Study on the Lipid Composition of Aging Fischer-344 Rat Lymphoid Cells: Effect of Long-Term Calorie Restriction

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Long-term calorie restriction (LCR) is widely known to increase the survival rate of laboratory rodents and appears to retard the aging and senescence process. The present study was undertaken in Fischer-344 male rats maintained on *ad libitum* (AL) or LCR (40% less food intake than AL starting at 6 weeks of age). Age-associated changes in the proliferative response of lymphoid cells to mitogenic stimuli were studied in relation to alterations in the fatty acid composition of adherent and non-adherent-enriched subpopulations of spleen cells. Increases in spleen cell long-chain highly unsaturated fatty acids (20:4, 22:4 and 22:5) were accompanied by decreases in linoleic acid (18:2) in aging AL-fed rats. However, LCR stabilized levels of 18:2 and prevented the rise in highly unsaturated fatty acids. In addition, LCR markedly modulated the fatty acid profiles of thymocytes and bone marrow cells. A 70% decline in concanavalin A (Con A) stimulated [^{3}H]thymidine uptake of spleen cells from AL animals was normalized by LCR. Splenic reduced glutathione (GSH), a potential modulator of the mitogenic response, was unaffected by age and nutritional regimen. Thus, normalization of lymphoid cell fatty acid composition by LCR parallels the preservation of mitogenic responsiveness to Con A.
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Long-term calorie restriction (LCR) is a powerful nutritional intervention that increases both the mean and maximum lifespan of laboratory rodents. LCR retards, attenuates or abolishes the age-related decline of many immunological (1–3) and physiological functions (4,5) as well as autoimmune disease and malignant diseases (6,7). The underlying mechanisms for prolongation of longevity by calorie restriction are still unknown. Recent reports have emphasized that LCR can influence age-associated changes in neuroendocrine hormones, free radicals, gene expression and several immune functions. Previous observations have demonstrated that LCR-induced delay in age-related loss of immune function correlates with preservation of lymphoid cell proliferative responsiveness in various strains of mice and rats (3,8,9).

Reactivity of lymphoid cells can be affected by dietary fatty acid composition (10–13). Therefore, we hypothesized that the age-related decline in immune response may be associated with age-related alterations in the lymphoid cell subsets and their composition of fatty acids and that LCR can reverse age-related immune deficiency by modulation of both Interleukin-2 (IL-2) receptors and IL-2 production (14,15). Our hypothesis is also based primarily on our recent finding that LCR can prevent age-related changes in subcellular membrane lipid composition of liver tissue (16). The present detailed studies show effects of diet and aging on lymphoid cell fatty acid composition, mitogenic response and GSH levels. Implications of age-related changes in membrane lipids and the role of LCR in maintaining immune function throughout the lifespan are further discussed in this paper.

MATERIALS AND METHODS

Animals and diet. Male specific-pathogen-free Fischer-344 rats (Charles River Lab, Portage, MI), 28 ± 2 days of age were maintained in a barrier facility. They were housed singly in plastic cages with wire mesh floors suspended on the Hazelton-Enviro Rack System (Hazelton Systems, Inc., Aberdeen, MD). The procedures for monitoring and operating the barrier facility and composition of the diets (Table 1) have been reported in detail previously (5).

All weanling rats were fed Diet A (Ralston-Purina, St. Louis, MO) *ad libitum* (AL) until six weeks of age. Afterwards, only rats belonging to the AL-fed group were continued on Diet A until they were sacrificed. Calorie restricted rats (LCR) were provided Diet B in daily allotments, 60% of the mean amount of diet consumed by the AL-fed group cohort, which was measured regularly from 6 weeks until 18 months of age. Thereafter, the caloric intake of the LCR rats was maintained at the 18-month level of intake. Food intake, body weight, organ weight and lifetime caloric intake did not differ significantly from previous reports (5). Diet B was identical to Diet A, except that the concentration of dietary supplements (vitamin, minerals and choline, Ralson Purina) was modified in order to provide both groups of rats with the same intake. Dextrin concentration was adjusted to meet the restricted caloric requirements. Both AL-fed and LCR rats were provided their daily food allotment about 1 hr before the start of the dark phase of a 12-hr light/12-hr dark

TABLE 1

Composition of Diets[a]

Dietary components	Diet A (%)	Diet B (%)
Casein (vitamin-free)	21.00	21.00
Sucrose	15.00	15.00
Dextrin	43.65	39.55
Corn oil	10.00	10.00
Mineral mix	5.00	7.64
Vitamin mix	2.00	3.33
Choline chloride	0.20	0.33
DL-methionine	0.15	0.15
Solka-Floc	3.00	3.00

[a]The concentration of the vitamin, mineral mixes and choline chloride in Diet B was modified to provide a daily intake that is the same as the mean intake of the rats fed on Diet A.

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Abbreviations: AL, *ad libitum*; Con-A, concanavalin A; FBS, fetal bovine serum; GSH, glutathione; IL-2, Interleukin-2; LCR, long-term calorie restriction; PBS, phosphate-buffered saline.

cycle in order to maintain their circadian-based normal feeding pattern throughout the study.

