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Effects of Dietary Fats on Nucleoside Triphosphatase Activity and Nuclear Membrane Fatty Acid Composition of Rats during Development

A. Ammouche[a, b]
Y. Youyou[a, b]
G. Durand[c]
J.M. Bourre[a]

[a] INSERM U-26, Hôpital Fernand-Widal, Paris, France;
[b] Institut National Agronomique (INA), Département de Technologie des I.A.A. et de Nutrition Humaine, Alger, Algérie, et
[c] Laboratoire de Nutrition et de Sécurité Alimentaire, INRA, Jouy-en-Josas, France

Abstract

The effect of various dietary fats on the nucleoside triphosphatase (NTPase) activity and nuclear membrane lipid composition of rat liver during development was assessed. Rats fed a fat-free diet exhibited higher specific activity of NTPase at all ages, compared with control animals. In rats fed a sunflower oil diet, the specific activity of NTPase was also found to be highest at all ages than was observed in the control group. In contrast, animals fed the fish oil diet or peanut-rapeseed oil diet showed a decrease in NTPase activity in comparison with the control group. The specific activity of NTPase was correlated positively with dietary ΣPUFA n–6 ($r = 0.03$, $p < 0.05$) and negatively with the dietary ΣPUFA n–3 ($r = -0.87$; $p < 0.05$). The fatty acid composition of liver nuclear membranes of rats fed a fat-free diet revealed high levels of 16:1 n–9, 18:1 n–9, and 20:3 n–9 acids. A dramatic decrease in 18:2 n–6, 20:4 n–6, and 22:6 n–3 acids was observed. Animals fed a sunflower oil diet showed highs level of n–6 fatty acids, particularly 22:4 n–6 and 22:5 n–6, and low levels of monounsaturated fatty acids. However, when rats were fed a fish oil diet, the liver nuclear membranes were highly enriched in 20:5 n–3, and 22:6 n–3 acids, and there was a simultaneous decrease in arachidonic acid. From these observations it is concluded that dietary fats induce changes not only in the fatty acid composition of the nuclear membrane lipids but also in the specific activity of NTPase involved in nuclear function.

Key Words
Nucleoside triphosphatase
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A. Ammouche
INSERM U-26, Hôpital Fernand-Widal
200, rue du Faubourg St-Denis
75475 Paris, Cedex 10 (France)

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Introduction

In recent years, evidence has accumulated for a dynamic view of nuclear membrane structure and function. Several authors have shown that the nuclei of many different mammalian cells contain appreciable quantities of lipids [1–4]. Most of these lipids are contained in the nuclear membranes [3], and are probably involved in regulating transport between the nucleus and the cytoplasm. The nuclear envelope is a bilayered membrane structure that separates the nucleoplasm and cytoplasm of eukaryotic cells [5–7]. It is widely considered to control nucleocytoplasmic exchange of large macromolecules including many protein and RNA species [8]. A source of high-energy phosphate is necessary for transport but not processing [9–11]. One high-energy phosphate bond is hydrolyzed to transport one nucleotide of mRNA. Studies indicate that the high-energy phosphate specificity is not highly selective, since ATP, UTP, CTP, and GTP are all effective [9–11]. The enzyme appears to be associated with the nuclear lamina and histocytochemical studies indicate that it is located throughout the nuclear envelope [11]. Others, however, have suggested that the enzyme resides in the nuclear pore complex [10]. It has been recently purified and has an apparent molecular weight of 40,000 D [12]. There is considerable evidence suggesting that a nuclear-membrane triphosphatase provides the energy for the transport of mRNA. Several ATPases are reported to be lipid dependent [13, 14]. The lipid dependency of nucleoside triphosphatase (NTPase) activity and efflux of RNA from isolated nuclei is controversial [10, 13]. In addition, a number of investigators have shown that alterations in the lipid composition of membranes can affect the activity of membrane-associated enzymes [14–17]. Feeding diets high or low in polyunsaturated to saturated fats induces differences in fatty acid composition of the nuclear envelope phospholipid in the rat liver, altering functions involved in hormone binding [18], mRNA synthesis, and nucleocytoplasmic exchange of macromolecules [19]. Additionally, the presence of binding sites for steroid and thyroid hormones on the nuclear envelope of target tissues indicates that the nuclear envelope may be involved in regulating entry of these hormones into the nucleus, and thereby mediates hormone action [18]. Thus, diet-induced change in the composition of this membrane alters functions central to the activity of the cell. But the significance of these changes in terms of the time course of alteration has not received much attention. Our study evaluates the effect of qualitatively different lipid dietary intakes on the fatty acid composition of liver nuclear membrane and NTPase activity in the rat liver during development.

