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Long-term effects of litter size in early postnatal period on metabolism, aging and life span in rats

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Abstract

Some indices of aging, metabolism and life span were studied in the male Wistar rats, raised during the suckling period (up to 21 days) in nests of 8–10 pups (control) and 2 pups/dam (experiment). The milk intake of the rat pups was controlled by adjusting litter size at birth. After weaning, the rats of both groups each received the same standard diet ad libitum. Postnatal overfed rats had higher values of body weight, epididymal fat pads and lipid metabolism (total cholesterol, triglycerides, etc.) throughout their whole life. Rats from small nests had increased levels of insulin, thyroxine and decreased proteinase activity of hepatic lysosomes. Overfeeding in the early postnatal period was found to influence the dynamics of mortality and survival rates. It may be concluded that the modification of nutrition in the early period of life may influence an organism's aging process and the dynamics of age-related changes in metabolism and its regulation during an animal's life.

Key words: Postnatal overnutrition; Metabolism; Hormone level; Lysosomal activity; Aging

1. Introduction

Aging and life span are determined by the whole course of an individual's development in relation to age. This general principle seems to be recognized by all. Moreover, the effect of a number of geroprotective means and, particularly, of various diets was shown to be even more expressed and significant when initiated at an earlier age (Wyndham et al., 1983; Goodrick, 1984; Drori and Folman, 1986; Beauchene et al., 1986). Nevertheless, an important fundamental and applied problem — the effect of the above factors at early stages of ontogenesis upon the dyna-

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mics of age changes throughout a life course — remained beyond the investigators' attention. Such a study would allow the establishment of the relationship between an organism's growth and aging, and show the onset of age-related pathology; and might suggest the principles of prevention of accelerated aging pertinent to the whole life of an organism.

Nutrition may be referred to as one of the most essential factors influencing an organism. Beginning with the classical works of C. McCay (McCay et al., 1956), the effect of calorie-restricted diet on life span has been studied with a special care (Grigorov and Kozlovskaya, 1982; Masoro, 1984; Weindruch et al., 1986).

The aim of this investigation was to study the effect of overnutrition, in the early postnatal period, on the dynamics of age-related changes throughout the whole life of the rats, on the organism's aging process and life span.

2. Materials and Methods

This study was performed on 430 male Wistar rats in litters raised during the suckling period (from 1 to 21 days): experimental group — for two (small nests), control group — for 8–10 pups/dam (normal tests). The condition of overnutrition was reached by decreasing the number of pups in litters during a suckling period (Wurtman and Miller, 1976; Aubert et al., 1980; Faust et al., 1980). Further, after weaning the rats were kept in conditions identical for the experimental and control groups, i.e. 3–5 animals/cage and a fed standard diet ad libitum. The biochemical analyses were made in groups of animals aged between 5–7 and 21–24 months; the rest were used for assessment of the survivorship and mortality rates.

In both groups, the body weight and mass of the epididymal fat pads, and lipid metabolism indices (total and alpha-cholesterol, triglycerides and apo-β-lipoproteins in serum) were determined (Kiryakov and Tinterova, 1979). In parallel, the concentrations of insulin, thyroxine and triiodothyronine were determined using the standard rio-Ins-PG-^{141}I, rio-T_4-PG and rio-T_3-PG assay kits. The intensity of protein turnover was measured both in fast and slowly renewing tissues (liver, skeletal muscle) by means of continuous constant infusion of L-[^{14}C]leucine (8880 MBq/mmol) and a further preparatory analysis of amino acids on Hitachi Analyzer (Waterlow et al., 1978). The rate of protein breakdown expressed in lysosomal proteinase activity in the liver (cathepsins A, B, C and D) was determined by the spectrofluorimetric method (Barrett and Hitt, 1980; Vasiljev et al., 1983) and total protein in the liver was also examined.

Statistical analysis of data obtained was performed using Student's t-test.

3. Results and Discussion

The modification of nutrition in the early postnatal period, caused by changing the litter size, influenced body weight, fat contents, lipid metabolism and its regulation, synthesis and catabolism of proteins throughout the ontogenesis. Animals from the experimental vs. control group had a significantly higher body weight during the whole lifespan (Fig. 1A). Differences of body weight between the groups were observed from the 1st to the 32nd month of age. They sharply diminished thereafter

mainly at the expense of decreased body weight of the experimental rats and by the 34th month they disappeared. One could assume that the increase in body weight of the experimental animals might result from changes that occurred in the nutritional behaviour and increased food consumption. Our investigations showed that rats of the experimental group suckled about 70% more than controls during the early postnatal period. But then, after weaning, they returned to feeding a standard fodder ad libitum and consumed the same amount of food as the control group, which agrees with other literature (Aubert et al., 1980; Drori and Folman, 1986).

