Journal of Gerontology: BIOLOGICAL SCIENCES
1998, Vol. 53A, No. 2, B94–B101

Protein Glycation in the Aging Male Sprague-Dawley Rat: Effects of Antiaging Diet Restrictions

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Protein glycation and accumulation of advanced glycosylated end-products (AGEs) are supposed to play an important role in the process of aging. Dietary restriction increases life span and delays the onset of most age-associated diseases. Age-dependent changes in glucose homeostasis and glycated plasma proteins and hemoglobin were determined, and AGEs formation was measured as fluorescence in skin and aortic collagens in male Sprague-Dawley rats fed ad libitum or subjected to every-other-day feeding or 40% food restriction. In aging control rats, skin and aortic collagen-linked fluorescence increased with a similar exponential curve (aortic values being always higher), whereas glycated plasma protein and hemoglobin decreased slightly. Dietary restrictions decreased glycated plasma proteins and fluorescent products in skin collagen of younger but not older rats, and did not affect glycated hemoglobin or aortic collagen fluorescence. In conclusion, our data indicate that age-related changes in glucose homeostasis do not play a substantial role in aging; and collagen-linked fluorescence increases significantly during aging, but it may not be sensitive to dietary intervention.

THE term *glycation* (nonenzymatic glycosylation) denotes a multireaction pathway whereby the free aldehyde group of sugars reacts with primary amino groups of proteins leading to the formation of an unstable Schiff-base type compound (aldimine). In long-lasting biological molecules only, the rearranged products may undergo further transformations that finally lead to the formation of irreversible complexes known as heterogeneous advanced glycosylation end-products (AGEs) (1). The Maillard reaction refers to all reactions encompassing the initial, intermediate, and advanced glycosylation end-product reactions; most available data have been generated by measurements of protein-linked fluorescence (2,3).

The main factors responsible for AGE product accumulation include the concentration of blood glucose, the time of exposure of proteins to the sugar (mainly depending on the protein turnover), and the rate of their removal by tissue macrophages (4,5). Not only the first, but also the last of these factors can be modulated by insulin (6). In addition, temperature could be important in determining the rate of protein modifications.

From a gerontological perspective, it is interesting that advanced glycation shows a progressive age-related increase in skin and tendon collagen (7), basement membrane proteins (8), and lens crystallin (9). It has been reported that the accumulation of fluorescent AGE products can alter the function of nucleic acids (10) and proteins, including collagen (11); it may be responsible for several age-associated diseases, and it also plays a role in normal aging processes (12). On the other hand, very little is known with regard to the effects of dietary restriction, a treatment that increases life span, retards a broad spectrum of age-related physiological changes, and delays or prevents many age-associated diseases (13).

We investigated the effects of aging on the accumulation of stable glycation products of certain proteins with different turnover rates, located in different compartments, namely collagen, plasma proteins, and hemoglobin. These proteins were studied in animals either fed ad libitum or subjected to two different types of food restriction, namely every-other-day feeding (14) and the more widely used 40% restriction (15). Despite their similar effectiveness on aging modulation, these two dietary regimens are characterized by different effects on carbohydrate metabolism and insulin function (16).

Materials and Methods

Animals.—Male Sprague-Dawley albino rats were purchased from Nossan (Milan, Italy) at 2 months of age, randomly divided into three groups, and subjected for various lengths of time to the following dietary regimens: (a) ad libitum feeding (AL, controls); (b) every-other-day feeding ad libitum (EOD); and (c) 40% food restriction (40% DR), i.e., this last group received 60% of the amount consumed by the ad libitum group. The animals were subjected to a controlled 12-h dark-light cycle and received a standard (Randoin Causeret) pellet diet. Food administration was adjusted weekly, and body weight was measured at 4-week intervals.

As to the mortality rate of the animals used in this study, the surviving rats in the AL, EOD, and 40% DR groups were 90%, 95%, and 100%, respectively, at 5 months of age; 80%, 92%, and 90%, respectively, at 12 months; 43%, 90%, and 81%, respectively, at 18 months; 35%, 80%, and 70%, respectively, at 24 months.

Preparation of tissues.—Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and blood samples were taken from the tail vein and collected in EDTA-treated tubes for the measurement of glycated hemoglobin and plasma glucose, insulin, and glycated proteins.

Samples of abdominal skin and segments of thoracic aorta were then excised, freed from extraneous tissue, flushed with isotonic saline, and frozen at –80°C until analysis.

