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Effect of dietary lipids on plasma lipoproteins and fluidity of lymphoid cell membranes in normal and leukemic mice

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Mice of the GR/A strain were fed four different isocaloric semipurified diets, enriched in either (1) saturated fatty acids (palm oil), or (2) polyunsaturated fatty acids (corn oil), or (3) palm oil plus cholesterol, or (4) a fat-poor diet containing only a minimal amount of essential fatty acids. We have studied the effects of these dietary lipids on the density profile and composition of the plasma lipoproteins and on the lipid composition and fluidity of (purified) lymphoid cell membranes in healthy mice and in mice bearing a transplanted lymphoid leukemia (GRSL). Tumor development in these mice occurred in the spleen and in ascites. While the fatty acid composition of the VLDL-triacylglycerols still strongly resembled the dietary lipids, the effects of the diets decreased in the order VLDL-triacylglycerols > HDL-phospholipids > plasma membrane phospholipids. Diet-induced differences in the latter fraction were virtually confined to the content of oleic acid and linoleic acid, and they were too small to affect the membrane fluidity, as measured by fluorescence polarization using the probe 1,6-diphenyl-1,3,5-hexatriene. Healthy mice were almost irresponsive to dietary cholesterol, but in the tumor bearers, where lipoprotein metabolism has been shown to be disturbed, the cholesterol diet caused a substantial increase in the low- and very-low density regions of both blood and ascites plasma lipoproteins. The cholesterol-rich diet also increased the cholesterol/phospholipid molar ratio and lipid structural order (decreased fluidity) in GRSL ascites cell membranes, but not in the splenic GRSL cell membranes. We conclude that the composition of plasma lipoproteins and cell membrane lipids in GR/A mice is subject to exquisite homeostatic control. However, in these low-responders to dietary lipids the development of an ascites tumor may lead to increased responsiveness to dietary cholesterol. The elevated level of membrane cholesterol thus obtained in GRSL ascites cells did not affect the expression of various cell surface antigens or tumor cell growth.

Introduction

The degree of lipid fluidity of the plasma membrane has been generally recognized to be important for the appropriate functioning of cells [1–3]. It may control various cell membrane properties such as membrane permeability and

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carrier-mediated transport [2–4], the binding of ligands to their cell surface receptors [3–7], and the functional coupling of the occupied receptor to effector enzymes in cell signal transduction, such a adenylate cyclase [8–11]. The degree of membrane fluidity may also play a role in tumor–host interactions of both humoral and cellular immunological types [1], the immunogenicity of tumor cells [12–14] and their sensitivity towards certain chemotherapeutic drugs or hyperthermia [15–17]. These findings have suggested the possibility that membrane lipid modification of immunocompetent cells (e.g. lymphocytes) and/or tumor cells in vivo could be a useful adjunct to currently available therapeutic modalities [1,16,17]. Therefore, it is very important to investigate in animal models how and to what extent cell membrane lipids can be modified and whether such alterations affect the membrane fluidity. The best way to perform such studies is by making use of well-defined semipurified diets that are enriched in certain types of lipids.

In the present study we used GR/A mice and a murine leukemia (GRSL), which had originated and was subsequently transplanted in this mouse strain. Previously, we have found that the plasma membrane lipid composition and fluidity of GRSL tumor cells strongly depends on their localization in the host [18]. Ascites cells show an extremely high fluidity in their plasma membrane, due to both very low cholesterol and sphingomyelin levels [19], while tumor cells located in the spleen exhibit a membrane fluidity which is slightly lower than in normal splenic lymphocytes [18]. Here we have investigated to what extent the lipid composition and fluidity of the plasma membranes of these cells can be modulated by using four different isocaloric diets, enriched in either (1) saturated fatty acids (palm oil), or (2) $(n-6)$ polyunsaturated fatty acids (corn oil), or (3) palm oil plus a high amount (1.9 wt%) of cholesterol, or (4) a fat-poor diet, containing only a minimal amount of essential fatty acids. Since plasma lipoproteins are the main carriers of (dietary) lipids through the body, we also examined their composition in normal and tumor-bearing mice as a function of the diets.

Materials and Methods

Diets. Four semipurified diets (compositions are given in Table I) were kindly provided by Unilever Research Lab (Vlaardingen, The Netherlands) and freshly prepared every 2 weeks. Immediately after arrival in our Institute the powders were stored in small packages (150 g) under nitrogen at $-20\,^{\circ}C$ until they were used. Feeding-throughs were cleaned and refilled every second day. Mice were put on the diets 3 weeks after birth and were given free access to food and tap water.

Animals and cells. Six experiments were carried out, each with four diet groups consisting of twenty male mice of the GR/A strain. In three of the experiments 6–8 weeks old mice were inoculated intraperitoneally with $5 \cdot 10^6$ GRSL 13 cells. The GRSL 13 tumor, which originated from a spontaneous thymus-derived lymphoid leukemia in the GR/A mouse strain, grew out in the peritoneal cavity as a cell suspension bathed in an ascites fluid, and in lymphoid organs such as the spleen, but more slowly than the GRSL 18 tumor which

TABLE I

COMPOSITION OF THE FOUR DIETS

The added fat components represent 30 energy% and 7 energy% of the fat-rich and fat-poor diets, respectively.