Materials and Methods

ATP, and deoxyribonuclease I (DNAse I; bovine pancreas, specific activity: 50 U/mg) were obtained from Sigma (La Verpillière, France) or Merck (Nogent-sur-Marne, France).

Animals and Diets

Experiments were performed on male weanling rats of the Sprague-Dawley strain weighing 40 ± 5 g purchased from Iffa Credo, l'Arbresle, France. Rats were divided into five groups of 30 animals each and maintained under standardized conditions of temperature (22 ± 1 °C) and relative humidity (70%) with a 12-hour light-dark cycle. The animals were fed from days 21 to 120 with a commercial rat diet ad libitum, or a semisynthetic diet containing no fat or 10% fat consisting of peanut-rapeseed oil (48–52%), sunflower oil diet or salmon oil. Fish oil was supplemented with 100 mg α-tocopherol/100 g oil. Diets were stored in the dark at ± 4 °C. The composition of diets and the fatty acid composition of commercial dietary fats are presented in tables 1, 2.

Table 1. Composition of the experimental diets

Ingredients, g/100 g	Diet				
	control	fat free	peanut-rapeseed oil	sunflower oil	fish oil
Casein	23	22.5	22.5	22.5	22.5
Glucose	58	63.5	53.5	53.5	53.5
Lipids	5	<0.1	–	–	–
Cellulose	6	6	6	6	6
Mineral mixture[1]	7	7	7	7	7
Vitamin mixture[2]	1	1	1	1	1
Peanut oil	–	–	4.8	–	–
Rapeseed oil	–	–	5.2	–	–
Sunflower oil	–	–	–	10	–
Salmon oil	–	–	–	–	10

[1] Mineral composition of the mixture (mg/100 g): KH_2PO_4 20.0, $CaCO_3$ 34.6, $CaHPO_4$ 26.55, NaCl 13.70, $MgSO_4 \cdot 7H_2O$ 3.42, $CuSO_4 \cdot 5H_2O$ 0.042, $MnSO_4 \cdot H_2O$ 0.27, $FeSO_4 \cdot 7H_2O$ 1.02, $ZnSO_4 \cdot H_2O$ 0.15, $CoCO_3$ 0.0008, KI 0.0016.

[2] Composition of vitamin supplement per kilogram (triturated in dextrose; United States Biochemical Corporation, Cleveland, Ohio, USA): thiamine-HCl 20 mg, riboflavin 15 mg, nicotinic acid 70 mg, pyridoxine-HCl 10 mg, inositol 170 mg, cyanocobalamin 0.05 mg, menadione 40 mg, *dl*-α-tocopherol 170 IU, choline chloride 1,360 IU (ergocalciferol).

Preparation of Nuclear Membranes

Liver nuclear membranes were prepared by extensive digestion of purified nuclei with DNase I. Nuclei were isolated by modification of the procedure of Widnell and Tata [20]. The resulting pellet of purified nuclei was used to prepare nuclear membranes by modifications of the procedure of Kay et al. [21]. The nuclei were resuspended in 0.25 *M* sucrose containing 1 m*M* $MgCl_2$ (pH 7.4) and centrifuged at 750 *g* for 5 min. The pellet was resuspended in digestion buffer (10 m*M* Tris HCl, 0.30 *M* sucrose, 0.1 m*M* $MgCl_2$ and 5 m*M* 2-mercaptoethanol, pH 8.5) to which freshly dissolved DNase I was added to a concentration of 10 µg/ml. The suspension was incubated at room temperature for 20 min. Following centrifugation (12,000 *g* for 5 min) the crude nuclear membranes were resuspended in 10 m*M* Tris HCl (pH 7.4). Nuclear membranes prepared in this manner are free of other subcellular fractions. Electron microscope examination indicated that the nuclear envelope preparations were intact, with inner and outer membranes well preserved. Protein content was estimated by the method of Lowry et al. [22], using bovine serum albumin as standard.