Assays.—Plasma glucose was assayed by a glucose-oxidase technique (Glucinet, Sclavo, Siena, Italy). Insulin was measured by radioimmunoassay in accordance with Herbert et al. (17) using rat insulin as a standard. The sensitivity and the coefficients of variation of the radioimmunoassay were as follows: detection limit 0.13 ng/ml, intraassay variation 3.1%, interassay variation 10.2%.

Measurement of glycated hemoglobin and plasma proteins was performed by boronic affinity chromatography (18), using a commercially available kit (Glycotest, Pierce Chemical, Rockford, IL).

Preparation of collagen digests.—The procedure described by Monnier et al. (19) was followed with minor modifications. On the day of analysis, skin samples were frozen in liquid nitrogen to allow an easier removal of epidermis and fat with a razor blade. The residual tissue was washed in 0.15 M saline, minced in 10 ml of cold phosphate-buffered saline (pH 7.4), and homogenized for 60 sec with an Ultra Turrax homogenizer (Janke & Kuntel KG, IKA-Werk, Staufen, Germany). The aortic samples were washed and then finely minced with dissecting scissors (20,21).

The samples were extracted with chloroform-methanol (2:1) for 24 h at 4°C to remove lipids and washed with methanol and water. Collagen digestion was carried out for 24 h at 37°C in shaking bath by using 250 U of type VII collagenase (Sigma, St. Louis, MO), which resulted in 96% solubilization of the collagen. The clear supernatant, resulting from centrifugation of the digest, was used for determination of fluorescence and hydroxyproline content, as indicated by hydroxyproline measurement of the re-digested pellet.

One milliliter of distilled water was added to the supernatant to make hydroxyprolin concentration range of 600–800 μg and fluorescence was measured against water, at an emission wave length of 440 nm on an excitation wave length of 370 nm, by a fluorescence spectrometer (Perkin-Elmer 650-10S).

All fluorescence values were corrected for collagenase blanks. Results were expressed in arbitrary fluorescence units per milligram of collagen. Collagen content was calculated on the base of hydroxyproline measurements in the digests assuming a hydroxyproline content of 14% in collagen by weight (22). Hydroxyproline was assayed in accordance with the method of Stegemann and Stalder (23), as modified by Maekawa et al. (24).

Statistical analysis.—Data are expressed as means ± *SEM*. Statistical analysis included determination of regression lines and relative correlation coefficients. The statistical significance of the effects of age and type of food restriction on plasma glucose and insulin levels, as well as their interactions, glycated plasma proteins and hemoglobin, and collagen-linked fluorescence were assessed by analysis of variance (ANOVA). Tukey post-test with multiple comparisons was also used.

A specific power analysis was presented for key non-significant results (25).

Results

Animals' body weight.—The changes in animals' body weights during the 22-month experimental period are illustrated in Figure 1. From our data, EOD and 40% DR rats weighed less than control rats by 28% and 36% on average, respectively.

Plasma glucose and insulin levels.—Table 1 shows plasma glucose and insulin levels in fed rats of various ages, subjected to different dietary regimens. The effect of age on plasma glucose concentrations was not significant [$F(3,61) = .47$, n.s.]. In EOD rats, glucose concentrations were similar to those of controls at all ages. Glycemic levels of 40% DR rats were significantly lower than controls after 3 months of dietary treatment (–30%) and remained stable at these lower values throughout their lifetime, as assessed by Tukey test between diets. A significant effect of age on plasma insulin concentrations was found [$F(3,59) = 9.82$, $p < .01$]. A significant effect of food restriction on insulinemia was also observed [$F(2,59) = 8.95$, $p < .01$], attributable mostly to 40% DR (Tukey test).

Table 2 summarizes the changes in plasma glucose and insulin levels in control and food-restricted rats after 24 h fasting. No statistically significant difference was found for plasma glucose values as a function of age [$F(3,60) = 2.24$, n.s.]. Glycemic values in controls and EOD were similar, and higher than in 40% DR rats (Tukey test). In regard to plasma insulin, the effects of age and food restriction were both significant [$F(3,54) = 32.51$, $p < .01$ and $F(2,54) = 4.47$, $p < .05$, respectively]. The interaction between age and food restriction was also significant [$F(6,54) = 2.57$, $p < .05$]. Indeed, plasma insulin levels were unchanged between 2 and 18 months and increased significantly at 24 months in control and EOD rats; in oldest 40% DR, insulinemia was significantly lower than in age-matched EOD animals (Tukey test).