The fat content of animals was judged from the epididymal fat mass. Both the total body weight and its epididymal content were significantly greater in the experimental vs. control rats (Fig. 1B). Besides, there was a strong direct correlation found

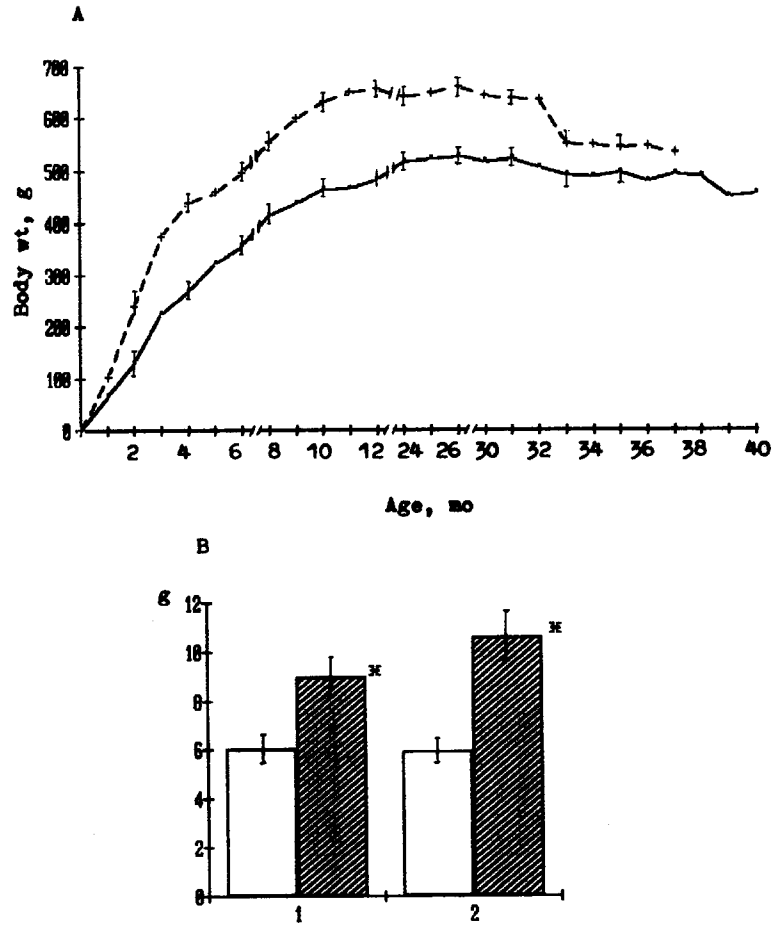

Fig. 1. Effect of litter size in the early postnatal period on dynamics of body weight (A) and on epididymal fat mass (B) of rats. Values represent means ± S.E.M. (A) abscissa, body wt., g; ordinate, age, months; solid line, control; broken line, experiment; (B) 1, adult (n = 36); 2, old (n = 48); white columns, control; dashed columns, experiment. *Significant difference between experiment and control ($P < 0.05$).

between the epididymal fat content and body weight ($r = \pm 0.75$; $P < 0.01$), which was sustained until late ontogenesis.

Rats are known to be resistant to factors that induce atherosclerosis. Still, the animals of the experimental group raised in small nests during the early postnatal period showed considerable shifts in the lipid metabolism, namely, increased levels of blood total cholesterol, triglycerides and apo-β-lipoproteins (Fig. 2A). With age, these differences were not only maintained, but also became even more pronounced. In the old vs. adult rats, the level of cholesterol increased 1.5-fold, triglycerides 3-fold, and apo-β-lipoproteins 1.9-fold. All this may suggest that the overnutrition-related metabolic changes which occurred during the postnatal phase were not only sustained for a prolonged period, but even aggravated with age.