Lymphoid cell isolation. Rats under anesthesia were sacrificed by exsanguination. Spleen and thymus were aseptically collected only from those rats which were visibly tumor-free. Connective tissue was removed and single cell suspensions were prepared. Briefly, the minced tissue was gently homogenized in complete RPMI-containing medium at room temperature and the cell suspension was centrifuged at 100 × *g* and washed three times in RPMI. The cell suspension free of debris was placed on plastic Petri dishes, that had been coated previously with 10% fetal bovine serum (FBS), and was incubated for 2 hr at 37°C, and the non-adherent cell fraction was obtained by washing with warm (37°C) phosphate-buffered saline (PBS). Adherent spleen cells were scraped off and washed in cold PBS or RPMI at 4°C. To obtain a bone marrow-enriched fraction, femurs were removed and cleaned, and the bone cavity was tightly fitted to a needle and slowly flushed with cold PBS. Large clumps of cells were further dispersed through a 27-gauge needle, and cells were washed as described before. For each cell fraction, red blood cells were lysed using Tris/ammonium chloride solution.

Assessment of mitogenic response. Concanavalin A (Con-A) in appropriate serial dilutions, was used to measure *in vitro* mitogenic responses of splenocytes in microtiter plates as described previously (15).

Glutathione assessment. Total and reduced glutathione (GSH) were assayed spectrophotometrically by the enzymatic method of Anderson (17) and by the chemical method of Moron *et al.* (18), respectively.

Fatty acid analysis. Lipids were isolated by chloroform/methanol (2:1, v/v) extraction according to the procedure of Folch *et al.* (19). Extracted lipids were redissolved in chloroform/methanol (2:1, v/v). Fatty acid methyl esters were obtained by transesterification with methanolic HCl (90°C, 2 hr) (Alltech-Applied Science, Deerfield, IL). The esters were extracted and then redissolved in hexane and analyzed on a Hewlett-Packard Model 5880A gas chromatograph equipped with a fused silica megabore column (DB-225, J&W Scientific, Folsom, CA). Oven temperature was 205°C, the flame ionization detector was set at 250°C, and helium served as carrier gas at a flow rate of 9 mL/min. Peaks were identified by comparison of retention times with those of fatty acid standards (Nu-Check-Prep, Elysian, MN).

Data analysis. All data were compared by using multiple analyses of variance. Post-hoc analysis of individual group means was performed by the Duncan multiple-range test.

RESULTS

The body weights of the AL-fed and LCR animals are presented in Figure 1. LCR rats maintained a significantly lower body weight compared to AL animals throughout their lifespan. Although calories were restricted by 40%, LCR rats consumed between 0.13 and 0.15 kcal/g body weight/day after 6 months of age, which was very close to the amount of food consumed by the AL-fed rats (4,5). Therefore, it is of interest to analyze the possible relationship between immune function and the fatty acid

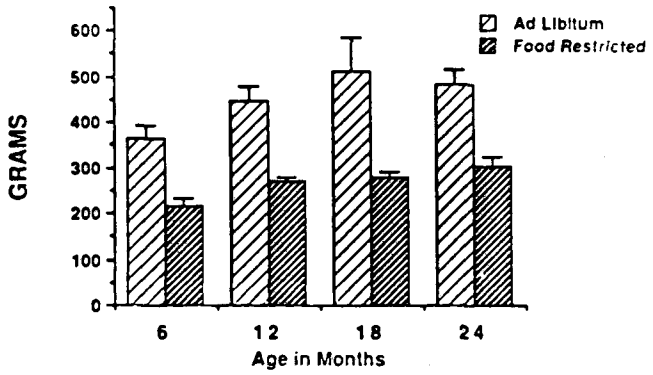

FIG. 1. Effect of aging and LCR on the body weights of rats.

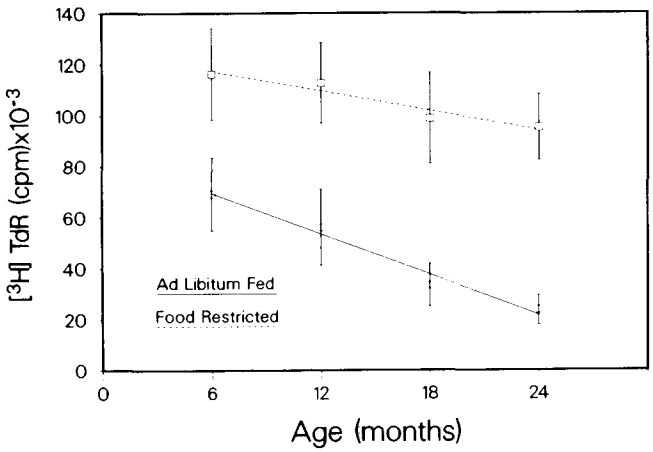

FIG. 2. Spleen cells from *ad libitum* and food restricted rats of various age groups were cultured at a concentration of 5 × 10^6 cells/mL for 48 hr in the presence of various levels of Con-A and pulsed with tritiated thymidine for a further 16 hr before harvest and thymidine uptake was determined. The results reported here are CPM obtained for optimal doses of Con-A (2.5 mg/mL) after subtracting background counts of medium alone, which ranged from 2000–4500 CPM.

composition of lymphoid cells from LCR rats and AL-fed controls (4,5).