	Corn oil diet	Fat-poor diet	Palm oil diet	Palm oil + cholesterol diet
Weight %				
Corn oil	14.5	–	–	–
Palm oil	–	–	13.3	11.2
Safflowerseed oil	–	2.6	1.2	1.4
Cholesterol	–	–	–	1.9
Corn starch	51.4	68.8	51.4	51.4
Cellulose	6.1	5.2	6.1	6.1
Casein	25.4	21.3	25.4	25.4
Salt mixture	2.2	1.8	2.2	2.2
Vitamin mixture	0.4	0.3	0.4	0.4
Major fatty acids (% of total FA)				
16:0	12.1	8.3	43.0	42.1
18:1	24.5	14.1	35.5	35.3
18:2	60.5	74.8	16.6	17.7

we have used in our previous experiments [18,20–23]. Normal and tumor-bearing mice were killed when they were 9–12 weeks old (16 days after tumor transplantation) and after an overnight fast. Tumor cells were harvested from the ascites fluid (see below) and from the spleen. Splenic lymphoid cells (normal or tumor cells) were separated from erythrocytes by centrifugation (20 min, $400 \times g$ at room temperature) of single-cell suspensions in Hanks' solution (Oxoid, London, U.K.) over a 17.5% metrizamide cushion.

Lipoproteins. Blood was collected in heparinized tubes and processed as described before [21]. Ascites fluid was collected by eluting the peritoneal cavity with 2 ml phosphate-buffered saline. Ascitic GRSL cells and extracellular membrane vesicles [20] were separated by centrifugation at $250 \times g$ for 10 min and at $105\,000 \times g$ for 60 min, respectively. The latter supernatant contained the diluted ascites plasma. Lipoproteins from the peripheral blood and the ascites plasma were fractionated by density gradient centrifugation, essentially according to Redgrave et al. [24], with some minor modifications [21]. Twelve fractions obtained by pipetting from the top of the gradients were analyzed for density by refractometry and for total cholesterol content. Fractions 1 + 2 containing VLDL and fractions 7 + 8 containing HDL were pooled for further analysis.

Isolation of plasma membranes. Splenic lymphoid cells or GRSL ascites cells were disrupted by pumping single-cell suspensions in Hanks' solution at 0–4°C through an air-driven cell disruptor (Stansted Fluid Power Ltd., Stansted, Essex, U.K.; model AO 612, disrupting valve 516) under conditions as described previously [18,23]. Plasma membranes were purified from $(1.95–945) \cdot 10^4 \times g \cdot \text{min}$ pellets of the cell homogenates by means of discontinuous sucrose gradients as described in detail before [25]. The purity of the plasma membrane preparations was ascertained routinely by electron microscopy and by marker assays, as described previously [25].

Chemical analysis. Lipoprotein fractions and membrane preparations were analyzed for their chemical composition. Triacylglycerol was measured by the method of Giegel et al. [26]. Free and esterified cholesterol were assayed enzymatically using a commercial kit (Merck, Darmstad, F.R.G.). In the lipoprotein fractions this was done without prior extraction. Phospholipid phosphorus [27] and protein [28] were measured by standard procedures. Fatty acid profiles were determined by gas-liquid chromatography [20] after lipid extraction [29], separation of lipid classes by thin-layer chromatography [30] and transesterification with boron trifluoride/methanol [31].

Membrane fluidity. 1,6-Diphenyl-1,3,5-hexatriene (DPH; Koch-Light Labs, Colnbrook, U.K.) was used as a probe for measuring the degree of lipid fluidity in the various plasma membrane preparations by steady-state fluorescence polarization at 25°C with an Elscint apparatus, model MV-1A (Elscint Ltd., Haifa, Israel) as described previously [25,32]. Polarization values mainly reflect the orientational constraint of the motions of the probe, and they are quantitatively related to order parameters in the membrane lipids [32]. High fluorescence polarization values represent high structural order or low membrane fluidity, and vice versa. Recently, we have described the quantitative contributions of the individual membrane lipid components to the fluorescence polarization value in detail [19].

Results

Growth of the mice appeared to be unaffected by the diets: 6 weeks after the beginning of the experiments all mice had similar body weights (23 ± 1 g). Also tumor-bearing mice did not show significant weight differences 16 days after inoculation of the tumor cells. The diets had no demonstrable effects on the development of the tumor, since the yield of GRSL cells in the ascites (about $5 \cdot 10^8$ cells) and the weight increase of the spleen (about 3-fold) were similar in the four groups.