To elucidate the mechanisms of disturbance of lipid metabolism in animals raised in small nests in the early ontogenesis, we determined a level of lipotropic hormones

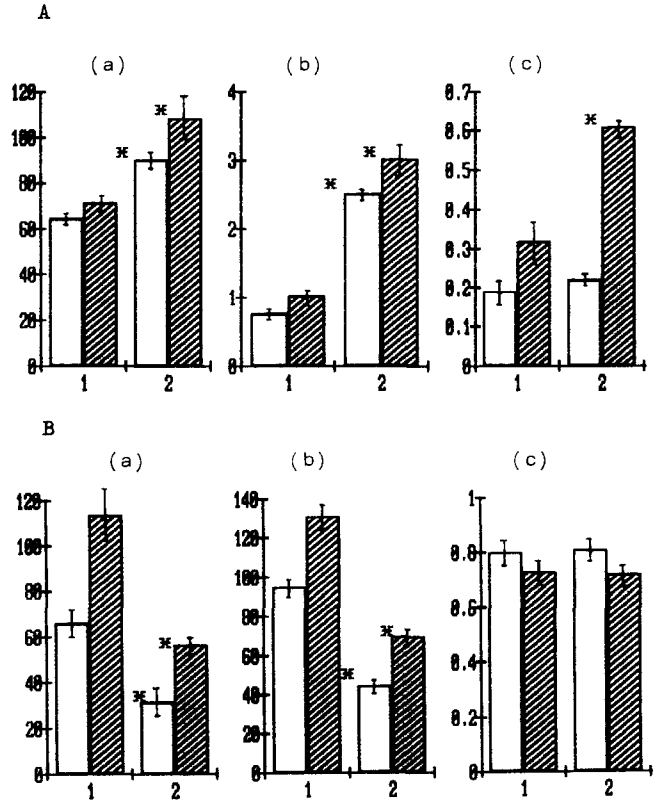

Fig. 2. Effect of litter size in the early postnatal period on lipid metabolism (A) and on blood serum hormones (B). Values represent means ± S.E.M. A: (a) cholesterol, mg/100 ml; (b) triglycerides, mmol/l; (c) apo-β-lipoproteins, mg/100 ml; (1) adult ($n = 20$), (2) old ($n = 24$); white columns, control; dashed, experiment. *Significant difference between old and adult rats within the same group ($P < 0.05$). B: (a) serum insulin, pmol/l; (b) thyroxine, nmol/l; (c) triiodothyronine, nmol/l; (1) adult ($n = 20$), (2) old ($n = 24$); white columns, control; dashed; experiment. *Significant difference between old and adult rats within the same group ($P < 0.05$).

in the blood (insulin, thyroxine and triiodothyronine). Compared to the control group, the experimental animals had an increased insulin content throughout their ontogenesis: by 70 and 80% in young and old rats, respectively (Fig. 2B). It is noteworthy that hyperinsulinemia is an established characteristic sign of obesity (Stern et al., 1972; Olefsky, 1976). The comparative study of overnutrition inducing obesity development showed that hyperinsulinemia in this case can not be linked with hyperphagia only. An increased insulin content in animals overfed during the early ontogenesis can be explained by increased resistance of adipocytes to insulin against the background of an increased body weight, as a consequence of both their hypertrophy, and hyperplasia. As is known, with an increased size of cells the number of receptors to insulin on their surface decreases (Stern et al., 1972; Sjostrom, 1972). There is a supposition that hyperinsulinemia in animals with an increased body weight may result from a reduced peripheral utilization of the substrates (Waterlow et al., 1978; Frolkis, 1982).

In both the adult and old rats from the experimental group, the thyroxine concentration was significantly higher vs. the control group (by 59 and 56%, respectively) against the background of an unchanged triiodothyronine concentration (Fig. 2B). Such dynamics of levels of thyroid gland hormones in rats which had been overfed during the early postnatal life may result from both disturbance of T_4 and T_3 conversion in the peripheral tissues, and the increase of their resistance to thyroxine and hence the rise of its concentration. Similar results have been obtained by other investigators (Macho et al., 1973; Aust et al., 1986) demonstrating that animals raised in small nests had an increased plasma thyroxine and a considerably decreased triiodothyronine against the background of a low level of labelled iodine incorporation in the thyroid. A reduced rate of T_4 and T_3 conversion may lead to slowing down the oxidation of fatty acids in tissues, to a decrease in the share of lipids involved in metabolic processes, and to an enhancement of the lipogenesis. These were our particular observations in animals of our experimental group. It was also noteworthy that with age, despite a total fall in hormone concentration, there were still differences found between the groups under study (Fig. 2B).