The splenocyte mitogenic response to Con-A in aging rats is illustrated in Figure 2. The proliferative response decreased linearly ($r=0.59$, $p<0.005$) in AL-fed rats between the ages of 6 and 24 months. Approximately 70% of the proliferative response was lost during this age interval. In contrast, the slight reduction in Con-A-induced incorporation of [^{3}H]thymidine in LCR rat splenocytes was not statistically significant from 6 to 24 months of age (Fig. 2).

The effect of LCR on whole spleen fatty acid composition was first analyzed in animals of both dietary groups at 12 and 18 months of age and the results are presented in Table 2 and Figure 3. Spleen cell fatty acids were affected by calorie restriction in both age groups. Specifically, linoleic acid (18:2) was higher, whereas polyunsaturated fatty acyls such as arachidonic (20:4), docosatetraenoic (22:4) and docosapentaenoic (22:5) acids were lower in LCR rats as compared to AL rats (ANOVA

$p<0.01$, <0.05, <0.05 and <0.01, respectively). The saturated fatty acids 16:0 and 18:0 were also significantly lower in LCR rats than in AL-fed rats at 12 and 18 months of age ($p<0.05$ and <0.01, respectively.).

These findings were further investigated in isolated splenic lymphoid cells obtained from rats aged 6, 12, 18 and 24 months. Data pertaining to non-adherent spleen cells are presented in Table 3. None of the saturated fatty acids were modulated either by age or by calorie restriction. However, percent composition of 18:2, 20:4, 22:4 and 22:5 were found to be significantly affected by age in the lymphocyte-enriched fraction of aged AL-fed rats. Linoleic acid content decreased between 12 and 24 months of age, whereas 20:4, 22:4 and 22:5 levels increased steadily during this period in AL-fed animals ($p<0.01$, <0.01, <0.01 and <0.001, respectively). These age-related changes in fatty acid composition were completely prevented by LCR. Accordingly, linoleic acid was higher at 18 and 24 months ($p<0.01$) and 20:4 was significantly lower in the lymphocyte-enriched fraction of 24-month-old LCR rats. Levels of 22:4 and 22:5 remained significantly lower in LCR rats after six months of age.

Fatty acids of the adherent cells were also altered by age. The data presented in Table 4 and Figure 4 show that 18:1 and 18:2 progressively decreased from 6 to 24 months ($p<0.005$ and $p<0.001$, respectively) and that 22:4 ($p<0.01$) and 22:5 ($p<0.005$) levels increased steadily between 12 and 24 months of age in the AL-fed rats. Although 18:2 was also decreased with age in the LCR rats, levels of this fatty acid were consistently higher than in AL-fed rats, at all ages studied (ANOVA, $P<0.05$). Arachidonic acid content was significantly lower in LCR rats at 12, 18 and 24 months of age (ANOVA, $p<0.025$). However, no significant diet-related differences in 22:4 or 22:5 were noted during this age interval.

Interestingly, the fatty acid composition of bone marrow cells was not markedly altered by age in the AL-fed rats, as described in Table 5. On the other hand, except for 16:0, all of the major fatty acids were strikingly modulated by long-term calorie restriction. Higher levels of oleic and linoleic acids were found in LCR rat bone marrow cells at all ages tested (ANOVA, $p<0.01$). Also, 18:0, 20:4, 22:4 and 22:5 were all significantly lower in LCR rats at all ages tested (ANOVA, $p<0.01$, <0.005, <0.01, <0.005, respectively) and differences in contents were more accentuated at 24 months of age.

Data pertaining to thymocyte fatty acids are reported in Table 6 and Figure 5. Proportions of 18:1 and 18:2 were dramatically increased with age in both dietary groups,

TABLE 2

Effect of Long-Term Calorie Restriction on the Fatty Acid Composition of Spleen from Aging Rats[a]

Fatty acid	12 months		24 months	
	AL	LCR	AL	LCR
16:0	21.98 ± 1.45	20.43 ± 0.03	24.20 ± 1.30	19.94 ± 0.90
16:1	1.05 ± 0.10	1.00 ± 0.08	1.15 ± 0.10	0.816 ± 0.09
18:0	16.08 ± 0.31	14.43 ± 0.46	17.29 ± 0.35	15.54 ± 0.25
18:1	9.97 ± 0.20	10.45 ± 0.38	10.76 ± 0.36	10.59 ± 0.38
18:2	9.99 ± 0.13	11.98 ± 0.40	9.78 ± 0.12	11.10 ± 0.50
20:2	1.65 ± 0.10	1.84 ± 0.11	1.12 ± 0.10	2.18 ± 0.15
20:4	19.89 ± 0.59	7.98 ± 0.61	19.49 ± 0.51	15.79 ± 0.53
22:4	3.63 ± 0.23	3.04 ± 0.08	4.15 ± 0.35	3.30 ± 0.14
22:5	1.03 ± 0.10	0.64 ± 0.07	1.38 ± 0.10	0.62 ± 0.08
22:6	0.97 ± 0.34	1.72 ± 0.50	0.43 ± 0.02	0.64 ± 0.20

[a]Values represent mean ± S.E.M. of 4–5 independent observations. Results are expressed as percent composition. AL, *ad libitum* fed rats; LCR, long term calorie restricted rats. Diet effect (two-way ANOVA): 16:0, 20:4, 22:4 ($p<0.05$); 18:0, 18:2, 22:5 ($p<0.01$). Age effect: non-significant.