NTPase Assay

The NTPase reaction was carried out according to Agguter and McCaldin [23] with some modifications. The standard assay mixture (final volume 2.4 ml) contained 25 m*M* tris HCl buffer (pH 8.0), 130 m*M* sucrose, 2.5 m*M* ATP, 2.5 m*M* $MgCl_2$, and 400 µl of nuclear suspension (142 µg protein). Mixtures were incubated at 37 °C, aliquots of 1 ml were taken after 0 and 15 min, and the inorganic phosphate released was determined according to the method of Cammer et al. [24]. The specific activity of the NTPase was 3.35 µmol ATP hydrolyzed/h/mg protein. Under these conditions the reaction was linear up to an incubation time of 25 min.

Lipid Analysis

Lipids were extracted from nuclear membranes using chloroform/methanol 2:1 (vol/vol) by the method of Folch et al. [25] as modified by Pollet et al. [26]. The lipid extracts were then methylated [27]. After extraction with hexane, the fatty acid methyl esters were analyzed by gas phase chromatography using a Carlo Erba (Rueil-Malmaison, France) chromatograph with an automatic on-column injector, a flame ioniza-

Table 2. Fatty acid composition of the experimental diets

Fatty acid	Diet[1]			
	control	peanut-rapeseed oil	sunflower oil	fish oil
14:0	1.08	1.06	0.22	7.48
16:0	20.70	6.02	8.01	15.30
17:0	–	0.06	–	0.88
18:0	11.63	4.27	7.28	3.41
20:0	0.49	0.84	0.44	–
22:0	0.90	1.51	1.00	3.32
24:0	–	–	0.65	–
ΣSFA	34.80	13.76	17.60	30.39
16:1 n–9	0.27	0.20	–	11.81
16:1 n–7	1.34	–	0.11	1.18
18:1 n–9	42.36	52.22	26.14	15.90
18:1 n–7	2.29	–	–	4.43
20:1 n–9	0.92	3.12	0.22	8.80
ΣMUFA	47.18	55.55	26.40	42.12
18:2 n–6	16.52	27.10	55.80	2.07
18:3 n–6	0.15	0.02	–	–
20:4 n–6	–	–	–	0.65
ΣPUFA n–6	16.67	27.12	55.80	2.07
18:3 n–3	1.29	3.00	0.10	0.65
18:4 n–3	–	–	–	1.91
20:5 n–3	–	–	–	10.16
22:5 n–3	–	–	–	2.41
22:6 n–3	–	0.55	–	9.63
ΣPUFA n–3	1.29	3.55	0.13	24.76
n–6 + n–3	17.96	30.67	55.90	27.48
n–6/n–3	12.92	7.63	558.02	0.10
DBI/SFA	2.47	9.37	7.80	6.02

SFA = Saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; DBI = double-bond index.

[1] Values represent the average obtained from at least three separate extractions and analyses of the respective diets and amounts are expressed as a percentage of the total identified fatty acids.

tion detector and capillary-type Carbowax CP Wax 52 CB column. The data were processed using a microcomputer, and fatty acid concentrations were reported as percent of total fatty acid content. The double-bond index (DBI) was calculated from the sum of the percentages of each unsaturated fatty acid times the number of double bonds in that acid.

Statistical Analysis

All data are presented as means ± SD. The effects of different dietary fats were examined by analysis of variance (ANOVA), and the effect of individual diets was compared for statistical significance ($p < 0.05$) using the unpaired Student t test [28]. Estimation of the correlation coefficient was determined by the regression test [29].