The intensity of processes of protein synthesis was judged from the data on relative specific radioactivity of tissues, having both high (liver) and low (skeletal muscle) level of protein synthesis. Analysis of age peculiarities of protein synthesis showed that under given experimental conditions (study method, animals' age) the level of protein synthesis in both liver and skeletal muscle remained unchanged in the control animals. Thus, the relative specific radioactivity in the liver was 66.4 ± 6.7% in adult and 95.7 ± 23.3% in old rats ($P > 0.05$); in the skeletal muscle −4.1 ± 0.6 and 3.9 ± 0.5%/24 h ($P > 0.05$), respectively. In the experimental group there was a significant rise of the level of protein synthesis in the liver: 59.0 ± 5.7% in adult and 98.4 ± 13.4% in old ($P < 0.05$), while in the skeletal muscle no differences were found: 4.4 ± 0.5% in adult and 4.7 ± 0.8%/24 h in old ($P > 0.05$). In our earlier studies we have shown that the differences in early postnatal nutrition did not influence protein synthesis either in liver or skeletal muscle (Petzke et al., 1988).

A study on the activity of lysosomal proteinases may shed light on age peculiarities of protein metabolism as they represent one of the cellular structures

which are most sensitive to effects of alimentary factors and provide for intracellular metabolism at the stage of catabolism of almost all biopolymers (Millward et al., 1981; Tuteliyan and Vasilyev, 1987; Ferland et al., 1992). The comparative analysis of age changes in the activity of lysosomal cathepsins demonstrates that they were mainly one-directional in character, this being expressed in an increased activity of most study proteinases of the liver observed by the 21st month of life. As is seen in Table 1, in the control group there was a significant rise in the activity of cathepsin B (by 109%) and cathepsin C (by 46%). In the experimental rats, which had been raised in small nests in the suckling period, apart from the mentioned proteinases, there was also a rise in cathepsin A activity, thus being increased correspondingly: A — by 128%, B — by 56% and C — by 60%. The higher activity of proteinases in 21-month-old rats was paralleled with the lower protein content of the liver: by 8% in control and by 34% in experiment. Differences in the early postnatal feeding also influenced the activity of lysosomal proteinases of the liver. In the rats of experimental group, vs control the activity of cathepsins was decreased: in young animals there was a statistically significant decrease in the activity of cathepsin A ($P < 0.001$); in old rats the activity of cathepsin A was decreased by 18% ($P < 0.1$), of cathepsin B by 33% ($P < 0.001$) and of cathepsin D — by 26% ($P < 0.05$) (Table 1). Thus, the early postnatal feeding influenced both protein synthesis and its catabolism.

The following values were determined to characterize the life span: the rates of survivorship and mortality, the average and maximal life span of animals fed during early postnatal period in nests with various numbers of pups. Age-related dynamics of mortality in control (1) and experiment (2), expressed by coordinates of Gompertz formula, was described by the following equations:

$$\ln R = -5.49 + 0.11\ t; \qquad r = 0.77 \tag{1}$$

$$\ln R = -5.56 + 0.14\ t; \qquad r = 0.92 \tag{2}$$

Table 1
Effect of litter size on the lysosomal proteinase activity and total protein in liver of adult and old rats (Mean ± S.D.)

Indexes	Adults		Old	
	Control (n = 18)	Experiment (n = 18)	Control (n = 24)	Experiment (n = 24)
Liver lysosomal cathepsins (μmol/min per g of tissue)				
Cathepsin A	124.3 ± 17.0	56.7 ± 3.5**	157.8 ± 14.1	129.4 ± 2.1*
Cathepsin B	9.4 ± 0.6	8.5 ± 0.3	19.7 ± 1.4*	13.2 ± 0.4* **
Cathepsin C	51.9 ± 4.7	51.7 ± 5.5	79.8 ± 8.4*	82.7 ± 7.6*
Cathepsin D	10.7 ± 1.7	8.6 ± 0.8	12.8 ± 1.2	9.4 ± 0.6**
Total protein (mg per g of tissue)	194.6 ± 9.3	269.2 ± 4.2**	178.3 ± 2.3	181.3 ± 2.1*

n, number of rats in the group.
*Statistically significant differences between old and adult animals within the same group ($P < 0.05$).
**Statistically significant differences between experimental and control group ($P < 0.05$–0.001).

where ln R is the natural logarithm of mortality, in months^{-1}, and t is the age, in months.