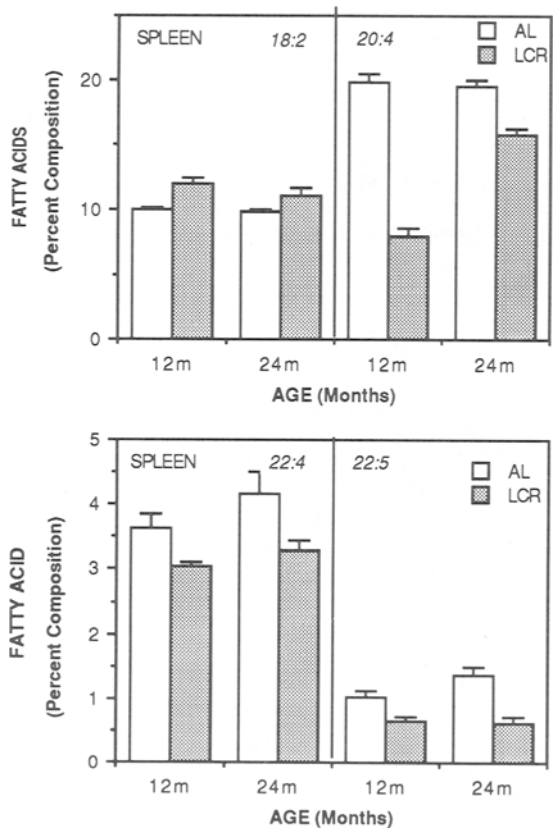

FIG. 3. Effect of age and LCR on the fatty acid composition of spleens of 12- and 24-month-old Fischer-344 rats.

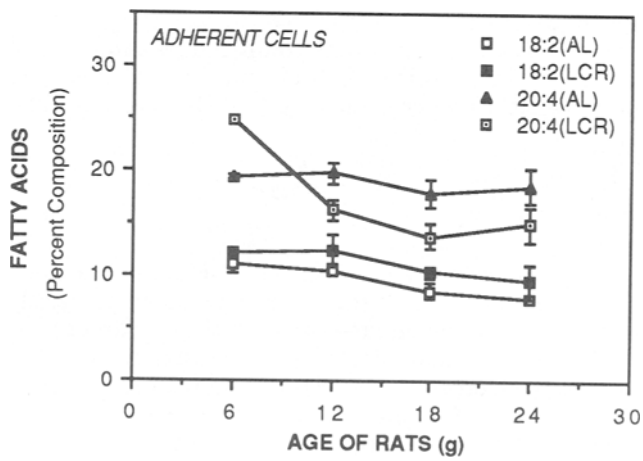

FIG. 4. Effect of LCR on 18:2 and 20:4 composition of splenic adherent cells in 6- to 24-month-old Fischer-344 rats.

TABLE 3

Effect of Long-Term Calorie Restriction on the Fatty Acid Composition of Splenic Non-Adherent Cells from Aging Rats[a]

Fatty acid	6 months		12 months		18 months		24 months	
	AL	LCR	AL	LCR	AL	LCR	AL	LCR
16:0	19.12 ± 1.28	20.63 ± 0.98	20.72 ± 0.65	19.98 ± 0.68	20.36 ± 0.93	18.85 ± 0.44	18.75 ± 1.12	20.71 ± 1.31
16:1	1.42 ± 0.24	1.26 ± 0.20	1.22 ± 0.27	0.87 ± 0.04	0.98 ± 0.06	0.93 ± 0.21	0.66 ± 0.04	0.71 ± 0.07
18:0	13.16 ± 0.58	16.15 ± 1.50	15.08 ± 0.65	15.19 ± 0.47	15.47 ± 0.56	15.92 ± 0.25	15.29 ± 0.75	15.72 ± 1.46
18:1	10.03 ± 1.04	10.05 ± 0.50	10.22 ± 0.39	10.07 ± 0.350	9.86 ± 0.24	10.18 ± 0.44	9.83 ± 0.51	11.00 ± 0.29
18:2	9.54 ± 1.48	9.94 ± 0.63	10.24 ± 0.76	10.34 ± 0.22	7.85 ± 0.13	10.34 ± 0.36	8.07 ± 0.16	9.22 ± 0.36
18:3	0.16 ± 0.04	0.00	0.70 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.11 ± 0.02	0.82 ± 0.03	0.00
20:2	1.37 ± 0.53	2.39 ± 0.19	1.48 ± 0.10	1.75 ± 0.28	1.33 ± 0.15	1.87 ± 0.12	1.45 ± 0.20	0.97 ± 0.92
20:4	19:36 ± 0.67	20.26 ± 1.18	19.03 ± 0.69	19.12 ± 1.08	20.78 ± 0.25	20.26 ± 1.46	21.57 ± 1.05	16.75 ± 1.63
22:4	3.59 ± 0.18	3.75 ± 0.50	3.61 ± 0.31	3.16 ± 0.48	4.65 ± 0.24	4.05 ± 0.18	6.39 ± 1.11	4.24 ± 1.13
22:5	0.97 ± 0.06	0.78 ± 0.08	0.92 ± 0.05	0.70 ± 0.08	1.41 ± 0.12	0.72 ± 0.06	2.40 ± 0.47	0.82 ± 0.20
22:6	0.57 ± 0.07	0.56 ± 0.15	0.82 ± 0.08	0.33 ± 0.01	0.44 ± 0.05	0.56 ± 0.23	0.71 ± 0.08	0.55 ± 0.12

