increased in the recuperated group by nearly 40% (P < 0.001) compared with control animals. No effect of maternal diet was seen on MnSOD protein expression in the PLP group compared with controls (Fig. 4A). Maternal diet tended to have an effect on protein expression of CuZnSOD (P = 0.05), with PLP animals having significantly increased CuZnSOD expression (P < 0.05; Fig. 4B). A significant effect of maternal diet was also observed on catalase protein expression (P < 0.01). The expression of catalase was significantly increased by nearly 40% in the PLP group (P < 0.01) compared with control animals. No effect was seen on catalase protein expression in recuperated animals compared with control offspring (Fig. 4C). There was no significant effect of maternal diet on the protein expression of GR in female kidney at 21 days of age (Fig. 4D). Maternal diet had a significant effect on GPX-1 protein expression (P < 0.001) with nearly a 40% increase in GPX-1 protein expression in PLP animals (P < 0.01) and a tendency for the GPX-1 protein expression to be decreased in recuperated animals (P = 0.06) compared with the control group (Fig. 4E).

**DISCUSSION**

The aim of this study was to investigate the mechanisms present in early postnatal life that may underlie the differences in the aging phenotype observed among rodents that experienced protein restriction in utero or during lactation. Here we have confirmed previous observations showing a significant effect of maternal protein restriction on the birth weight of the offspring (22). At birth, pups of mothers fed the low-protein diet (recuperated group) were significantly smaller than both controls and PLP animals. Recuperated animals underwent catch-up growth, so by day 14 the body weights of control and recuperated groups were comparable. In contrast, by 7 days of age the PLP offspring were significantly smaller than the control group. PLP animals remained significantly smaller than controls throughout the adulthood (48). Smaller body size is a striking characteristic of most long-lived mutants. The negative correlation of body size and longevity has been reported in laboratory rats and humans (41, 43). Smaller size might therefore be a phenotypic marker of some developmental/metabolic factor(s) that predispose to extended life span. However, reduced body size per se does not increase life expectancy, as, for example, an increase in body size of Snell dwarf mice did not shorten their life span (54).

To assess insulin sensitivity, fasting glucose and plasma insulin concentrations were measured in parallel with expression of proteins in insulin/IGF-1 signaling pathway. Recuperated offspring had increased glucose concentrations, but insulin concentrations were comparable to those of the control group at 22 days of age, suggesting that in utero growth restriction followed by accelerated postnatal growth is detrimental to glucose homeosta-

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**Table 2. Fasted blood glucose and serum insulin concentrations in female rats at weaning**

<table>
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<tr>
<th></th>
<th>Control</th>
<th>PLP</th>
<th>Recuperated</th>
</tr>
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<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.2±0.4</td>
<td>6.0±0.4</td>
<td>8.5±1.0*</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>34 (15–50)</td>
<td>5 (3–11)*</td>
<td>17 (12–35)</td>
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</tbody>
</table>

Glucose data are means ± SE. Insulin data are log transformed before examination and are shown as geometric mean (confidence intervals); n = 8 per group. *P < 0.001 when compared to controls.
sis. Hyperglycemia in these animals at a very young age of 22 days clearly suggests the presence of early defects perhaps at the level of pancreas, kidney, muscle, or liver tissue. These could not only have detrimental effects on long-term health but also on longevity. Conversely, PLP animals had significantly lower concentrations of insulin and similar concentrations of glucose compared with controls indicating that these animals were more insulin sensitive than control offspring. Increased renal insulin sensitivity is also suggested by the striking increases in expression of proteins in the insulin/IGF-1 signaling pathway in PLP animals.

Both IR-β and IGF-1R expression were significantly upregulated in the PLP group alongside downstream components of IR/IGF-1R signaling pathway: Akt1, Akt2, and phospho Akt Ser473 compared with control animals. There was no significant difference in the expression of PI3-kinase p85α and PKCζ. Reduced insulin levels and improved whole body insulin sensitivity maintaining a glucose concentration comparable to those of control animals is an important phenotypic characteristic of PLP animals.

