Longevity, Hepatic Lipid Peroxidation and Hepatic Fatty Acid Composition of Mice Fed Saturated or Unsaturated Fat-Supplemented Diets

The greater the degree of unsaturation of a fat, the more susceptible it is to oxidation, with the production of fatty acid peroxides[1]. These peroxides have been shown to have many deleterious effects, among them a damaging effect on membranes and subcellular structures[2] and on proteins[2], including mitochondrial oxidative enzymes[3]. The free radical and peroxide products of fat oxidation have been implicated as initiating and/or accelerating factors in ageing[4], carcinogenesis[4,5], atherosclerosis[6] and liver damage[7]. Vitamin E and selenium have been shown to inhibit the damage to biological membranes and proteins due to lipid peroxidation[2]. In other studies long term administration of antioxidants to mice has been shown to increase their lifespan and to inhibit the development of spontaneous cancer[8].

If the ingestion of increased amounts of unsaturated fat results in the ingestion of more oxidation products, or an increased in vivo production of the latter substances, the organism, according to the above evidence, would be expected to exhibit an earlier and higher incidence of degenerative and neoplastic disease, with a resultant decreased lifespan. The present investigation was designed to test this hypothesis by feeding several large groups of mice lifelong diets of different fat content and degree of unsaturation.

Materials and methods. One hundred and eighty male LAF_1J mice and 180 male C3H/HeJ mice (Jackson Laboratory, Bar Harbor, Maine) were obtained at 4 weeks of age, divided into 6 groups of 60 each and fed the following diets: groups I (LAF_1J) and IV (C3H/HeJ) – stock diet (Purina Chow) + 15% sucrose, groups II (LAF_1J) and V (C3H/HeJ) – stock + 15% hydrogenated coconut oil (HCNO), groups III (LAF_1J) and VI (C3H/HeJ) – stock + 15% safflower oil (SFO). The sucrose and oils were blended with the powdered diet in an electric mixer. Fresh diets were prepared weekly and stored in the refrigerator. The Purina Laboratory Chow contained 8.17×10^{-5} g of butylated hydroxyanisole/pound of Laboratory Chow, an amount sufficient to retard oxidation in the 5% fat in the Chow, but insufficient for the added safflower oil. Neither of the oils contained added antioxidants, and the diets were not covered with nitrogen while stored. The sucrose, HCNO and SFO were added to the stock diet in 5% increments every 2 weeks until the 15% level was reached. The mice were then maintained on these diets for their entire lifespan. Weights were taken at monthly intervals. When pre-terminal (12–24 h before anticipated death), the mice were killed by i.p. injection of sodium pentobarbital. Portions of liver were obtained and assayed for lipid peroxidation in vitro as described by BARBER[9]. The livers were homogenized in 0.01 *M* phosphate buffer, pH 7.0, containing 0.15 *M* NaCl, and incubated for 90 min in air at 37 °C. After addition of 1.0 ml of 30% trichloracetic acid and 1.0 ml of 0.75% 2-thiobarbituric acid, the mixture was heated at 100 °C for 15 min, cooled, centrifuged and the optical density read at 530 nm in a Beckman Model DB spectrophotometer. Malonyl dialdehyde prepared by hydrolysis of 1,1,3,3-tetraethoxypropane was used as a standard[10].

Portions of the livers were also extracted in methylal-methanol 4:1, the total lipids isolated, purified, hydrolyzed and methylated, and the major fatty acids analyzed by gas-liquid chromatography using diethylene glycol

Table I. Longevity, body weights, hepatic peroxide values and hepatic fatty acid compositions of LAF_1J mice on different fat-containing diets

Group	I	II	III
Diet	Stock + 15% sucrose	Stock + 15% HCNO	Stock + 15% SFO
Age at death (mos.) [a]	20.2 ± 4.2	21.4 ± 3.1	21.1 ± 3.4
Weight at death (g) [a]	34.5 ± 5.1	36.2 ± 6.3	37.5 ± 6.6
Peroxide value [b]	68 ± 6	63 ± 5	64 ± 5
Fatty acids [c]			
16:0	30.4 ± 8.0	27.3 ± 4.6	17.1 ± 7.9
16:1	4.5 ± 2.1	2.6 ± 1.0	2.0 ± 0.5
18:0	12.3 ± 4.6	16.1 ± 4.1	10.2 ± 6.9
18:1	27.2 ± 9.1	24.3 ± 8.5	13.9 ± 6.4
18:2	16.6 ± 3.2	18.3 ± 2.8	44.6 ± 10.3
20:4	9.0 ± 4.0	11.5 ± 5.0	12.2 ± 5.7

[a] Results are expressed as mean of all animals ± standard deviation. Abbreviations: HCNO, hydrogenated coconut oil; SFO, safflower oil. [b] Peroxide values are expressed as μgms malonyl dialdehyde/gm liver. [c] Percentages of total fatty acids. Peroxide values and fatty acids are the means of 30 determinations on livers of preterminal mice at the mean ages indicated ± standard deviations.

Table II. Longevity, body weights, hepatic peroxide values and hepatic fatty acid compositions of C3H/HeJ mice on different Fat-containing diets [a]

Group	IV	V	VI
Diet	Stock + 15% sucrose	Stock + 15% HCNO	Stock + 15% SFO
Age at death	16.8 ± 4.5	17.3 ± 3.9	15.8 ± 3.8
Weight at death	30.8 ± 3.4	28.6 ± 3.7	29.0 ± 3.9
Peroxide value	65 ± 7	70 ± 6	64 ± 4
Fatty acids			
16:0	26.6 ± 5.7	24.5 ± 6.2	22.2 ± 4.9
16:1	5.6 ± 1.1	4.2 ± 0.9	1.3 ± 0.5
18:0	13.8 ± 2.4	14.0 ± 3.1	16.5 ± 3.2
18:1	31.9 ± 4.8	32.2 ± 2.5	8.9 ± 1.3
18:2	12.7 ± 2.0	14.0 ± 1.9	37.2 ± 6.9
20:4	9.4 ± 2.5	11.0 ± 2.1	13.9 ± 4.3

[a] For explanation see Table I.

