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Aging and Food Restriction: Effect on Lipids of Cerebral Cortex

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TACCONI, M. T., L. LLIGOÑA, M. SALMONA, N. PITSIKAS AND S. ALGERI. *Aging and food restriction: Effect on lipids of cerebral cortex.* NEUROBIOL AGING **12**(1) 55–59, 1991.—In experimental animals dietary restriction reduces the body weight increase due to aging, increases longevity and delays the onset of age-related physiological deterioration, including age-related changes in serum lipids. Little is known about the influence of food restriction on brain lipids, whose concentration and composition have been shown to change with age. We studied whether some biochemical and biophysical parameters of rat brain membranes, known to be modified with age, were affected by a diet low in calories, in which 50% of lipids and 35% of carbohydrates have been replaced by fibers. The diet was started at weaning and maintained throughout the animal's entire life span. Animals fed the low calorie diet survived longer and gained less body weight than standard diet fed rats. Age-related increases in microviscosity, cholesterol/phospholipid and sphingomyelin/phosphatidylcholine ratios were reduced or restored to the levels of young animals in cortex membranes of 32 old rats fed the low calorie diet, while the age-related increase in mono- to polyunsaturated fatty acid ratios in phospholipids was further raised. In conclusion we have shown that a diet low in calories and high in fibers affects lipid composition in the rat brain, in a direction opposite to that normally believed to reduce age-related deterioration of brain functions.

Aging Food restriction Brain lipids Microviscosity Survival

SEVERAL studies in animals have shown that various regimens of food restriction increase longevity, reduce body weight gain due to aging and delay the onset of age-related physiological deterioration as compared to ad lib feeding (15,28). In rodents, limited calorie intake delays the onset or reduces the incidence of a variety of pathological conditions, including tumors (29,30), renal diseases (3), bone loss (7), and slows a number of age-associated changes in biochemical parameters in tissues (11, 12, 19, 27), including serum lipids (1,14). Little is known, however, about the influence of food restriction on age-induced modifications of brain lipids, which are not only structurally but also functionally important in the activity of brain membranes (25). To this end we studied whether age-induced biochemical and biophysical changes in rat brain membranes were affected by a dietary regimen, started at weaning, in which 50% of lipids and 35% of carbohydrates of a standard pellet diet were replaced by fibers.

METHOD

Male rats CD-COBS (Charles River Italy, Calco, Italy), housed in Makrolon cages (35 × 45 × 20 cm), 2 rats per cage, with 12-h light/dark cycle, with free access to food and water, were used. Animals were fed a pelleted standard diet (STD), normal rodent chow, Dott. Piccioni, Gessate, Italy, or a low calorie diet (LCD) in which 50% of lipids and 35% of carbohydrates had been replaced by fibers. Detailed compositions of the two diets are given in Table 1. Care was taken to remove from STD nutrients rich in calories, without changing too much the composition of essential amino acids (see Table 1) and fatty acids [the only differences in fatty acid composition of LCD were in oleic acid (27% in LCD versus 23% in STD) and linoleic acid (41% in LCD versus 44% in STD, respectively)]. Diets were started at weaning and maintained up to 32 months of age or until the death of the animals. Body weight, date and possible causes of death were recorded.

Food intake was periodically recorded by putting a preweighed amount of chow in the food reservoir of the cage and weighing the remainder 24 hours later. As each cage housed two rats, this evaluation, although made on a large number of animals, is not a precise measure. To get a more reliable measurement we evaluated spillage and found that it was not greater than 5% and similar in both populations. In addition the determination was also made on a group of ten rats 24 months of age placed singly in metabolic cages.

Groups of six animals/diet were killed at 4, 15 and 32 months of age; brains were removed; different brain regions were dissected (6) and used for various analysis. In our study cerebral cortexes (telencephalon without striatum, approximately 400 mg fresh weight) were used as a first approach; in fact our research was part of a broader experimental design aimed to evaluating the effect of low calorie diet on various aspects of brain functions (18). On the other hand cerebral cortex functions show major impairment with age. They were homogenized in 1 ml cold saline; aliquots of the homogenates (10–20 μl) were used for protein determination (13) and fluorescence polarisation measurements (as an index of membrane fluidity), with a Microviscosimeter Elscint MV 1 (Haifa, Israel), using 1,6 diphenylhexatriene as fluorescent probe (22).

