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Plasma protein's glycation is decreased in Sprague Dawley rats under caloric restriction

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Abstract

Different dietary regimens were applied to three cohorts of rats. The first was fed ad libitum every day (AL), the second was fed ad libitum every other day (EOD) and the third was fed a diet equivalent to 60% of the caloric intake (60% CI) of the AL cohort. Levels of stable early glycation products in plasma proteins were then measured according to two different methods. Glycation of plasma proteins progressively increased in AL animals belonging to the 2–12 month age interval, while it showed a less pronounced age-dependent increase in EOD and 60% CI animals. The lowest degree of glycation was detected 2–3 months after the beginning of caloric restriction. After 12 months of age a lower level of glycation was detected in 60% CI rats than in EOD animals. Body weight was lower in restricted animals than in AL animals and was lowest in 60% CI rats. During the life span, glycemia was lower in fasting 60% CI than in EOD or AL rats.

Keywords: Caloric restriction; Glycation; Glycemia; Aging

1. Introduction

Caloric restriction (CR) increases either average or maximum life span in different animal species (Weindruch et al., 1986). It is interesting to note that CR is also able to decrease the incidence and severity of chronic degenerative diseases peculiar to

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old age (Weindruch et al., 1986). Furthermore, CR has been shown to modulate critical biochemical functions such as DNA repair which may exert anti-aging effects (Licastro et al., 1988). Thus, CR is at the moment one of the strategies that can reduce mortality rates and prolong survival. These data have led to the assumption that it may be possible to delay the effect of aging and extend life span in humans through the modification of dietary regimen (Walford et al., 1992).

Mechanisms by which the restriction of the caloric intake affects life span are still unknown. For instance, the metabolic rate of the CR animals has been found to be reduced (Gonzales-Pacheco et al., 1993) or unchanged (McCarter et al., 1985). It has been proposed that the restriction of caloric intake may retard aging by affecting the rate of macromolecule deterioration caused by non-enzymatic glycosylation (Masoro et al., 1989). In fact, glucose, once considered biologically inert, can induce detrimental biological effects by excessive non-enzymatic glycosylation of enzymes, membrane proteins, regulatory proteins and DNA molecules (Cerami, 1985; Vlassara et al., 1986). The process involving protein modification by this sugar is also known as glycation. This reaction leads to the formation of a Schiff base, followed by further rearrangement resulting in Amadori products and stable ketoamines (Monnier, 1990) which can lead to cross-linking of proteins and other macromolecules (Eble et al., 1983). With time, glycated products dehydrate and rearrange to irreversibly form compounds called advanced glycosylation end-products (Pongor et al., 1984; Dunn et al., 1989).

High concentrations of glycation products are present in diabetes (Kaneshige, 1987) and may be involved in some of the disease complications (Wolff and Dean, 1987). Furthermore, it is interesting that age-related alterations such as cataract and atherosclerosis occur early in the life of diabetic patients (Brownlee, 1991). However, possible relationships between glucose metabolism and accelerated senescence in this disease are not yet understood.

With reference to the above, we thought it of interest to investigate whether CR could affect the glycation of proteins. Here we have investigated the presence of stable glycation products in plasma proteins from rats of different ages and subjected to different diet regimens.

2. Materials and methods

Male Sprague Dawley rats were divided into three groups. The first had free access to food (Purina lab chow pellets). The second was fed ad libitum every other day. The third received 60% of the daily food intake of unrestricted animals and was given daily access to food in the morning. Dietary manipulations were started 2 months after birth.

Animals were bled between 09:00 and 11:00 h after 24 h of fasting to obtain sterile blood samples. Plasma was then separated and stored at −80°C. Glucose plasma levels were measured by the glucose oxidase method (Glucinet, Sclavo, Italy).

Glycated proteins were detected by two different methods. The first assay measured ketoamine products in plasma proteins according to the Glyco.Gel B assay purchased from Pierce Chemical Company (USA). The second assay measured acid-

stable glucose adducts by the thiobarbituric acid assay (TBA) (Dolhofer and Wieland, 1981; Ney et al., 1981; Murtishaw et al., 1983), modified as previously described (Davis et al., 1989, 1992).

3. Results

The body weight of 2–24 month old rats under 60% CI diet was slightly lower than that of EOD animals, and it was lower in these cohorts of rats than in AL fed animals (Fig. 1). In particular, the increment in body weight observed in young AL rats (2–6 months of age) was less pronounced in rats under caloric restriction and a slight decrement was observable after they reached the middle age (12 months).