[a]Values represent mean ± S.E.M. of 5–6 independent observations. Results are expressed as percent composition. AL, *ad libitum* fed rats; LCR, long-term calorie restricted rats. Age effect in 12- to 24-month-old AL-fed rats (one-way ANOVA): 18:2, 20:4, 22:4 ($p<0.01$); 22:5 ($p<0.01$); 22:5 ($p<0.001$). Age effect in LCR rats: non-significant. Diet effect (two-way ANOVA and multiple-range test): 18:2 (at 18 and 24 months, $p<0.01$): 20:4 (at 24 months, $p<0.025$); 22:4 (from 12 to 24 months, $p<0.025$); 22:5 (at 12 months, $p<0.025$; at 18 and 24 months, $p<0.001$).

TABLE 4

Effecct of Long-Term Calorie Restriction on the Fatty Acid Composition of Splenic Adherent Cells from Aging Rats[a]

Fatty acid	6 months		12 months		18 months		24 months	
	AL	LCR	AL	LCR	AL	LCR	AL	LCR
16:0	15.37 ± 2.06	16.50 ± 2.47	17.86 ± 1.58	20.37 ± 1.62	16.90 ± 1.10	15.31 ± 1.90	18.44 ± 1.30	20.54 ± 1.75
16:1	0.94 ± 0.18	0.48 ± 0.03	1.01 ± 0.16	1.35 ± 0.35	1.06 ± 0.16	2.08 ± 0.72	1.12 ± 0.20	1.39 ± 0.03
18:0	15.57 ± 0.82	20.25 ± 1.76	15.63 ± 0.72	16.09 ± 0.75	16.56 ± 0.81	15.16 ± 1.30	15.46 ± 0.81	16.30 ± 0.76
18:1	12.89 ± 1.31	13.51 ± 0.80	10.54 ± 0.75	10.83 ± 1.92	9.77 ± 0.73	9.82 ± 0.77	9.00 ± 0.56	11.95 ± 2.20
18:2 (6)	11.17 ± 0.96	12.23 ± 0.43	10.50 ± 0.55	12.39 ± 1.60	8.58 ± 0.67	10.36 ± 0.49	7.78 ± 0.46	9.46 ± 1.60
20:2 (6)	2.01 ± 0.45	4.22 ± 1.70	1.92 ± 0.50	2.68 ± 0.53	1.55 ± 0.16	1.96 ± 0.40	±0.25 ± 0.25	±0.20 ± 0.20
20:4 (6)	19.32 ± 0.25	24.70 ± 1.14	19.74 ± 1.00	16.22 ± 1.22	17.83 ± 1.21	13.73 ± 2.60	18.52 ± 1.65	14.96 ± 2.60
22:4 (6)	3.77 ± 0.49	6.95 ± 0.27	3.40 ± 0.15	3.55 ± 0.18	4.33 ± 0.53	3.12 ± 0.59	5.29 ± 0.35	6.76 ± 0.09
22:5 (3)	0.96 ± 0.12	1.62 ± 0.18	1.04 ± 0.09	0.89 ± 0.15	1.48 ± 0.42	0.69 ± 0.09	1.90 ± 0.30	0.86 ± 0.11
22:6 (3)	0.96 ± 0.14	0.00	0.66 ± 0.10	0.00	0.00	1.10 ± 0.37	0.00	0.00

[a]Values reprsent mean ± S.E.M. of 5–6 independent observations. Results are expressed as percent composition. AL, *ad libitum* fed rats; LCR, long-term calorie restricted rats. Age effect in Al-fed rats (one-way ANOVA): 18:1, 18:2, (from 6 to 24 months, $p<0.005$): 22:4, 22:5 (from 12 to 24 months, $p<0.01$); 20:4, non-significant. Age effect in LCR rats: 18:2 (from 12 to 24 months, $p<0.05$). Diet effect (two-way ANOVA): 18:2 (from 6 to 24 months, $p<0.05$): 20:4 (from 12 to 24 months, $p<0.025$); 22:4, 22:5, non-significant.