Lipids were extracted from the remaining homogenates according to the method described by Folch et al. (4), in the presence of hydroquinone as antioxidant. On the lipid containing chloroform phases the following analysis were done: total choles-

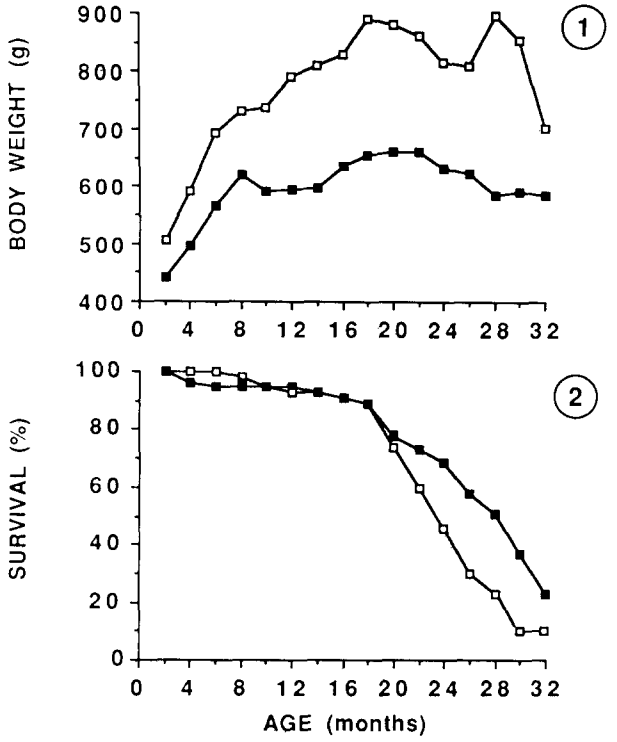

FIG. 1. Body weight and survival of rats fed standard □, or low calorie diet ■.

terol (reaction of Lieberman and Burchard), phosphorus in phospholipids (PL) after acidic digestion (26), both on total PL and on fractions separated by TLC, and composition of fatty acids in the major PL by gas-chromatographic analysis.

PL fractionation was performed on silica gel 60 plates (with concentration zone, Merck), using chloroform/ethanol/triethylamine/water, 30/34/35/8, as developing mixture. Different PL, visualized with 0.1% rhodamine in methanol and identified with the aid of corresponding authentic standards run in parallel, were scraped from plates; PL were extracted with methanol and phosphorus assay was performed as previously described.

Portions of extracts containing phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) were used for gas-chromatographic analysis of their fatty acids, after alkaline transmethylation (5). Fatty acid methylesters were injected into a 3200 Carlo Erba gas-chromatograph, connected with a flame ionization detector and a SP 4290 integrator (Spectra-Physics). Fatty acid methylesters were separated with a WCOT fused silica column, 10 m long, 0.53 mm internal diameter, using helium as carrier gas. The flow was 5 ml/min. The temperature was programmed as follows: after 3 min at 170°, temperature was raised to 220°, in increments of 2°/min. After identification of individual fatty acids by comparing their retention times with those of corresponding standards, percent composition was calculated.

The ANOVA 2-way test and Tukey's test for multiple comparison were used for statistical analysis (8).

RESULTS

The low calorie regimen resulted in a reduced body weight gain with time during the first 4–5 months; afterwards rats on STD continued to gain weight up to 18–20 months, when a tendency to body weight loss appeared. Rats on LCD, instead, maintained their body weight more or less constant until the end of the experiment, being approximately 200 g lighter than control rats (Fig. 1, panel 1). This difference was attained although the rats fed LCD ate more food, and their calculated daily calorie intake was not as low as might have been expected from the calorie content of the two diets; for example at 18 months the average food intake was 36 g/rat/day in LCD fed rats and 28 g in STD fed ones (Table 1). Total calorie reduction ended up as not more than 15% in the LCD group: for example at 18 months of age calorie intake was 104 kcal/day/rat in STD fed animals and 87 kcal/day/rat in LCD ones. Together with the decrease in body weight, this dietary regimen caused an evident increase in survival: at 24 months of age 70% of the rats kept on LCD were still alive while in the population of normally fed rats only 45% were

TABLE 1

COMPOSITION OF DIETS AND AVERAGE NUTRIENT INTAKE IN RATS (g/DAY/RAT)

	Standard Diet		Low Calorie Diet	
	% Dry Weight	g/Day/Rat*	% Dry Weight	g/Day/Rat*
Protein	21.0	5.9	17.9	6.5
Lipid	4.8	1.3	2.4	0.85
Carbohydrates	61.5	17.2	40.7	14.4
Fiber	4.2	1.2	27.9	10.1
Ash	8.5	2.4	11.1	4.0
Essential F.A.	2.6	0.72	1.62	0.58
Histidine	0.50		0.35	
Lysine	1.20		0.75	
Tryptophane	0.27		0.20	
Isoleucine	1.02		0.61	
Valine	1.10		0.74	
Leucine	1.62		1.10	
Threonine	0.78		0.55	
Methionine + Cystine	0.75		0.60	
Phenylalanine	0.99		0.80	
Arginine	1.27		1.02	
Calcium	1.30		2.20	
Phosphorus	0.92		0.24	
Sodium	0.57		0.54	
Potassium	0.65		0.83	
Iron ppm	150.00		130.00	
Copper ppm	25.00		13.00	
Iodine ppm	2.00		1.80	
Manganese ppm	86.00		85.00	
Zinc ppm	60.00		42.00	
Energy content kcal/kg	3930		2557	

*Values are calculated on the basis of daily food intake of 18-month-old rats (standard diet fed rats = 28 g, low calorie diet fed rats = 36 g).