Glycated plasma proteins and hemoglobin.—The levels of the Amadori products that accumulated in the plasma proteins of experimental animals are shown in Table 3. By ANOVA, the effects of age and that of food restriction were both significant [$F(3,60) = 2.9$, $p < .05$ and $F(2,60) = 15.05$, $p < .01$, respectively]. In particular, in food-restricted rats the levels of glycated plasma proteins decreased transiently after 3 months of dietary regimen, without any significant difference between the two types of dietary restriction, as assessed by Tukey test.

The percentages of glycated hemoglobin in control and food-restricted rats are shown in Table 4. Unlike the case of plasma proteins, total glycated hemoglobin increased by approximately 33% after 2 months of age. Thereafter, values remained unchanged until 18 months and declined significantly at 24 months (Tukey test). The effect of food restriction was not significant.

Advanced glycosylation end products (AGE products).—The accumulation of AGE products, a possible biomarker

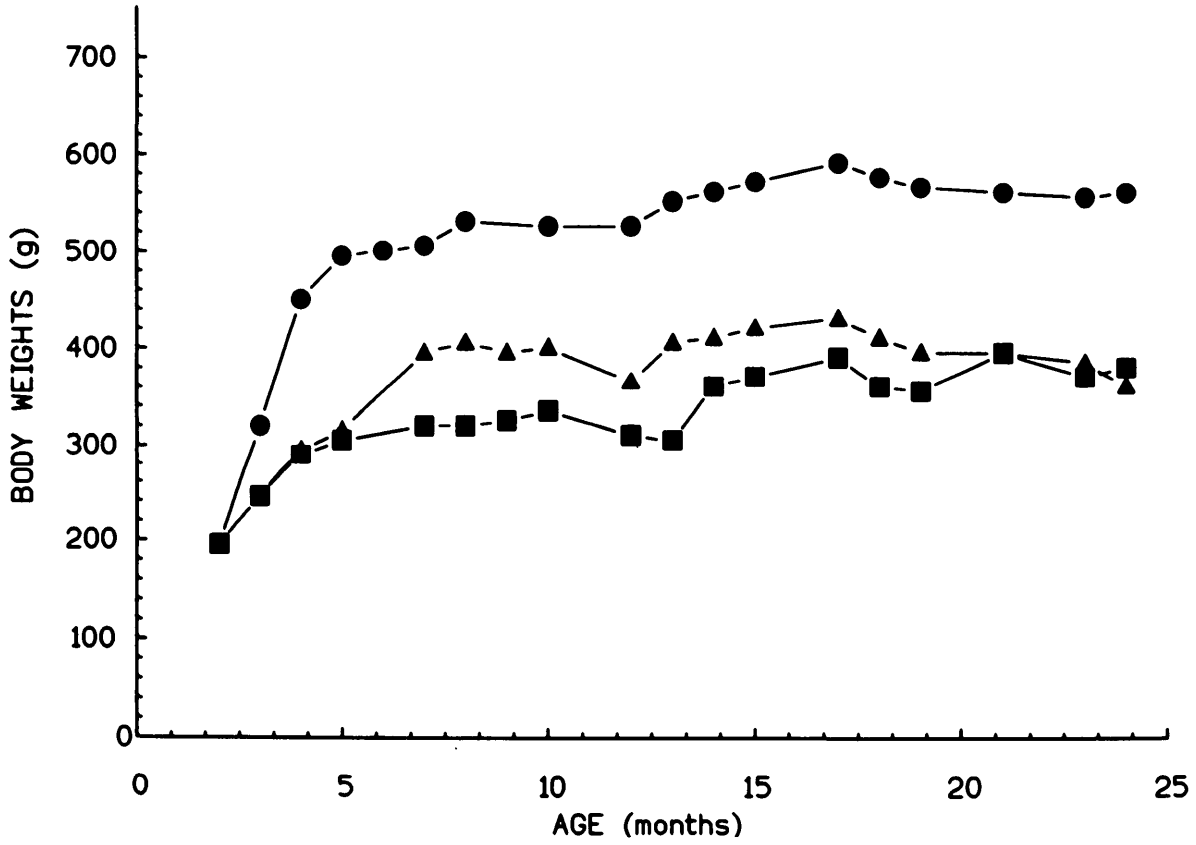

Figure 1. Body weights of control (●), every-other-day feeding (▲), and 40% food restricted (■) Sprague-Dawley rats.