TABLE 5

Effect of Long-Term Calorie Restriction on the Fatty Acid Composition of Bone Marrow Cells from Aging Rats[a]

Fatty Acid	6 months		12 months		18 months		24 months	
	AL	LCR	AL	LCR	AL	LCR	AL	LCR
16:0	21.28 ± 0.59	19.91 ± 1.06	19.86 ± 1.10	20.01 ± 0.34	20.78 ± 0.54	20.07 ± 0.36	18.51 ± 1.83	20.49 ± 0.75
16:1	1.54 ± 0.12	2.50 ± 0.23	1.17 ± 0.11	1.36 ± 0.13	1.38 ± 0.08	1.27 ± 0.06	0.97 ± 0.15	1.54 ± 0.24
18:0	12.49 ± 0.33	10.80 ± 1.11	13.60 ± 0.56	12.60 ± 0.70	12.88 ± 0.70	13.25 ± 1.11	16.21 ± 0.06	10.71 ± 1.00
18:1	16.54 ± 0.53	21.55 ± 0.70	13.37 ± 0.80	16.05 ± 1.25	15.57 ± 1.36	15.80 ± 1.46	13.86 ± 0.42	18.06 ± 2.00
18:2	16.13 ± 0.68	19.54 ± 1.03	13.16 ± 0.80	16.50 ± 1.26	14.80 ± 1.25	16.63 ± 1.30	13.38 ± 0.46	18.43 ± 2.80
18:2	0.23 ± 0.01	0.26 ± 0.04	0.25 ± 0.05	0.25 ± 0.07	0.37 ± 0.10	0.26 ± 0.06	0.07 ± 0.05	0.33 ± 0.08
20:2	1.09 ± 0.05	0.74 ± 0.04	0.96 ± 0.16	0.93 ± 0.04	0.88 ± 0.09	1.08 ± 0.12	1.30 ± 0.09	0.56 ± 0.06
20:4	15.50 ± 0.42	11.75 ± 0.70	18.43 ± 1.23	15.75 ± 1.00	15.70 ± 1.23	15.10 ± 1.58	19.83 ± 0.37	9.53 ± 0.60
22:4	2.72 ± 0.10	1.90 ± 0.09	2.88 ± 0.14	2.40 ± 0.13	2.41 ± 0.26	2.45 ± 0.26	3.26 ± 0.27	1.85 ± 0.24
22:5	0.87 ± 0.03	0.65 ± 0.03	1.03 ± 0.08	0.71 ± 0.03	1.10 ± 0.08	0.68 ± 0.03	1.90 ± 0.20	0.49 ± 0.07
22:6	0.51 ± 0.13	0.35 ± 0.04	0.51 ± 0.10	0.33 ± 0.02	0.77 ± 0.23	0.19 ± 0.01	0.42 ± 0.03	1.25 ± 0.43

[a]Values reprsent mean ± S.E.M. of 5–6 independent observations. Results are expressed as percent composition. AL, *ad libitum* fed rats; LCR, long-term calorie restricted rats. Age effect in AL-fed rats (one-way ANOVA): 22:5 (from 6 to 24 months, $p<0.001$); all other fatty acids, non-significant. Diet effect (two-way ANOVA from 6 to 24 months): 18:0, 18:1, 18:2, 22:4 ($p<0.01$); 20:4, 22:5 ($p<0.005$).

TABLE 6

Effect of Long-Term Calorie Restriction on the Fatty Acid Composition of Thymocytes from Aging Rats[a]

Fatty Acid	6 months		12 months		18 months		24 months	
	AL	LCR	AL	LCR	AL	LCR	AL	LCR
16:0	22.56 ± 0.43	22.18 ± 0.58	22.27 ± 1.03	20.65 ± 1.50	22.05 ± 2.26	20.80 ± 1.84	26.81 ± 1.68	22.37 ± 1.16
16:1	1.58 ± 0.17	1.69 ± 0.21	2.20 ± 0.35	2.07 ± 0.33	1.52 ± 0.21	2.05 ± 0.34	2.31 ± 0.23	2.39 ± 0.36
18:0	15.41 ± 0.53	14.95 ± 0.61	8.76 ± 1.73	11.22 ± 1.33	9.10 ± 1.20	10.47 ± 1.95	6.16 ± 0.05	8.99 ± 0.63
18:1	12.66 ± 0.54	12.28 ± 0.43	22.12 ± 2.10	18.73 ± 1.14	22.66 ± 0.06	22.39 ± 2.25	26.85 ± 1.46	26.23 ± 1.47
18:2	8.57 ± 0.46	8.12 ± 0.32	22.65 ± 2.66	16.57 ± 1.10	23.90 ± 1.92	20.16 ± 2.43	25.52 ± 1.70	20.01 ± 1.60
18:3	0.00	0.00	0.22 ± 0.06	0.26 ± 0.05	0.21	0.68 ± 0.00	0.15 ± 0.01	0.40 ± 0.13
20:2	2.46 ± 0.31	2.06 ± 0.04	0.61 ± 0.10	1.44 ± 0.18	0.91 ± 0.26	0.57 ± 0.10	0.02 ± 0.01	0.00
20:4	20.47 ± 1.00	19.31 ± 1.06	8.22 ± 2.10	11.81 ± 0.94	6.98 ± 2.31	3.14 ± 0.48	3.34 ± 0.06	3.44 ± 0.93
22:4	1.89 ± 0.16	2.00 ± 0.18	1.52 ± 0.30	1.71 ± 0.26	2.95 ± 1.17	0.76 ± 0.10	1.15 ± 0.07	0.83 ± 0.24
22:5	0.51 ± 0.06	0.45 ± 0.03	0.47 ± 0.10	0.51 ± 0.20	0.83 ± 0.07	0.34 ± 0.13	0.47 ± 0.02	0.35 ± 0.04
22:6	0.00	0.00	0.12 ± 0.03	1.36 ± 0.39	0.53 ± 0.04	0.16 ± 0.07	0.28 ± 0.04	0.24 ± 0.05