Raw components common to both diets: corn, barley, wheat and soy flour, powdered meat, dehydrated alfalfa, minerals and vitamin mixture.

Raw components unique to standard diet: powdered milk, wheat germ, powdered fish, herring meal, dried yeast and maize oil.

Raw components unique to low calorie diet: carob, wheat bran, solka floc (cellulose and lignocellulose).

Both diets were prepared according to Good Laboratory Practice and did not contain nitrosamines, antibiotics, hormones or other contaminants.

TABLE 2

EFFECT OF A LOW CALORIE DIET ON MICROVISCOSITY OF CORTEX MEMBRANES OF RAT

Age Months	Rat Cortex Microviscosity (Poise ± SEM) Standard Diet	Low Calorie Diet
4	2.17 ± 0.008	2.18 ± 0.008
15	2.29 ± 0.009*	2.26 ± 0.003*§
32	2.33 ± 0.014*	2.27 ± 0.009*†‡

n = 6.
*$p<0.01$ versus rats of 4 months fed the standard diet.
†$p<0.05$ versus rats of 32 months fed the standard diet.
‡$p<0.01$ versus rats of 4 months fed the low calorie diet.
§$p<0.05$ versus rats of 4 months fed the low calorie diet.

still surviving (Fig. 1, panel 2).

Membrane microviscosity in cortex increased slightly but significantly with age in both groups of animals, but at different rates: at 32 months microviscosity was lower in LCD fed rats than in STD fed ones ($\mu = 2.33 \pm 0.01$ poise versus $\mu = 2.27 \pm 0.009$ poise, respectively) (Table 2). These differences although small are consistent due to the low variability of the microviscosity measurements.

Rats fed the STD diet showed an age-related increase in cortex cholesterol (Chol) content, Chol/PL and sphingomyelin/(Sph)/PC ratios, while total PL content was unchanged (Table 3). In LCD fed rats we observed a similar rise in Chol levels with age but PL tended to increase too; this resulted in a significantly lower Chol/PL ratio in 32-month-old rats fed LCD compared to the STD fed rats of the same age; similarly the Sph/PC ratio was lower in 32-month-old LCD fed animals, on account of a slight rise observed in PC content (data not shown).

We compared the fatty acid percentages in main cortex PL (PC, PE and PS) of 4- and 32-month-old rats receiving the two diets. Polyunsaturated fatty acids decreased with age in both diets, as shown by the higher ratios of mono- to polyunsaturated fatty acids (Table 4); however, the effect was much more marked in rats on LCD, especially in the PS fraction.

TABLE 4

EFFECT OF LOW CALORIE DIET ON MONO- VERSUS POLYUNSATURATED FATTY ACIDS OF SOME CORTEX PHOSPHOLIPIDS

Age Months	Cortex Phospholipids ± SEM Mono/Polyunsaturated Fatty Acids PC	PE	PS
4 Standard Diet	2.81 ± 0.13	0.400 ± 0.015	0.481 ± 0.050
4 Low calorie diet	2.68 ± 0.08	0.431 ± 0.049	0.564 ± 0.061†
32 Standard diet	2.88 ± 0.29	0.477 ± 0.040†	0.608 ± 0.018†
32 Low calorie diet	3.01 ± 0.55	0.547 ± 0.040*†‡	0.807 ± 0.086*†‡

n = 5–6.
*$p<0.05$ versus rats of 32 months fed the standard diet.
†$p< 0.05$ versus rats of 4 months fed the standard diet.
‡$p<0.01$ versus rats of 4 months fed the low calorie diet.
PC = phosphatidylcholine, PE = phosphatidylethanolamine, PS = phosphatidylserine.