Table 1. Plasma Glucose and Insulin Levels in Nonfasted Sprague-Dawley Rats of Various Ages, Subjected to Different Dietary Regimens

	Controls		EOD		40% DR	
Age (months)	Plasma Glucose* (mg/dl)	Plasma Insulin† (ng/ml)	Plasma Glucose (mg/dl)	Plasma Insulin (ng/ml)	Plasma Glucose (mg/dl)	Plasma Insulin (ng/ml)
2	128 ± 2	4.3 ± 1.0	—	—	—	—
5	122 ± 10	4.5 ± 0.7	123 ± 5	2.8 ± 0.9	90 ± 5	3.0 ± 0.9
12	120 ± 4	6.3 ± 1.2	128 ± 9	6.0 ± 0.4	96 ± 6	1.6 ± 0.2
18	125 ± 4	5.3 ± 0.5	115 ± 8	4.0 ± 0.8	100 ± 8	1.2 ± 0.2
24	125 ± 9	8.3 ± 0.8	132 ± 6	7.8 ± 0.4	98 ± 12	7.5 ± 2.4

Notes: Results are expressed as means ± *SEM* of 4–9 observations. In plasma glucose, differences between controls versus 40% ad libitum (DR) and dietary restriction (EOD) versus 40% DR were significant; difference between controls versus EOD was not significant. In plasma insulin, difference between controls and 40% DR only was significant; differences between following ages were significant: 5 versus 24 mo; 12 versus 24 mo; 18 versus 24 mo (Tukey test).

*Plasma glucose: F(age) = 0.47 (df = 3, 61) n.s. (power = 74%; δ = 20 ng/ml, ϕ = 1.5, SD = 21.3); F(diet) = 18.6 (df = 2, 61) $p < .01$; F(interaction) = 0.43 (df = 6, 61) n.s.

†Plasma insulin: F(age) = 9.82 (df = 3, 59) $p < .01$; F(diet) = 8.95 (df = 2, 59) $p < .01$; F(interaction) = 1.13 (df = 6, 59) n.s.

of aging, was investigated in the long-lived protein, collagen, from two different locations. Figure 2 shows the effect of aging on fluorescence values of insoluble subcutaneous collagen in rats fed ad libitum or subjected to dietary restrictions. In control rats, skin fluorescence levels increased following an exponential curve ($y = 6.26e^{0.080x}$, $r = .827$, $p < .01$). Actually, the values of skin collagen fluorescence were unchanged during the first 7 months of age, then rose, showing two sharp increments, the first between 9.5 and 12 months, and the second between 18 and 24 months. At this latter age, subcutaneous collagen-linked fluorescence was sixfold that of 2-month-old animals. It may be interesting to notice that the real fluorescence value obtained at 12 months of age was much higher than the expected value of the best fit curve.

In both 40% DR and EOD rats, a similar age-related increase in fluorescence occurred, best fitted by exponential curves ($y = 5.79e^{0.078x}$, $r = .855$, $p < .01$ in EOD and $y = 5.43e^{0.080}$, $r = .857$, $p < .01$ in 40% DR rats). A significant effect of food restriction on fluorescence was observed

Table 2. Plasma Glucose and Insulin Levels Measured After 24 h Fasting in Sprague-Dawley Rats of Various Ages, Subjected to Different Dietary Regimens

Age (months)	Controls		EOD		40% DR	
	Plasma Glucose* (mg/dl)	Plasma Insulin† (ng/ml)	Plasma Glucose (mg/dl)	Plasma Insulin (ng/ml)	Plasma Glucose (mg/dl)	Plasma Insulin (ng/ml)
2	115 ± 6	2.7 ± 0.4	—	—	—	—
5	111 ± 8	1.9 ± 0.1	96 ± 7	1.9 ± 0.3	96 ± 11	1.4 ± 0.4
12	96 ± 4	2.5 ± 0.6	103 ± 5	2.5 ± 0.5	88 ± 8	1.6 ± 0.3
18	104 ± 5	2.7 ± 0.8	102 ± 5	1.4 ± 0.2	81 ± 3	1.8 ± 0.2
24	119 ± 8	5.2 ± 0.6	107 ± 6	6.6 ± 0.5	92 ± 7	3.7 ± 0.4

Notes: Results are expressed as means ± *SEM* of 4–9 observations. In plasma glucose, differences between controls versus 40% DR and EOD versus 40% DR were significant; difference between controls versus EOD was not significant. In plasma insulin, difference between EOD and 40% DR only was significant; differences between following ages were significant: 5 versus 24 mo; 12 versus 24 mo; 18 versus 24 mo (Tukey test).