Fig. 1. The effect of maternal protein restriction on renal insulin receptor-β (IR-β; A) and IGF-1 receptor (IGF-1R; B) and downstream insulin signaling molecules: phosphatidylinositol 3-kinase (PI3-kinase) p110β (C), Akt1 (D), Akt2 (E), and Akt phosphorylation at Ser473 (F), PKCζ (G), and PI3-kinase p85α protein expression (H); n = 8 per group. PLP, postnatal low protein. Data are %mean of control group ± SE. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. control.

Fig. 2. Effect of maternal protein restriction on renal SIRT1 protein expression; n = 8 per group. Data are %mean of control group ± SEM. ***P < 0.001 vs. control.

Fig. 3. Effect of maternal protein restriction on renal telomere length; n = 8 per group. PSL, photo-stimulated luminescence.
A similar phenotype is shared by both CR animals and long-lived mutants ranging from yeast to rodents and monkeys (42, 52). The association between improved insulin sensitivity and prolonged life span has also been proven to exist in humans, insulin sensitivity normally declines with age but centenarians have been shown to have greatly enhanced sensitivity to insulin compared with younger subjects (38, 39).

The significant increase in Akt1 and Akt2 expression and phosphorylation of Akt at Ser473 in PLP animals is consistent with an increased longevity phenotype. Akt is a serine/threonine protein kinase that acts as major signal transducer downstream of activated PI3-kinase and via multiple mechanisms can regulate cell metabolism, survival, and proliferation (25, 46). Akt has been shown to exert anti-apoptotic actions via numerous pathways including direct phosphorylation of the FOXO subfamily of Forkhead transcription factors (18, 46). The upregulation of Akt in PLP animals could potentially indicate that these animals benefit from increased protection from apoptosis. Although in recuperated animals there was increased expression of both Akt1 and Akt2, there was no increase in phosphorylated Akt. This could indicate impaired Akt phosphorylation in these animals. Disruption of Akt2 signal transduction could lead to impaired glucose metabolism in these animals. Knockout of Akt2 in mice is known to lead to decreased insulin resistance and diabetes (12).

Sir2, the NAD+-dependent histone deacetylase, has been shown to modulate the life-extending effects of CR in Saccharomyces cerevisiae and Caenorhabditis elegans (23, 50). SIRT1, the mammalian homologue of Sir2, has been also shown to be an important modulator of the mammalian aging process (19). Here we show that maternal nutrition can affect the expression of SIRT1 in the offspring at weaning. SIRT1 expression was significantly increased in the PLP group, while there was no significant difference in SIRT1 levels between the control and recuperated groups. There are several ways via which the increased expression of this deacetylase could contribute to the prolonged life of the PLP animals. Firstly, SIRT1 has been shown to act on transcription factors including the FOXO family (8), p53, and NF-κB (57), therefore, affecting survival through preventing apoptosis and increasing stress resistance. Secondly, SIRT1 can enhance DNA repair activity via formation of complexes with Ku70 (21). Thirdly, SIRT1 has been shown to control hepatic glyconeogenic/glycolytic pathways in response to nutrients via transcriptional coregulator peroxisome proliferator-activated receptor gamma co-activator (PGC)-1α. SIRT1-mediated deacetylation of PGC-1α led to an increase in hepatocyte nuclear factor-4α, a transcription factor that activates gluconeogenic genes and hence glucose production (40). It remains to be seen if a similar PGC-1α-mediated mechanism is involved in gluconeogenesis in the kidney.