Table 3. Fatty acid composition of liver nuclear membranes of rats fed various dietary fats for 3 months

Fatty acid	Diet				
	control	fat free	peanut-rapeseed oil	sunflower oil	fish oil
14:0	1.01±0.01	0.70±0.01	0.60±0.02	0.40±0.01	0.62±0.02
15:0	0.33±0.02	0.63±0.02	0.62±0.01	0.40±0.01	0.50±0.04
16:0	20.35±0.12	27.70±0.02	22.30±0.03	20.04±0.06	25.44±0.01
16:1 n–9	1.17±0.04	1.22±0.01	1.65±0.01	0.74±0.01	1.10±0.03
16:1 n–7	1.38±0.02[a]	3.00±0.07[b]	1.80±0.05[c]	0.92±0.02[d]	2.45±0.09[e]
17:0	0.94±0.01[a]	2.90±0.01[b]	1.20±0.01[c]	0.91±0.05[a]	0.67±0.01[a]
18:0	19.96±0.44	17.10±0.06	19.80±0.03	24.40±0.04	19.80±0.02
18:1 n–9	11.78±0.15[a]	26.20±0.10[b]	15.10±0.02[c]	8.60±0.01[d]	13.70±0.03[e]
18:1 n–7	2.84±0.03[a]	5.30±0.01[b]	4.10±0.10[c]	3.22±0.03[d]	4.35±0.04[e]
18:2 n–6	13.32±0.22[a]	4.24±0.07[b]	6.10±0.02[c]	11.10±0.01[d]	3.30±0.05[e]
18:3 n–6	trace	0.22±0.01	0.20±0.01	0.30±0.02	0.21±0.01
18:3 n–3	trace	0.42±0.02	0.20±0.01	–	0.30±0.04
20:0	0.28±0.02	0.55±0.01	0.30±0.01	0.40±0.02	0.20±0.01
20:1 n–9	0.55±0.04	0.63±0.01	0.36±0.01	0.82±0.03	0.60±0.02
20:1 n–7	0.08±0.00	0.36±0.03	0.12±0.01	0.10±0.00	0.14±0.02
20:2 n–6	trace	–	0.33±0.01	0.80±0.04	–
20:3 n–9	0.32±0.01[a]	1.25±0.01[b]	0.52±0.02[c]	0.40±0.03[a, c]	0.50±0.03[c, d]
20:3 n–6	0.80±0.00	0.50±0.01	0.63±0.02	0.65±0.01	0.52±0.01
20:4 n–6	16.67±0.17[a]	1.70±0.01[b]	12.16±0.03[c]	19.97±0.09[d]	6.30±0.04[e]
20:5 n–3	0.39±0.03[a]	0.23±0.01[b]	2.64±0.05[c]	0.20±0.02[d]	6.44±0.01[d]
22:0	0.67±0.01	0.75±0.04	0.40±0.02	0.32±0.01	0.20±0.01
22:1 n–9	0.28±0.08	0.84±0.04	0.41±0.02	–	0.20±0.01
22:4 n–6	–	–	0.35±0.01	0.91±0.01	0.21±0.02
22:5 n–6	0.05±0.01[a]	0.22±0.03[b]	0.10±0.01[a, b, d]	1.94±0.06[c]	0.20±0.01[b, d]
22:5 n–3	1.21±0.01	–	0.90±0.02	0.10±0.01	2.00±0.05
22:6 n–3	3.17±0.09[a]	0.60±0.01[b]	5.10±0.07[c]	1.12±0.03[d]	8.90±0.04[e]
24:0	1.27±0.05	1.94±0.02	1.40±0.01	1.22±0.03	0.40±0.03
24:1 n–9	0.11±0.01	0.62±0.03	0.10±0.00	trace	0.53±0.02
24:1 n–7	0.05±0.01	trace	0.10±0.01	–	0.20±0.01

Results are expressed as percent of total fatty acids (mean ± SD, n = 6). For each fatty acid, values not bearing the same superscript are significantly different at $p < 0.05$. If no superscript letter appears, values are not significantly different.

Results

Fatty Acid Composition of Nuclear Membranes

Substantial changes occurred in the fatty acid patterns of liver nuclear-membrane lipdis among the various groups (tables 3, 4). A fat-free diet induced in the liver nuclear membranes an increase in oleic (18:1 n–9), palmitoleic (16:1 n–7) and 5,8,11-eicosatrienoic (20:3 n–9) acids. A concomitant decrease in linoleic (18:2 n–6), arachidonic (20:4 n–6), and docosahexaenoic (22:6 n–3) acids was observed, as compared to the control group. The

Table 4. Indices of lipid differences in liver nuclear membranes from rats fed various dietary fats