*Plasma glucose: F(age) = 2.24 (df = 3, 60) n.s. (power = 85%; δ = 20 ng/ml, ϕ = 1.8, SD = 17.9); F(diet) = 6.1 (df = 2, 60) $p < .01$; F(interaction) = 1.03 (df = 6, 60) n.s.

†Plasma insulin: F(age) = 32.51 (df = 3, 54) $p < .01$; F(diet) = 4.47 (df = 2, 54) $p < .05$; F(interaction) = 2.57 (df = 6, 54) n.s.

Table 3. Glycated Plasma Protein Levels in Sprague-Dawley Rats of Various Ages Subjected to Different Dietary Regimens

Age (months)	Glycated Plasma Proteins (%)		
	Controls	EOD	40% DR
2	2.10 ± 0.23 (8)		
5	1.92 ± 0.37 (4)	0.97 ± 0.10 (5)	0.81 ± 0.26 (4)
12	2.13 ± 0.11 (5)	1.47 ± 0.11 (4)	1.24 ± 0.08 (4)
18	1.70 ± 0.12 (7)	1.38 ± 0.08 (5)	1.38 ± 0.07 (5)
24	1.70 ± 0.21 (11)	1.47 ± 0.33 (9)	1.34 ± 0.12 (6)

Notes: Glycated plasma protein levels are expressed as percentages of total plasma proteins. Results are expressed as means ± SEM of the number of observations indicated in parentheses. F(age) = 2.9 (df = 3, 60) $p < .05$; F(diet) = 15.0 (df = 2, 60) $p < .01$; F(interaction) = 1.73 (df = 6, 60) n.s.

The following differences between ages were significant: 5 versus 12 months; 5 versus 18 months (Tukey test). Differences between controls versus EOD and controls versus 40% DR were significant; difference between EOD versus 40% DR was not significant (Tukey test).

Table 4. Glycated Hemoglobin Levels in Sprague-Dawley Rats of Various Ages Subjected to Different Dietary Regimens

Age (months)	Glycated Hemoglobin (%)		
	Controls	EOD	40% DR
2	1.9 ± 0.07 (10)		
5	2.5 ± 0.07 (6)	2.4 ± 0.08 (6)	2.5 ± 0.09 (6)
12	2.5 ± 0.06 (12)	2.5 ± 0.05 (7)	2.5 ± 0.06 (7)
18	2.6 ± 0.09 (9)	2.5 ± 0.07 (5)	2.5 ± 0.08 (10)
24	2.1 ± 0.13 (18)	1.9 ± 0.20 (11)	2.0 ± 0.13 (11)

Notes: Glycated hemoglobin levels are expressed as percentages of total hemoglobin. Means ± *SEM* of the number of observations indicated in parentheses. F(age) = 12.72 (df = 3, 89) $p < .01$; F(diet) = 0.36 (df = 2, 89) n.s. (power = 88%, δ = 0.4%, ϕ = 2.0, SD = 0.43); F(interaction) = 0.11 (df = 6, 89) n.s. Differences between controls versus EOD, controls versus 40% DR and EOD versus 40% DR were not significant. The following differences between ages were significant: 5 versus 24 months; 12 versus 24 months; 18 versus 24 months (Tukey test).

[$F(2,108) = 6.99$, $p < .01$], the fluorescence values in EOD and 40% DR rats being similar (Tukey test between diets).

In aortic collagen, the age-dependent accumulation of AGE products showed an exponential trend similar to that of skin collagen ($y = 9.07e^{0.081x}$, $r = .933$, $p < .01$) (Figure 3). Fluorescence values of aortic collagen were always higher than those of skin collagen as indicated by the higher "A" value in the exponential regression. A similar age-related accumulation of aortic fluorescence occurred in food-restricted animals ($y = 8.81e^{0.079x}$, $r = .941$, $p < .01$; $y = 8.70e^{0.082x}$, $r = .984$, $p < .01$ in EOD and 40% DR, respectively). Interestingly, unlike the case of the skin, collagen fluorescence values were not affected significantly by diet restrictions.

Discussion

It is known that changes in the levels of both plasma glucose and insulin might influence the glycation process (6,26). Hence, age-dependent modification in glucose homeostasis was explored in detail in AL and in CR rats.

With regard to glucose, data in AL rats are in agreement with several reports documenting that levels of circulating glucose do not change with increasing age (27,28,29). In EOD animals, plasma glucose was similar to control levels. On the other hand, in 40% DR rats a decrease in circulating glucose was usually observed (30). A reduction in the average daily plasma concentrations of glucose was observed in food-restricted Sprague-Dawley rats (16). Similar results were obtained by Masoro et al. (31) on 40% dietary restricted male F344 rats.