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succinate on Anakrom AB 110/120 at 180 °C. All the lipid methods have been previously described[11].

The probabilities that differences in the data were due to chance were calculated by the *t* test.

Results and discussion. As indicated in Tables I and II, although the long term administration of a 15% safflower oil-containing diet resulted in a 2–3-fold increase in the percentage of linoleic acid in the livers of both the LAF_1J and the C3H/HeJ strains of mice, the levels of thiobarbituric acid reacting substances (expressed as malonyl dialdehyde) were not increased as compared to the groups on the hydrogenated coconut oil-containing diet or the groups with no added fat. The longevity of the safflower oil fed mice was also not significantly affected as compared to the other 2 groups.

In one previous investigation the addition of oxidized hog fat to the diet of rats resulted in a significant weight loss[12]. The weight curves of the mice in the present experiment and the terminal body weights given in Table I indicate that the safflower oil-containing diet did not result in a lower rate of weight gain or a loss of weight in either of the mouse strains.

The results of this investigation, therefore, do not support the hypothesis that a moderate increase in dietary unsaturated fat has a deleterious effect on the organism. The effects of the administration of high concentrations of fatty acid oxidation products to animals, as has been done in some previous studies[5,7,12] cannot justifiably be extrapolated to implicate unaltered dietary unsaturated fat as producing increased neoplastic and degenerative disease and decreased longevity. Although the tissue levels of unsaturated fat are increased, they either do not have an increased susceptibility to oxidation in vivo, as has been suggested[13], or if peroxides and free radicals are formed they are so rapidly metabolized so as not to have an adverse effect[14].

Zusammenfassung. Mäuse, die während des ganzen Lebens Nahrung mit 15% Safblumenöl aufnahmen, zeigten eine Steigerung des Linolsäuregehaltes der Leber. Dieses Futter blieb ohne Wirkung auf die Höhe der Oxydationsprodukte, Gewichtskurven oder Langlebigkeit der Versuchstiere, dies im Gegensatz zu Mäusen, die im Futter 15% gehärtetes Kokosnussöl oder kein zusätzliches Fett bekamen.

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Zur Kenntnis der Blattlipide von *Acer platanoides* L. während der herbstlichen Vergilbung

Die herbstliche Vergilbung gewisser Laubbäume ist mit einer chemischen Veränderung der Blattlipide verbunden, welche nach den bisherigen Untersuchungen durch zwei Erscheinungen gekennzeichnet ist. Einerseits werden neue Verbindungen gebildet, die im grünen Blatt nicht vorkommen. Zu diesen gehören eine Reihe von Xanthophyllestern[1–3]. Anderseits beobachtet man eine deutliche Verminderung gewisser Lipidkomponenten, wie des β-Carotins und der ungesättigten Fettsäuren[4] oder sogar ein vollständiges Verschwinden wie im Falle des Chlorophylls. Verschiedene Beobachtungen lassen darauf schliessen, dass das Chlorophyll zwar entfärbt, aber nicht vollständig abgebaut wird. FISCHER et al.[5] und HROMATKA et al.[6] haben nämlich seinerzeit gefunden, dass beim Abbau des Chlorophylls der Phytolrest erhalten bleibt, konnten aber über dessen Bindungsform keine weiteren Angaben machen.

Im Zuge unserer Untersuchungen über die Lipide vergilbender Blätter haben wir uns mit dem weiteren Schicksal des Phytols näher befasst. Bei der Auftrennung von Lipidextrakten aus gelben Blättern von *Acer platanoides* L. auf DC-Platten konnten wir freies Phytol nur nach vorheriger alkalischer Verseifung nachweisen. Dies bedeutet, dass Phytol in diesen Blättern nur als Ester vorliegt. Zu seiner Identifizierung bedienten wir uns eines natürlichen Präparates[7], wobei die Alkohole in freier Form und als 3,5-Dinitrobenzoate auf DC-Platten chromatographiert wurden. Durch präparative Trennung des Lipidextraktes auf Dünnschichtplatten gelang es, die phytolhaltige Verbindung anzureichern. Nach alkalischer Verseifung wurden die Hydrolyseprodukte identifiziert und quantitativ bestimmt. Die Alkoholkomponente, zur Hauptsache aus Phytol bestehend, wurde nach der Methode von WEIGEL[8] bestimmt. Die Säurekomponente wurde als Methylester im Phasenumkehrverfahren auf DC-Platten und gaschromatographisch untersucht, und war identisch mit Linolensäure ($\Delta^{9,12,15}$-Octadecatriensäure). Zur quantitativen Bestimmung wurde ein von ROTHBLAT et al.[9] für Squalen verwendetes und nach unseren Erfahrungen auf Polyensäuren übertragbares Verfahren verwendet.

Da die beiden Komponenten fast rein und in nahezu äquimolaren Mengen vorliegen (Tabelle I), schliessen wir, dass dieser Ester aus Phytyllinolenat besteht.

Nachdem in Herbstblättern wiederholt Ester der Linolensäure nachgewiesen wurden[1–3], die in grünen

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