Index	Diet				
	control	fat free	peanut-rapeseed oil	sunflower oil	fish oil
ΣSFA	44.81 ± 0.68[a]	52.27 ± 0.19[b]	46.62 ± 0.14[a,c,d]	48.09 ± 0.29[c]	47.83 ± 0.15[c,d]
ΣMUFA	18.24 ± 0.38[a]	38.17 ± 0.29[b]	24.14 ± 0.15[c]	14.40 ± 0.11[d]	23.27 ± 0.26[c,e]
Σn–6	30.84 ± 0.39[a]	6.88 ± 0.12[b]	19.87 ± 0.11[c]	36.00 ± 0.24[d]	10.74 ± 0.14[e]
Σn–3	4.77 ± 0.13[a]	1.25 ± 0.04[b]	8.84 ± 0.15[c]	1.42 ± 0.06[d,b]	17.64 ± 0.14[e]
ΣPUFA	35.93 ± 0.53[a]	9.39 ± 0.17[b]	29.23 ± 0.28[c]	37.49 ± 0.33[d]	28.88 ± 0.31[c,e]
n–6/n–3	6.46 ± 0.05[a]	5.50 ± 0.02[b]	2.24 ± 0.05[c]	25.32 ± 0.10[d]	0.64 ± 0.03[e]
DBI/SFA	3.07 ± 0.01[a]	1.26 ± 0.01[b]	3.00 ± 0.02[a]	30.1 ± 0.03[a]	3.25 ± 0.09[a,c]
20:3 n–9/20:4 n–6	0.02 ± 0.00[a]	0.73 ± 0.01[b]	0.04 ± 0.01[a,c]	0.02 ± 0.01[a]	0.08 ± 0.01[c]
20:3 n–6/18:2 n–6	0.06 ± 0.01[a]	0.11 ± 0.01[b]	0.10 ± 0.01[a]	0.06 ± 0.02[a]	0.16 ± 0.01[b]
20:4 n–6/20:3 n–6	20.83 ± 0.10[a]	3.40 ± 0.01[b]	19.30 ± 0.10[c]	31.23 ± 0.19[d]	12.11 ± 0.23[e]

For each index, values not bearing the same superscript are significantly different at $p < 0.05$. If no superscript appears, values are not significantly different. SFA = Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated acids.

DBI saturated fatty acid (SFA) ratio was lower ($p < 0.05$) than that of the control group. Higher ratios of 20:3 n–9/20:4 n–6, 18:1 n–9/18:0 [which are commonly used as an index of essential fatty acid (EFA) deficiency], and 20:3 n–6/18:2 n–6 indicate a Δ9- and Δ6-desaturase stimulation, respectively. When the animals were fed the sunflower oil diet, n–6 series fatty acids were increased, particulary 22:4 n–6, and 22:5 n–6, and 22:6 n–3 decreased, resulting in an increase of the n–6/n–3 molar ratio (25.32 vs. 6.46 in the nuclear membrane of rats fed the standard diet), but the total amount of polyunsaturated fatty acids (PUFA) was not altered. The total amount of the monounsaturated fatty acids (MUFA), of the SFA, and DBI/SFA ratio were not altered. Animals fed a peanut-rapeseed oil diet showed similar fatty acid patterns in the nuclear membrane to those of control animals. The animals fed the fish oil diet exhibited a decrease in the proportion of the major PUFA of the n–6 series (20:4 n–6, 22:4 n–6, 22:5 n–6), and an increase in those of the n–3 series, particulary 22:5 n–3 and 22:6 n–3, resulting in a decreased n–6/n–3 ratio (0.64 vs. 6.46 in the nuclear membrane of rats fed the standard diet). Lower ratios of 20:3 n–6/18:2 n–6 and 20:4 n–6/20:3 n–6 could indicate that Δ6- and Δ5-desaturase enzymes were inhibited. The total amount of the PUFA and SFA, and the DBI/SFA ratio were not altered (table 4).