In older control Sprague-Dawley rats, both oral and intravenous glucose tolerance tests appear to be altered (32,33), the hyperglycemic effects of glucocorticoid administration are higher (34), and postprandial accumulation of glycogen in muscle decreases (35). These age-related changes in

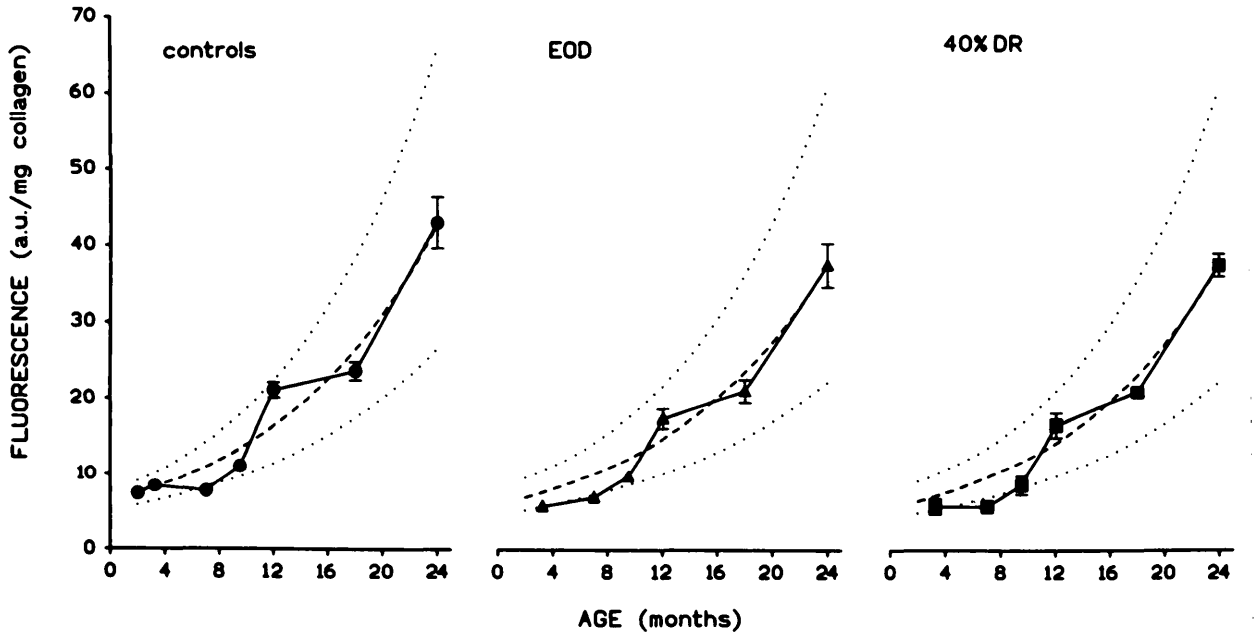

Figure 2. Effect of age and dietary regimens on accumulation of fluorescence in skin collagen of Sprague-Dawley rats. Results are given as means ± *SEM* of 4–14 observations. F(age) = 127.66 (df = 5, 108) $p < .01$; F(diet) = 6.99 (df = 2, 108) $p < .01$; F(interaction) = .64 (df = 8, 70) n.s. Difference between EOD and 40% DR was not significant (Tukey test). EOD = every-other-day feeding; 40% DR = 40% food restriction.