It follows from (1) to (2) that in the experimental animals there is an increase in the aging rate against some decrease in the mortality (initial vulnerability) during early ontogenesis compared to the intact animals. Thus, within the first two months of life 3.7% of animals died in the experimental group and 17% in the control group. At the same time, at the closing stages of ontogenesis the mortality rate in the experimental group was higher vs. the control group. Their maximal life span was shorter, making thus 1043 ± 20.4 days in the experimental and 1116 ± 30.3 days ($P < 0.1$) in the control rats (of last 10% of animals of both groups) (Fig. 3). No significant differences in an average life span were found between the experimental and control group. It may be thought that changes in the metabolism caused by overfeeding during milk suckling influenced favourably their survivorship at early and mature ages.

Our study shows that an overfeeding during the early postnatal life does influence dynamics of age changes in the metabolism, its hormonal regulation throughout life, and the course of aging processes in an organism. This is evidenced from data on the dynamics of age changes in the body weight of rats, contents of epididymal fat, total and alpha-colesterol, triglycerides, concentrations of insulin, thyroxine and triiodothyronine, values of protein metabolism, and rates of survivorship and mortality of the animals.

The authors of the known works dealing with overnutrition during the postnatal period (Macho et al., 1973; Wurtman and Miller, 1976; Faust et al., 1980) observed shifts during only a short period of life, several months. Our study extended the observation period from birth to death of the rats of both groups. An essential learning in this studies was that modification of nutrition during the early postnatal period does not merely sustain differences that develop during overnutrition, but also leads to changing age-dependent dynamics throughout life, with changes in the pattern of aging. Indeed, if the differences of body weight between the experimental

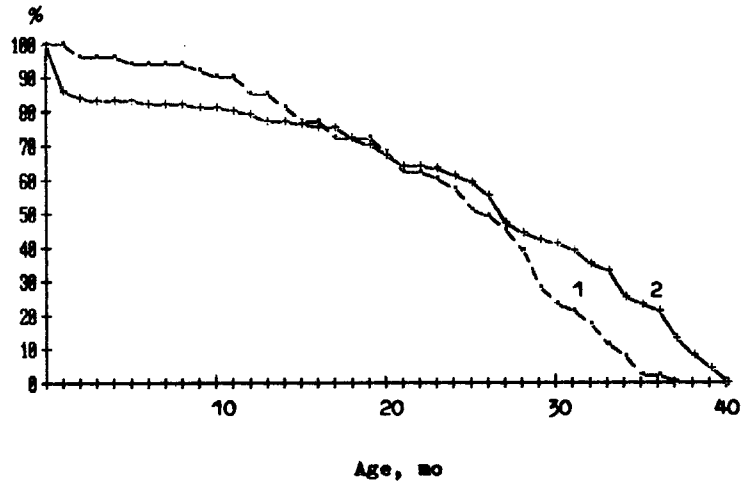

Fig. 3. Effect of litter size in the early postnatal period on survivorship of rats. Abscissa, survivorship, %; ordinate, age, months; 1, experiment (n = 60); 2, control (n = 60).

and control animals during the first month of life were 30 g, they reached 120 g by the fourth month and 180–209 g by 21–24 months. The epididymal fat content at the age of 5 months was 8.99 ± 0.84 g in experiment compared to 6.06 ± 0.69 g in control ($P < 0.05$); at 21 months: 10.58 ± 1.04 g and 5.95 ± 0.66 g ($P < 0.001$), respectively. The same regularity of increase in the differences was found in other study parameters like the concentrations of total cholesterol, triglycerides, apo-β-lipoproteins, blood serum levels of insulin and thyroxine, and the activity of the liver lysosomal proteinases.

All this may suggest that changes in the metabolism and its regulation caused by differences of feeding rat pups during the early postnatal period are not only sustained, but also progress with age. This points to the effect of overfeeding during the early postnatal period on some quantitative and qualitative manifestations of the organism's aging. Hormones, amino acids, etc. at early states of ante- and postnatal development are known to bring out significant changes: sex formation, level of separate hormones, enzymes, etc. The question is of the imprinting (Gupta et al., 1990; Jane et al., 1991), a genetic retaining, possibility of influencing considerably the genome at certain stages of gene regulation formation. In turn, the gene-regulatory changes determine the rate of organism's aging (Frolkis, 1981, 1982; Richardson et al., 1985). That is why the whole dynamics of age development and the rate of age-related changes can be influenced, in a given study, by the overfeeding at early stages of ontogenesis. There is hope that further study in this field will lead to establishing recommendations concerning one's lifestyle and the role of nutrition during early stages of ontogenesis, which may positively influence the entire course of life. With this approach it may be possible to guide an organism's development through nutrition and positively influence an individual's ontogenesis.

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