Levels of stable ketoamines measured by Glyco.Gel B were higher ($P < 0.01$) in 5 months and 12 months old unrestricted rats than in animals under EOD or 60% CI diets (Fig. 2). Moreover, the concentration of ketoamines adducts was slightly higher in EOD animals than in 60% CI rats. In older animals (24 months) a slight but statistically significant reduction ($P < 0.02$) of plasma ketoamines was found only in rats fed the 60% CI diet. The plasma concentration of glycated proteins detected by TBA assay was higher ($P < 0.03$) in young (3–5 months) AL fed rats than in animals on EOD or 60% CI diets (Fig. 3). In 12 months old or older animals, statistically significant differences ($P < 0.04$) were present only between ad libitum and 60% CI regimens.

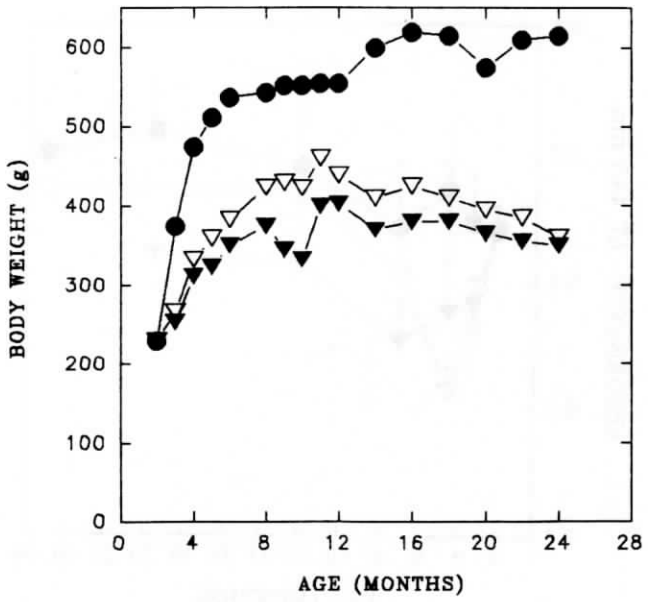

Fig. 1. Age related variations of body weights in rats under ad libitum (●), EOD (▽) and 60% CI (▼) diets. Data are presented as mean ± S.E.

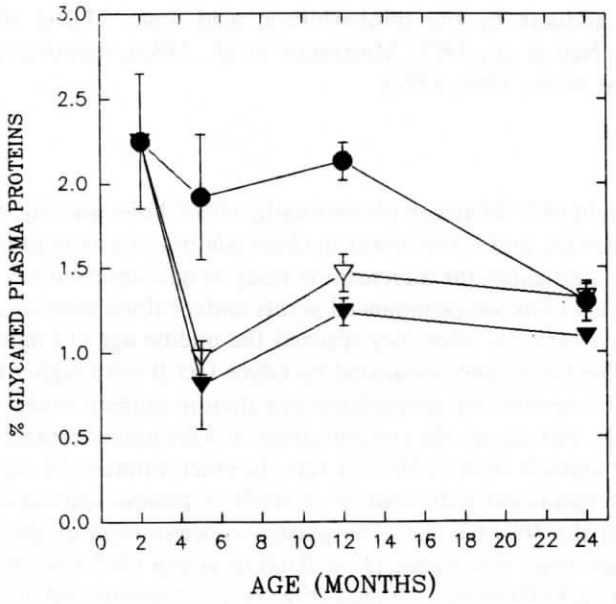

Fig. 2. Age associated changes in percentage of glycated plasma proteins from rats under ad libitum (●), EOD (▽) and 60% CI (▼) diets as measured by Gyco.Gel B. Data are presented as mean ± S.E.

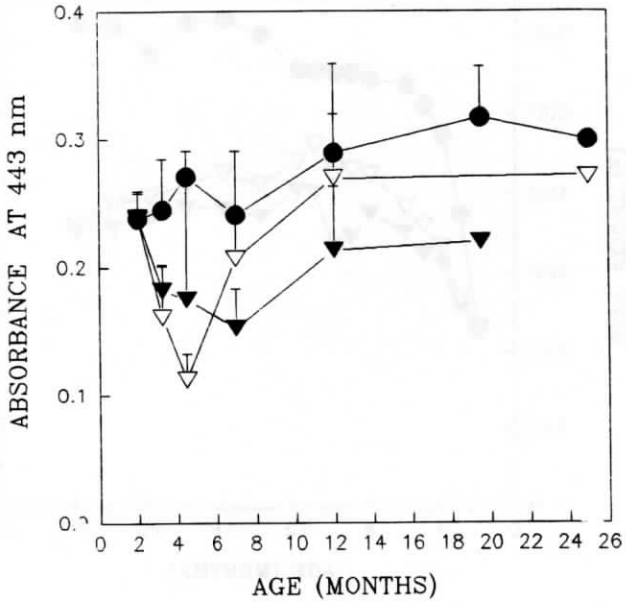

Fig. 3. Age associated changes of glycated plasma proteins from rats under ad libitum (●), EOD (▽) and 60% CI (▼) diets as measured by TBA assay. Data are presented as mean ± S.E.