It is well documented that the aging process is associated with shortening of telomeres and that telomere shortening can be accelerated by oxidative stress. We did not find any significant differences in telomere length between groups at 22 days. We have assessed the effect of maternal diet on the ability of the offspring to neutralize ROS in the kidney by analyzing protein expression of antioxidant enzymes: mitochondrial MnSOD, peroxisomal catalase and cytoplasmic GR, CuZnSOD, and GPX-1. MnSOD and CuZnSOD are responsible for dismutation of O2− into O2 and H2O2, while catalase and GPX-1 catalyze the breakdown of H2O2 into H2O and O2. We have observed a striking increase in the protein expression of catalase, GPX, and CuZnSOD in the PLP group. Of interest is that observed changes in the expression of antioxidants seen in the PLP group at 22 day of age are also present in adulthood. At 3 mo of age, PLP animals have significantly upregulated protein.
expression of GPX-1 and GR compared with the control group (48). The upregulation of antioxidant defenses could be interpreted in two ways. Either antioxidant enzyme defenses are upregulated because PLP animals encounter greater oxidative damage or upregulation of these enzymes prevents damage occurring in the first place enabling PLP animals to catalyze H$_2$O$_2$ into H$_2$O and O$_2$ more efficiently than the other two groups. Reduced levels of insulin and increased insulin sensitivity seen in PLP animals would be expected to lead to reduced oxidative metabolism, characteristics that are seen in various models of long-lived mutant mice including Ames dwarf and Swell dwarf mice (4). This is supported by the observation that a strong negative correlation exists between serum insulin (and glucose) concentrations and catalase activity (7, 56). The increase in GPX-1 protein expression in the PLP animals is puzzling. Mice overexpressing GPX-1 develop hyperglycemia and hyperinsulinemia and have reduced insulin sensitivity, accompanied by downregulation of insulin-mediated phosphorylation of Akt protein (29, 33). Such a phenotype is very different to that observed in PLP animals. As GPX-1 has been shown to protect against diquat-induced cell death, but promote peroxynitrite-induced cell death, its toxicative action may vary depending on the nature of the stressor (ROS or reactive nitrogen species related), the insult level, and the duration (16, 29). Although several studies reported that increased CuZnSOD has only a slight effect on longevity (51), the absence of CuZnSOD has been associated with elevated oxidative stress, loss of muscle mass, and acceleration of normal age-related sarcopenia (35). Taken together, the increased protein expression of the antioxidant enzymes in the PLP kidney may protect it from the age-related telomere shortening and age-associated renal damage.

We found mitochondrial MnSOD protein expression to be significantly increased in the recuperated animals compared with both control and PLP groups. Again this could indicate that either there is more oxidative damage taking place or that elevated oxidative enzyme prevents damage from occurring. Recently we have shown that mitochondrial abnormalities are present in the kidneys of 12-mo-old recuperated animals due to functional deficit of the mitochondrial cofactor coenzyme Q$_9$ (CoQ9) that leads to reduction in activity of complex II-III of electron transport chain (45). It remains to be seen if this reduction is accompanied by changes in mitochondrial ROS and MnSOD expression. However, it has been shown that hyperglycemia increases the generation of the superoxide anion (O$_2^-$) by interfering with the flow of electrons along the mitochondrial electron transport chain (36) and that glucose-induced ROS generation can lead to the activation of apoptosis (26). It has been reported that in utero protein restricted rats do not show alterations in oxidative injury markers at 18 mo of age (28); however, as glucose metabolism or protein expression of antioxidant enzymes were not assessed in this study, it would be of interest to investigate these parameters at an older age. The findings of this study are in agreement with Hormesis Hypothesis, which has been proposed to explain the life-extending action of calorie restriction. The hypothesis postulates that a low-intensity biological stressor exerts defense responses in the organism that help protect it against the causes of aging. The enhanced coping with intense stressors and restriction of senescent deterioration lead to retardation of age-associated diseases and increased longevity (32). It seems that a similar process may underlie the association between nutrition and aging.

**Perspectives and Significance**

Aging and life span are controlled by complex molecular networks. Here we show that both nutrition during fetal life and nutrition during lactation can alter the components of major pathways associated with the aging process in the kidney. Animals growth restricted during pregnancy but who underwent catch up growth during suckling (recuperated group) displayed hyperglycemia at a very young age and parameters associated with accelerated aging phenotype. In contrast animals, which grew slower during the suckling period had improved insulin sensitivity, increased SIRT1 protein expression and elevated protein expression of the antioxidant enzymes, all of which are associated with prolonged life span. Therefore it can be concluded that the susceptibility to oxidative damage and telomere shortening might be already defined at a very young age and may affect the rate and phenotype of aging. Further studies are needed to determine whether similar alterations are present in other organs and tissues. Investigations into the molecular mechanisms via which diet influences longevity of the offspring so profoundly may ultimately enable us to slow the aging process.

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