NTPase Activity

The specific activities of NTPase in nuclear membranes of rats fed various diets are shown in figure 1. It can be seen that the specific activity of NTPase increased from day 21 to day 60. In rats fed a fat-free diet, the specific activity of NTPase at 120 days of age was about 2-fold that at 21 days (108.22 ± 3.16 vs. 64.62 ± 0.60 nmol P_i/mg protein/min), and increased by 16% compared with control animals at the same age (93.33 ± 3.33 nmol P_i/mg protein/min). In rats fed a sunflower oil

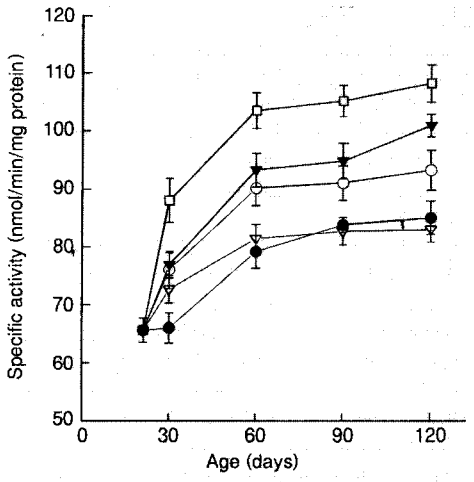

Fig. 1. Specific activity of NTPase in liver nuclear membranes of rats fed various dietary fats during development. Values are the mean ± SD of 6 animals; experiments were done in triplicate. □ = Fat-free diet; ▼ = sunflower oil diet; ● = peanut-rapeseed oil; ○ = control diet; △ = fish-oil diet.

Table 5. Effects of dietary fats on the specific activity of NTPase in rat liver nuclear membrane at 120 days, and the correlation coefficient between the dietary ΣPUFA n–6, ΣPUFA n–3, DBI/SFA, and the NTPase activity

Diet	NTPase activity, nmol P_i released/min/mg protein
Control	93.33 ± 3.33[a,b]
Fat free	108.22 ± 3.16[b]
Peanut-rapeseed oil	85.19 ± 2.88[a]
Sunflower oil	100.93 ± 1.92[b]
Fish oil	83.11 ± 2.15[a]
Correlation coefficient (r)	
Σn–6	0.03
Σn–3	–0.87
DBI/SFA	0.30

NTPase values are expressed as the mean ± SD for 6 animals. Those not bearing the same superscript are significantly different at $p < 0.05$.

diet, the specific activity of NTPase was also found to be higher at all ages than in the control group. The specific activity at 120 days was 1.6-fold that at 21 days (100.93 ± 1.92 vs. 64.62 ± 0.60 nmol P_i/mg protein/min), and increased by 10% compared to the control animals at 120 days of age (93.33 ± 3.33 nmol P_i/mg protein/min). In contrast, animals fed the fish oil or peanut-rapeseed oil diet showed a decrease in ATPase activity of approximately 11% in comparison with the control group (83.11 ± 2.15 vs. 93.33 ± 3.33 nmol p_i/mg protein/min). The values of the correlation factors given for the five diets are shown in table 5: the specific activity of NTPase was correlated positively with dietary Σn–6 ($r = 0.03$; $p < 0.05$). There was also a positive correlation between the DBI/SFA ratio and the specific activity of NTPase ($r = 0.30$; $p < 0.05$). In contrast, the dietary Σn–3 level was negatively correlated with the specific activity of NTPase ($r = -0.87$, $p < 0.05$).

Discussion

The present study is the first, to our knowledge, to examine the rate of alteration in nuclear membrane NTPase activity of developing rats fed various dietary fats. The data presented in this work have shown that it is possible to manipulate the fatty acid composition of the nuclear membrane in rats using diets suitably enriched in given fatty acids. The specific activity of NTPase was altered by a diet-induced change in the composition of the nuclear membrane, clearly indicating lipid dependency. The difference of conformation between the n–3 and n–6 PUFA in the nuclear membrane certainly plays a role. A high level of n–6 PUFA gives a less packed (more fluid) lipid apolar core [30, 31]. The changes in NTPase activity observed could be explained in terms of alterations in physical