Table 1
Plasma levels of glucose (mg/dl) measured after 24 h of fasting in rats of different ages and under different diets

Cohort	Age (months)				
	4	7	12	20	25
AL	98 ± 5	129 ± 3	92 ± 2	85 ± 7	77 ± 2
EOD	106 ± 5	105 ± 9	95 ± 3	77 ± 4	75 ± 2
60% CI	99 ± 5	103 ± 5	68 ± 3	65 ± 3	63 ± 1

Data represent mean ± S.E. (minimum four different animals for each age group).

Age dependent levels of glucose in the blood of fasting rats under different diet regimens are reported in Table 1. Levels of blood glucose decreased through the life span of rats from the three cohorts, glycemia being the lowest in 60% CI animals.

4. Discussion

The mechanism or mechanisms responsible for aging and senescence are still largely unknown. However, the rate of aging is quite different among individuals and usually does not follow chronological age, especially during early phases of senescence. This observation implies that mechanisms responsible for aging or those with anti-senescence effects are numerous and some might involve simple basic metabolic mechanisms.

Glucose is one of the major components of metabolic chains leading to energy production and synthesis of more complex compounds. Glycation is a potentially harmful process where glucose is added to any of several sites along protein molecules (Cerami et al., 1987). This random chemical addition triggers biochemical reactions which may lead to the accumulation of irreversible cross-linking of proteins (Eble et al., 1983).

Here we have shown that the restriction of caloric intake affected the glycation rate of macromolecules with a short half life such as plasma proteins. Our data indicated that the maximum effect of caloric restriction on glycation of plasma proteins was obtained early during the life span, i.e. a few months after the initiation of dietary manipulation, with a minor but still evident effect detectable in restricted adult and old rats.

Plasma protein glycation patterns obtained with the two assays were partially overlapping, and the lowest level of glycation products was observed in young adult restricted animals, although the first assay (Glyco.Gel B) appeared to yield a lower intra-experimental variability than that observed using the second assay (TBA method).

Slight differences in the amount of glycated circulating proteins were also found between the two cohorts of dietary restricted rats. For instance, glycated plasma pro-

teins were lower in 12 months old or older 60% CI rats than in age matched EOD animals. These results could be explained by taking into account the fact that glucose plasma levels were lower at all ages in 60% CI rats than in unrestricted or EOD animals. Thus, assuming that the amount of glycation may depend on the concentration of glucose, it is reasonable to expect a decreased glycation of short half life plasma proteins in animals subjected to diets able to maximally decrease blood glucose levels throughout the life span.

It is interesting to note that differences in glycation levels in 20 months old or older animals subjected to the three different diets were lower than those observed in young animals. In particular, no statistically significant difference was found between EOD and ad libitum fed 12–14 months old or older rats. Once again, these results might be related to blood glucose levels, since glycemia decreased in these two cohorts of animals after they reached adulthood.

The present data parallel those previously reported showing that glycemic and insulinaemic patterns were partially different in EOD and 60% CI rats (Bergamini et al., 1990).

Our findings confirm and extend previous observations which showed that the restriction of caloric intake affected the age related level of Schiff base in cytosolic hepatic proteins from mice under severe caloric restriction (Davis et al., 1992). Furthermore, Masoro et al. (1989) showed that dietary restriction influenced the glycation rate of blood hemoglobin in young rodents.

At present it is not clear whether the variation of glycated products is coincidentally rather than casually linked with aging. However, it has been hypothesized that glycation might influence the rate of aging by inducing progressive and irreversible modification of intracellular and extracellular macromolecules (Cerami et al., 1987).

Results presented here reinforce the notion that caloric restriction affects glycation of extracellular macromolecules by increasing the efficiency of mechanisms counteracting the formation of glycated plasma proteins. Further studies will clarify whether the restriction of caloric intake might result in a decrement of adduct formation on plasma proteins, in a more efficient clearance of glycated proteins from the circulation, or in a combination of these two mechanisms.

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