DISCUSSION

Several authors reported increases in survival in rodents when their calorie intake was restricted by various means (10–12). The dietary protocol used in the present study, consisting in substituting fibers for 50% of the calories in the diet, resulted in a theoretical 12–22% reduction of calorie intake, with an eight-fold enrichment in fibers. This dietary restriction, continued from weaning to the end of life, increased survival and limited the body weight gain usually observed with aging in the strain of rats employed. The striking reduction in body weight gain, in spite of the relative small reduction in calorie intake in LCD fed rats, is probably the result of two combined effects: a 12–22% reduction in calorie intake for a very long period, and the high fiber content of the LCD, which have been described to reduce intestinal lipid absorption (9,24). On the other hand, that such calorie restricted regimen can affect age-induced rise in lipid content is supported by data obtained in our institute in the same strain of animals. Plasma and liver triglycerides, for example, were reduced in 32-month-old rats fed LCD in comparison to STD ones

TABLE 3

EFFECT OF A LOW CALORIE DIET ON PROTEIN, CHOLESTEROL (Chol) AND PHOSPHOLIPIDS (PL) OF RAT CORTEX

Age Months	Protein mg/g ± SEM	Chol µmol/g Fresh Tissue ± SEM	Pi in PL	Chol/PL Molar Ratio ± SEM	SPH/PC
4	78.8 ± 1.2	28.8 ± 1.2	37.4 ± 0.7	0.77 ± 0.03	0.157 ± 0.02
15	82.5 ± 2.4	33.4 ± 0.8†	35.6 ± 0.6	0.93 ± 0.03†	0.182 ± 0.01†
32	81.4 ± 1.4	35.2 ± 1.2‡	37.9 ± 0.5	0.97 ± 0.02†	0.215 ± 0.01†
		Low Calorie Diet			
4	81.2 ± 1.2	28.8 ± 0.8	37.3 ± 0.7	0.82 ± 0.01	0.149 ± 0.01
15	82.9 ± 1.5	32.8 ± 0.6†§	39.7 ± 0.5	0.85 ± 0.03	0.160 ± 0.01
32	83.4 ± 1.1	34.7 ± 0.9‡§	42.3 ± 0.2*†§	0.82 ± 0.03*	0.167 ± 0.01*†§

N = 6. Chol = cholesterol, PC = phosphatidylcholine, SPH = sphingomyelin.
*$p<0.05$ versus rats of 32 months fed the standard diet.
†$p<0.05$ versus rats of 4 months fed the standard diet.
‡$p<0.01$ versus rats of 4 months fed the standard diet.
§$p<0.05$ versus rats of 4 months fed the low calorie diet.

(plasma triglycerides = 48 ± 7 mg/dl versus 71 ± 9, liver triglycerides = 530 ± 81 mg/dl versus 1086 ± 218, respectively) (Bizzi A., personal communication).

Lipid content and composition are parameters involved in the homeostasis of membrane fluidity: membranes tend to maintain a certain degree of fluidity, specific for each membrane, by regulating a variety of biochemical parameters (protein, cholesterol and PL content, PL and fatty acid composition) (21). It was described that aging reduced brain membrane fluidity (2,23) by affecting lipid composition. In our study we found that some parameters involved in membrane fluidity homeostasis were changed in 32-month-old rats fed LCD toward a more fluid state than that of STD fed rats of the same age (i.e., cholesterol/PL and Sph/PC ratios were reduced), while others (mono/polyunsaturated fatty acid ratios in PL) were increased suggesting a tendency toward rigidification. These latter results are in line with the observation that, in the liver, a restricted diet slows the metabolic cascade from linoleic to arachidonic acid, with the consequence of reducing the arachidonate content in PL (1).

The direct measurements of cortical membrane fluidity showed that membranes of 32-month-old rats fed LCD had a tendency toward a more fluid state of membranes in comparison to STD fed rats of the same age. This may indicate that changes in lipid composition induced by dietary restriction limit the age-related rigidification of brain membranes.

These data are interesting if one considers the important role played by the fluidity and phospholipid composition of brain membrane on membrane functions and neurotransmitter activities. For example, PL may modulate binding to the receptor and release from the synapse of a number of neurotransmitters (2, 20, 25). Age-related modifications of membrane biochemical and biophysical parameters may therefore be related to the loss of neurotransmitter functions or binding capacity. Age-related changes in neurochemical markers vary with genetic strain, sex and even brain region of the same animals. In rat cortex cholinergic system seems involved in age-induced damage: choline acetyltransferase activity, Ca^{++}-dependent acetylcholine release and muscarinic binding sites were reduced with age (12, 16, 17) and seemed positively influenced by restricted feeding (12). In addition, behavioral tests done on the same animals showed that cognitive deficiency observed in aged rats fed STD was reduced in LCD ones (18). It is difficult to establish a direct relationship between membrane status and cognitive impairment. However, alteration in lipid content is an important mechanism that modulates nervous tissue membrane functions with age.

In conclusion, we have found that LCD feeding counteracts the age-related increase in Chol/PL and Sph/PC ratios of cortex membranes, affecting the age-related reduction in membrane fluidity. These lipid modifications may play some role in the antiaging effects of restricted diets.

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