characteristics of the nuclear membrane due to an alteration in the phospholipid fatty acid profile. Phospholipid fatty acid composition has previously been reported to be altered in nuclei and nuclear envelopes prepared from hepatoma cells [32]. Our results support the observation of Venkatraman et al. [18], who found that the specific activity of NTPase was significantly higher in liver nuclear-envelope preparations from mice fed high-P/S-ratio compared with low-P/S diets. At least three different explanations can be advanced to explain the increase of NTPase activity in rats fed a fat-free diet: (a) an increase in the number of enzyme molecules per milligram protein; (b) an increase in the specific activity of the individual NTPase molecules, and (c) a change in the physical state of the membrane containing the NTPase molecules that affects the activity. However, the present results on NTPase activity in the nuclear membrane tend to support the role of membrane lipid fluidity in allosteric modification of NTPase activity. Additionally, it is conceivable that an increase in the specific activity of NTPase in liver nuclear membranes of rats fed the fat-free diet could favor increased mRNA transport associated with this enzyme. Supporting this view, it has been shown that modification of the phospholipid component of the nuclear membrane substantially modulated thyroid hormone physiology [33]. In contrast, the reduced NTPase activity in liver nuclear membrane from rats fed the fish-oil diet indicates different modes of lipid influence on the enzyme activity. Dietary lipids can affect chemical and physical properties of the membrane, and fatty acid composition changes of membrane phospholipids can be related to changes in either their packing or bilayer fluidity, or both [34]. Moreover, activities of membrane-bound enzymes can be modulated by particular phospholipids and/or changes of membrane fatty acid composition [35]. On the other hand, enzyme activities may also change as a consequence of changes in metabolic-pathway rates [35–37].

In conclusion, the results of this study indicate a relationship between the specific activity of NTPase and the unsaturation index of the nuclear-membrane fatty acids. A change in the unsaturation index of the nuclear-membrane fatty acids is indicative of a change in the fluidity of the nuclear membranes.

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References

1 Gurr MI, Finean JB, Hawthorne JN: Phospholipids of liver-cell fractions. 1: Phospholipid composition of liver-cell nucleus. Biochim Biophys Acta 1963;70:406–416.
2 Konigs AWT, Loomeijer FJ: Lipid composition of isolated rat-thymus nuclei. Biochim Biophys Acta 1970; 202:216–218.
3 Khandwala AS, Kasper CB: Fatty acid composition of individual phospholipids from rat-liver nuclear membrane and nuclei. J Biol Chem 1971;246:6242–6246.
4 Hilderson HJ, Lagraw A, Dierick W: Nuclear lipids of bovine hypertrophic thyroid. Biochim Biophys Acta 1974;337:385–389.
5 Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD: The cell nucleus; in: Molecular Biology of the Cell. New York, Garland, 1983, pp 385–482.
6 Franke WW: Structure, biochemistry, and function of the nuclear envelope; in Bourne GH, Danielli JF (eds): International Review of Cytology. Academic Press, 1974, pp 71–236.