[a]Values represent mean ± S.E.M. of 5–6 independent observations. Results are expressed as percent composition. AL, *ad libitum* fed rats; LCR, long-term calorie restricted rats. Age effect in AL-fed rats (one-way ANOVA from 6 to 24 months): 18:0, 18:1, 18:2, 20:2, 20:4 ($p<0.001$). Age effect in LCR rats: 18:0, 18:1, 18:2, 20:2, 20:4, 22:4 ($p<0.001$). Diet effect (two-way ANOVA from 12 to 24 months): 18:0, 18:2 ($p<0.005$).

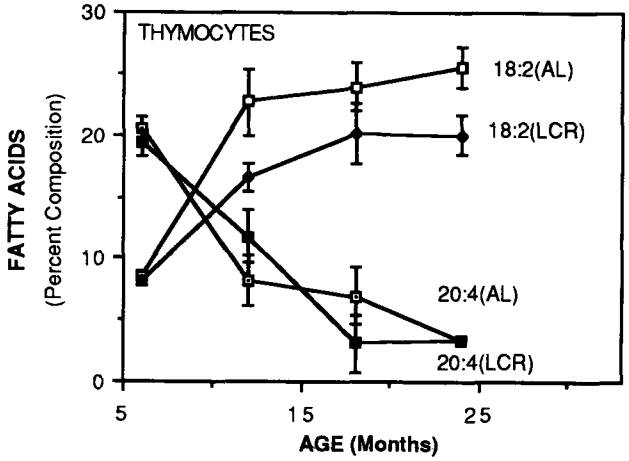

FIG. 5. Influence of LCR on 18:2 and 20:4 levels in thymocytes of 3- to 24-month-old Fischer-344 rats.

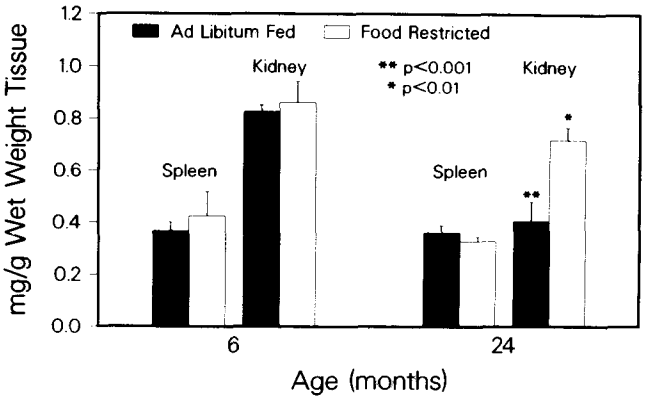

FIG. 6. Effects of age and food restriction on glutathione reductase activity in spleen and kidney tissues obtained from 6- and 24-month-old Fischer-344 male rats maintained on *ad libitum* and food restricted dietary regimens.

whereas 18:0, 20:2 and 20:4 were decreased (one-way ANOVA, $p<0.001$ for each fatty acid). No age-related change in proportions of 22:4 or 22:5 were observed in AL-fed rats, although 22:4 was significantly decreased with age in LCR rats. Diet-related changes in 16:0, 18:0, 18:1 and 20:4 failed to reach significance. However, 18:2 content was significantly lower in thymocytes of 12-, 18- and 24-month-old LCR rats.

Because reduced GSH modulates blastogenesis, we decided to measure GSH levels in the whole spleen supernatant fraction. The data presented in Figure 3 indicate that reduced GSH concentration, which was measured at 6 and 24 months of age in both dietary groups, remained constant at approximately 0.35 mg/g wet weight of spleen. This finding was not replicated in the kidney as shown in Figure 6. Reduced GSH concentration was lowered by 50% in the kidney supernatant of 24-month-old AL-fed rats. In contrast, reduced GSH was only slightly lowered ($p>0.05$) in LCR rat kidney. These results strongly suggest that regulation of spleen GSH concentration is well sustained in aged Fischer-344 male rats as opposed to the aging kidney in the same rats.

DISCUSSION

Our results confirm and extend previous observations that aging alters fatty acid distribution in organs and cells. Our studies of major lymphoid organs indicate that, except in thymocytes, the aging effect showed the following pattern of change: an increase in long chain highly unsaturated fatty acids concomitant with losses in linoleic acid levels. LCR counteracted the age-related loss of 18:2 in spleen cells and bone marrow cells. Therefore, long-term calorie restriction stabilized the fatty acid content of aging animals, and forestalled the loss of mitogenic responsiveness in splenocytes of Fischer-344 rats. Our results confirm the findings of Cheung and Richardson (20), who described the loss of immune function with age in Fischer-344 rats. Hence, alterations of splenocyte fatty acids may underlie the modulation of the response to mitogens in aging rats.