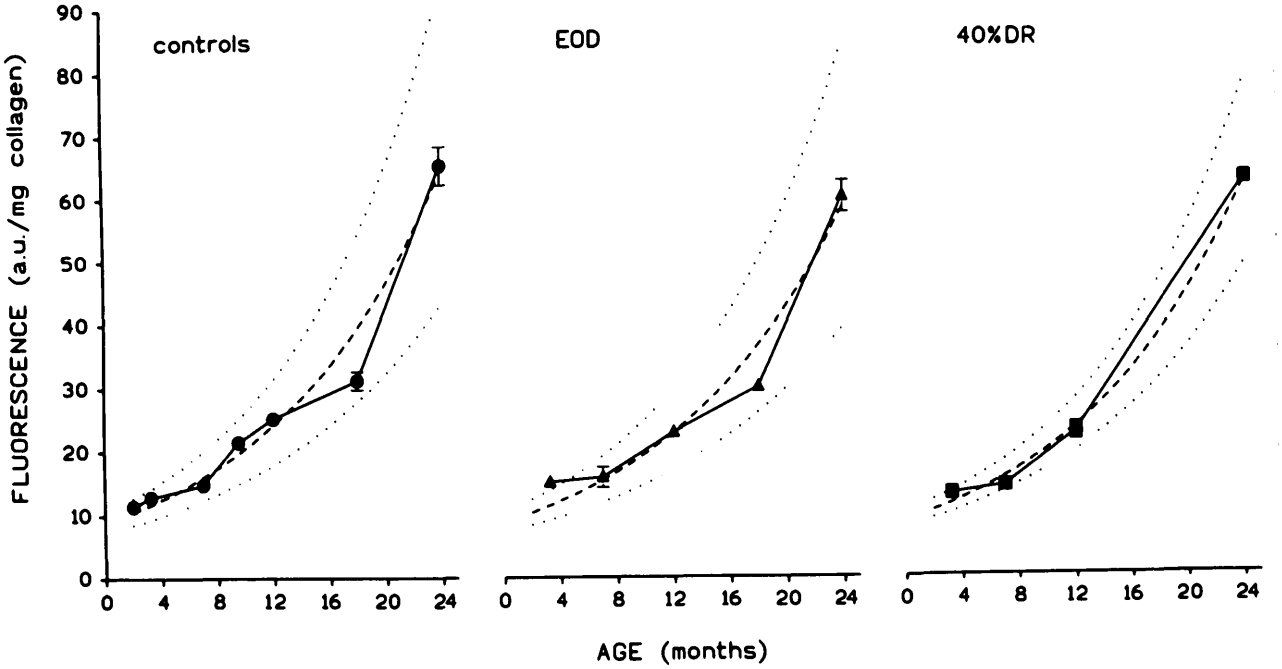

Figure 3. Effect of age and dietary regimens on accumulation of fluorescence in aortic collagen of Sprague-Dawley rats. Results are given as means ± *SEM* of 4–7 observations. F(age) = 411.25 (df = 4, 70) $p < .01$; F(diet) = 1.45 (df = 2, 70) n.s. (power = 75%; δ = 14 AUF, ϕ = 1.6, *SD* = 20); F(interaction) = .64 (df = 8, 70) n.s.

sugar metabolism are retarded at least in part by diet restriction. No significant age-related alterations appear to occur in plasma protein glycation in these animals, and most age-related changes in sugar metabolism in control rats are observed under unphysiological conditions.

Our data show that a significant decrease in the levels of glycated plasma proteins may be observed in food-restricted rats, the maximum effect being observed a few months after the initiation of the dietary manipulations. A decrease in plasma protein glycation (by the fructosamine assay) was reported by Cefalu et al. (30) in 40% food-restricted female Brown-Norway rats. The decrease of plasma protein glycation is similar in EOD and 40% DR rats and is not dependent on age. The rather surprising fact

that the two diets, which have different effects on daily plasma sugar and insulin levels, have a similar decreasing effect on glycated plasma proteins leads us to speculate that the plasma protein turnover might be affected.

Glycated hemoglobin values are unaffected by food restriction. Perhaps red blood cell permeability to glucose might be an important factor, as no significant changes were observed in the concentration of glucose within red blood cells of rats of different ages, subjected to different regimens (data not shown). It may be that levels of glycated hemoglobin, a commonly used indicator of long-term glycemic control in diabetic subjects, are not very sensitive to lower plasma glucose concentrations.

Plasma insulin levels remain substantially unchanged during aging, both in the fed and in the fasted state, with the remarkable exception of 24-mo-old animals, which show significantly higher concentrations. This late increase is not prevented by anti-aging diet restriction. Insulin levels in EOD rats are similar to those of controls. On the other hand, in 40% DR rats, circulating insulin levels are much lower, and smaller differences are observed between fed and fasted rats.

On the whole, in view of the widely accepted hypothesis that higher levels of plasma glucose should increase the rate of protein glycation and that higher levels of plasma insulin should help the disposal of glycated proteins, expectations are that changes in the inner micro-environment may not favor accumulation of glycated protein in older controls and that food restriction might cause a slight decrease.

Age-related accumulations of different unrelated glycated proteins were measured in each individual rat, and they show divergent kinds of behavior. As regards plasma proteins, which are shorter-lived, no significant change in glycation was observed from 2 to 24 months of age, although a tendency to decline was apparent in the oldest age group. In older rats, some urinary loss of protein may occur (36). Loss of plasma proteins with urine may be associated with lower accumulation of glycated plasma proteins (37).