7 Harris JR, Agutter PS: The isolation and characterization of the nuclear envelope; in Maddy AH (ed): Biochemical Analysis of Membranes. New York, Wiley, 1976, pp 132–173.
8 Herlan G, Giese G, Wunderlich F: Influence of nuclear-membrane lipid fluidity on nuclear-RNA release. Exp Cell Res 1979;31:305–309.
9 Ishikawa K, Sato-Odani S, Ogata K: The role of ATP in the transport of rapidly-labeled RNA from isolated nuclei of rat liver in vitro. Biochim Biophys Acta 1978;521:650–654.
10 Agutter PS, McCaldin B, McArdle HJ: Importance of mammalian nuclear-envelope nucleoside triphosphatase in nucleocytoplasmic transport of ribonucleoproteins. Biochem J 1979;182:811–819.
11 Clawson GA, James J, Woo CH, Friend DS, Moody D, Smuckler EA: Pertinence of nuclear-envelope nucleoside triphosphatase activity to ribonucleic-acid transport. Biochemistry 1980;19:2748–2756.
12 Schroder HC, Rottmann M, Bachmann M, Muller WEG: Purification and characterization of the major nucleoside triphosphatase from rat liver nuclear envelopes. Biol Chem 1986;261:663–668.
13 Agutter PS, Sucking KE: The fluidity of the nuclear envelope lipid does not affect the rate of nucleocytoplasmic RNA transport in mammalian liver. Biochim Biophys Acta 1982; 696:308–314.
14 Brivio-Haugland RP, Louis SL, Musch K, Waldeck N, Williams MA: Liver plasma membranes from essential fatty acid deficient rats: Isolation, fatty acid composition and activities of 5′-nucleotidase, ATPase and adenylate cyclase. Biochim Biophys Acta 1976;433:150–163.
15 Jarnefelt J: Lipid requirements of functional membrane structures as indicated by reversible inactivation of $(Na^+ + K^+)$-ATPase. Biochim Biophys Acta 1972;226:91–96.
16 Sanderman H Jr: Regulation of membrane enzymes by lipids. Biochim Biophys Acta 1987;515:209–237.
17 Bourre JM, François M, Youyou A, Dumont O, Picciotti M, Pascal G, Durand G: The effect of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance of learning tasks in rats. J Nutr 1989;119:1880–1892.
18 Venkatraman JT, Lefebvre YA, Clandinin MT: Diet fat alters the structure and function of the nuclear-envelope – Modulation of membrane fatty acid composition, NTPase activity. Biochem Biophys Res Commun 1986;135:655–661.
19 Venkatraman JT, Clandinin MT: Ribonucleic acid efflux from isolated mouse liver nuclei is altered by diet and genotypically determined change in nuclear envelope composition. Biochim Biophys Acta 1988; 940:33–42.
20 Widnell CC, Tata JR: A procedure for the isolation of enzymically active rat-liver nuclei. Biochem J 1964;92:313–317.
21 Kay RR, Frazer D, Johnston IR: Method for rapid isolation of nuclear membranes from rat-liver: Characterization of membrane preparation and its associated DNA polymerase. Eur J Biochem 1972; 30:145–154.
22 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.
23 Agguter PS, McCaldin B: Inhibition of ribonucleic acid efflux from isolated SV 40-3T3 cell nuclei by 3′-deoxyadenosine (cordycepin). Biochem J 1978;180:371–378.
24 Cammer W, Sirota SR, Zimmerman TR Jr, Norton WT: 5′-nucleotidase in rat brain myelin. J Neurochem 1980;35:367–376.
25 Folch JR, Lees M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
26 Pollet S, Ermidou S, Le Saux F, Monge M, Baumann N: Microanalysis of brain lipids: Multiple two dimensional thin-layer chromatography. J Lipid Res 1978;19:916–921.
27 Morrison WR, Smith LM: Preparation of fatty acid methyl esters and dimethyl-acetals from lipids with boron fluoride-methanol. J Lipid Res 1964;5:600–608.
28 Snedecor TW, Cochran WJ: Statistical Methods, ed 6. Ames, Iowa State University Press, 1967.
29 Schwartz E: Méthodes statistiques à l'usage des médecins et des biologistes. Paris, Flammarion, 1975.
30 Brenner RR: Effect of unsaturated acids on membrane-structure and enzyme-kinetics. Prog Lipid Res 1984;23:69–96.
31 Ehringer H, Belcher D, Wassal SR, Stillwell W: A comparison of the effects of linolenic and docosahexaenoic acids on phospholipid-bilayers. Chem Phys Lipids 1990;54: 79–88.
32 Belcher D, Stillwell W, Langsford CA, Wassal SR: Effect of fish-oils on rat plasma-lipoproteins. Biochem Biophys Res Commun 1988;150: 1063–1068.
33 Spindler BJ, McLeod KM, Ring J: Thyroid hormone receptors: Binding characteristics and lack of hormonal dependency for nuclear localization. J Biol Chem 1975;250: 4113–4119.
34 Rajamoorthi K, Trouard TP, Salmon A, Brown MF: NMR-studies of phospholipid-bilayers containing docosahexaenoic and arachidonic acids. Biophys J 1980;57:273–280.
35 Yamaoka S, Urade R, Kito M: Mitochondrial-function in rats is affected by modification of membrane phospholipids with dietary sardine-oil. J Nutr 1988;118:290–295.
36 Wong SH, Nestel PS, Trimble RP, Storer GB, Illman RJ, Topping DL: The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. Biochim Biophys Acta 1984; 792:103–109.
37 Simopoulos AP: Summary of the conference on the health effects of polyunsaturated fatty acids in seafoods. J Nutr 1986;116:2350–2354.