This postulate is in agreement with the paradigm that immune cell reactivity is directly influenced by the enrichment of lymphoid cell membranes with particular fatty acids (20,21). Specifically, membrane enrichment in linoleic acid was associated in various instances with immunosuppression (22–24), as seen in dietary-supplemented multiple sclerosis patients (21,25), experimental allergic encephalomyelitis (26) and rejection of skin allografts (23). Immunosuppression is most likely to occur through the subsequent increase in the content of membrane phospholipid arachidonic acid, which usually results from high linoleic acid supplementation (27). Arachidonic acid is the principal desaturated-elongated derivative of the essential fatty acid, linoleic acid. It is released from membrane phospholipids by the action of phospholipase A_2 (28,29), and is then taken up as precursor for the 2 series prostaglandins (PG) (30).

The PG suppresses blastogenesis, lymphokine production and cytotoxicity (31), especially in the spleen, which is a major site for the production of immunologically active PG (32). In the aging AL-fed rats, increasing membrane content of 20:4 may augment precursor availability for PG release, which may interfere with mitogenic responsiveness of non-adherent spleen cells. Conversely, maintenance of adequate 20:4 content in aging LCR rat lymphoid cells appears to regulate the synthesis of PG, and thereby prevent or delay the fall in splenocyte immune responsiveness.

Most importantly, modulation of cellular fatty acids by LCR was most prominent in the aging bone marrow. The mechanism underlying this finding is not clear, but the bone marrow is the major source of all lymphoid cells for distribution to the organism. Thus, changes in the fatty acid composition of bone marrow lymphoid precursors may be one of the mechanisms by which LCR regulates the lipid composition of mature lymphoid cell subsets, particularly the populations which reside in the spleen.

Because of the strikingly similar trend in the changes reported previously in liver subcellular membrane fatty acids (15,33) and the present results that desaturation of 18:2 into long-chain polyunsaturated derivatives is accelerated with age, this may be a general phenomenon that is inhibited efficiently by chronic calorie restriction. It has been shown earlier that serum 18:2 concentration decreased with age in AL-fed rats (34). Long-term calorie restriction has also been found to alter the profile of serum fatty acids in aging Fischer-344 rats and to reduce the loss of serum 18:2 levels with age (34). The effects of long-term calorie restriction on the fatty acid composition of lymphoid cells is of particular interest since approximately the same amount of food is consumed per gram of body weight by both the dietary groups throughout their lifespan (5,7). The present study further supports the evidence that age-related changes in serum fatty acids are also reflected in the lymphoid cell fatty acid composition, and this finding is in agreement with previous reports (21,25,35).

The marked increase in 18:2 content observed after six months of age for thymus gland reflects the gradual accumulation of adherent cells and surrounding fat tissue which is rich in 18:1 and 18:2 that accompanies the early onset of involution and degeneration of this organ (36). Interestingly, the age-related increase in 18:2 was significantly blunted in LCR rats, in agreement with the previous view that either the function of the thymic tissues or its age related involution may have been forestalled by long-term protein and/or calorie restriction (37–39).

Although the role of reduced GSH in immune activity is still unresolved (40), an increase in lymphocyte proliferation to mitogen stimulation has been reported after enhancing intracellular GSH levels (41). Furthermore, Chaplin and Wedner (42) have shown that depletion of intracellular GSH inhibited [^{3}H]thymidine incorporation and lymphocyte blastogenesis. Therefore, we speculated that possibly higher levels of splenic reduced GSH might influence the sustained proliferative response of aging LCR rats (Fig. 1). A previous report also showed that calorie restriction was able to blunt the age-related loss of reduced GSH in liver (43). Our data suggest that reduced GSH alone probably may not account for diminished lymphoid responsiveness to Con A as lymphoid cells from LCR and AL animals had similar levels of reduced GSH, but markedly different mitogenic responsiveness. However, in the present study, a significant age-related loss of kidney GSH was noted for AL rats which was markedly prevented by calorie striction (Fig. 6). Indeed, caloric restriction is known to reduce renal disease both in Fischer-344 rats as well as in short-lived autoimmunity prone mice (5–7). It appears that reduced GSH may play a role in modulating the renal disease, but more defined studies are required to confirm such a possibility in caloric restricted animals.

In conclusion, our study has provided new evidence that lymphoid cell membrane fatty acids are characteristically altered with age and offers clues regarding one of the possible mechanisms by which long-term calorie restriction prevents the age-dependent decline in lymphoid cell immune reactivity. Calorie restriction may prevent or forestall the increase of suppressor PG production in adherent cell populations. Another consideration (possibly related) is that LCR rats show a higher rate of induction for high affinity IL-2 receptors than AL-fed rats (15). The changes in fatty acid composition measured in this study for non-adherent cells may also relate to preservation of IL-2 receptors and their functions (14,15).

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