Levels of glycated hemoglobin are stable between the ages of 5 and 18 months. Lower levels are found in 2-month-old rats, whose erythrocyte profile may be characterized by a high percentage of young red cells (38). Lower levels are also found in older rats. In senescent animals, an age-related reduction in red blood cell life span has been reported (39,40).

The third protein investigated here, collagen, is an important target for the Maillard reaction. In view of the longer life of this protein, accumulation of AGE products can take place (1). Fluorescence values are different in the skin and in the aortic tissue, perhaps due to differences in the type of collagen, glucose concentration, and/or tissue temperature. In regard to aging, a similar exponential increase in the levels of glycated collagen was observed in the two different tissues.

An exponential increase with age in collagen-linked skin fluorescence has been reported in Wistar rats (29). More recently, similar results were obtained using the 335nm/385nm fluorescence measurements related to pentosidine-like molecules (26,41).

In the perspective of comparative gerontology, values of collagen fluorescence in rodents may increase with age with different exponential temporal patterns in rats and mice (42). In diabetic and in control human subjects, the age-related accumulation of collagen-linked fluorescence increases with age linearly (19). More extensive investigation may be warranted in dogs and horses, as preliminary data did not show any significant increase with age in fluorescence linked to skin collagen (42).

As regards the mechanism, taking into account that glycemia, glycation of plasma protein and hemoglobin are unchanged or lower in older rats, it can be argued that the increase in glycation might be the consequence of age-related modification(s) of the collagen metabolism (alteration in tissue protein composition and/or the rate of protein turnover), and that because of hyperinsulinemia (6), changes in the rate of removal of glycated collagen molecules by tissue macrophages may also play a role.

It has been suggested that treatments such as diet restriction, which affect longevity, could provide useful insight into the mechanisms of the process of aging. In principle, all age-related changes relevant to longevity should be delayed by this kind of treatment. In this research, in order to improve the level of discrimination, we compared the effects of two different regimens of dietary restriction on metabolism (e.g., on glucose homeostasis). In general, our results do not support the hypothesis that age-related changes in glucose homeostasis have a primary role in causing aging. Effects on blood glucose and insulin plasma levels were observed only with 40% diet restriction and were age-unrelated. A significant increase in plasma insulin levels was observed in both ad libitum-fed and food-restricted 24-mo-old rats.

Glycated hemoglobin values were not affected by dietary restrictions. The effect of diet restriction on glycated hemoglobin has been previously addressed by Masoro et al. (43) and Cefalu et al. (30) with conflicting results. In F344 rats of 4–6 months of age subjected to 40% caloric food restriction, Masoro and colleagues reported a decrease in the levels of glycated hemoglobin (assessed by the phenyl-boronate method), which disappeared with advancing age (40). Using the HPLC method, Cefalu et al. showed a sudden increase in glycated hemoglobin in female Brown-Norway animals between 11 and 17 months; this increase was prevented by diet restriction. We may mention here that we obtained consistent results with the assay of total glycated hemoglobin tested by phenyl-boronate method and with the HPLC measurement of HbA_{1c} (18).

The accumulation of fluorescence linked to collagen in skin and aorta exhibits major age-related changes and may deserve a longer discussion. In our hands, both types of dietary restriction had a similar significant decreasing effect on age-related fluorescence accumulation in skin collagen, and had lower, nonsignificant effects on aortic collagen. It should also be taken into account that diet restriction decreases body temperature (44,45). However, as regards the general mechanism, diet restriction does not affect the exponential rate of fluorescence increment during aging.

A significant effect of dietary restriction on the age-related accumulation of fluorescence and pentosidine in skin collagen was reported by Miksik et al. (46) and Cefalu et al. (30) using different rat strains and by Reiser (47)

using mice. Unlike our results, Miksik et al. (46) also showed that the age-related increase in aortic collagen-linked fluorescence was significantly lower in 50% diet-restricted animals. According to Reiser, 40% diet restriction did not affect pentosidine content of mouse aortic collagen.

In conclusion, the accumulation of fluorescence linked to collagen appears to be a specific, systemic consequence of aging, and may provide a useful biomarker of chronological age in rodents. On the other hand, it appears that the accumulation of fluorescence may not be sensitive to intervention in aging, and further research may be warranted to support the hypothesis that collagen glycation might be relevant to longevity.

ACKNOWLEDGMENTS

This research was supported in part by grants from MURST (40% and 60%) and CNR (Progetto Finalizzato Invecchiamento).

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Received June 27, 1996
Accepted April 30, 1997