Fig. 1 shows the lipoprotein profiles of normal blood plasma and of leukemic blood and ascites plasma as characterized by the concentration of total cholesterol in density gradient fractions. In healthy mice no differences were found between the diet groups, and even the mice fed a high-cholesterol diet showed a normal lipoprotein pattern. In tumor-bearing mice, however, this cholesterol-rich diet caused a substantial increase of total cholesterol in the low and very-low den-

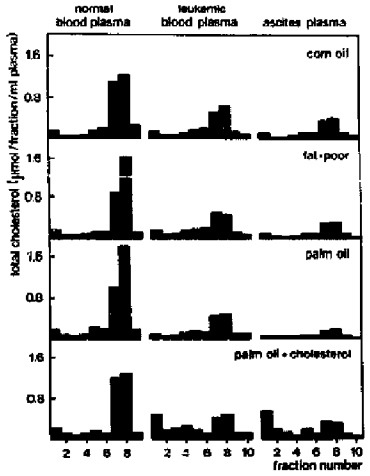

Fig. 1. Lipoprotein profiles as characterized by the concentration of total cholesterol in density gradient fractions of blood plasma of normal mice and of blood and ascites plasma of leukemic mice, as a function of dietary lipids.

sity regions, in blood as well as ascites plasma. In tumor bearers HDL appeared to be reduced to the same extent in all four diet groups, in ascites plasma even more so than in blood plasma. This reduction of HDL was not as dramatic as we have found previously for mice bearing the more rapidly growing GRSL 18 tumor [21].

The composition of the major blood plasma lipoprotein classes, VLDL and HDL, is presented in Table II. Particularly the cholesteryl ester content of VLDL appeared to be influenced by the diets: it was reduced as a result of the corn oil diet and increased at the expense of triacylglycerol by the cholesterol-rich diet. No major differences were observed between VLDL from normal and tumor-bearing mice, or between the compositions of HDL in the four diet groups. Only the content of unesterified cholesterol in leukemic HDL was slightly increased for all diets, and the leukemic VLDL in the fat-poor diet group contained a higher percentage of phospholipids mainly at the cost of the triacylglycerols.

The fatty acid compositions of some lipoprotein components are shown in Table III. Only major fatty acids are listed, accounting for 85–90% of the total fatty acids of VLDL-triacylglycerol and 80–86% of those of HDL-phospholipids. The fatty acid compositions of VLDL-triacylglycerol reflected more or less those of the diets (see Table I). No differences were found between normal and leukemic triacylglycerol, except in cholesterol-fed mice: the dietary cholesterol-induced increase of VLDL in tumor-bearing mice was accompanied with an increase of palmitic acid (16:0) and a relative decrease of linoleic acid (18:2) in this lipoprotein class. The fatty acid compositions of VLDL-cholesteryl esters resembled those of the triacylglycerols (not shown). Diet-dependent differences in the fatty acid composition of HDL-phospholipids were less pronounced. The order of a decreasing content of linoleic acid in the diets (corn oil ≫ fat-poor > palm oil ± cholesterol) was hardly reflected in the HDL-phospholipids. On the other hand, in this same order the phospholipids showed a clear decrease of stearic acid (18:0) and an increase of oleic acid (18:1). When compared with normal HDL, palmitic acid was proportionally increased in leukemic HDL of palm oil (± cholesterol)-fed mice, while stearic acid was increased in leukemic HDL of all diet groups.

The fatty acid composition of the plasma membrane phospholipids of GRSL ascites cells is given in Table IV. Marked differences were observed only in the oleic:linoleic acid ratios. With the corn oil and fat-poor diets the membranes could be enriched in linoleic acid at the expense of oleic acid, while by the palm oil diets (± cholesterol) a higher oleic acid content could be effectuated at the expense of linoleic acid. In the plasma membranes of splenic lymphocytes from normal and tumor-bearing mice similar diet-dependent shifts in the oleic:linoleic acid ratio were found, although the effects observed were even less pronounced (results not shown).

Whether these rather small variations in the fatty acid composition of the phospholipids had any influence on the lipid fluidity of the membranes was investigated by DPH-fluorescence polarization measurements. Results are presented in Table V. Fluorescence polarization of DPH in the isolated plasma membranes of normal spleen cells or splenic tumor cells appeared to be the same for all diet groups. Polarization values in

TABLE II

COMPOSITION OF VLDL AND HDL OF NORMAL AND LEUKEMIC BLOOD PLASMA AS A FUNCTION OF DIETARY LIPIDS

Values are expressed in wt% ± S.E. of three different experiments. TG = triacylglycerol, CE = cholesteryl esters, C = cholesterol, PL = phospholipids, Pr = protein.

		Corn oil diet	Fat-poor diet	Palm oil diet	Palm oil + cholesterol diet
Normal VLDL	TG	71.0±1.8	73.1±2.4	65.7±6.0	54.7±1.8
	CE	3.1±0.1	4.6±0.3	7.5±3.0	14.2±2.3
	C	4.3±0.1	4.7±0.6	5.1±1.6	6.6±1.3
	PL	15.6±2.2	11.0±1.9	14.9±2.4	17.0±1.5
	Pr	6.0±0.3	6.6±1.8	6.8±1.8	7.6±0.8
Leukemic VLDL	TG	69.8±1.8	63.9±2.1	66.0±4.0	52.8±2.7
	CE	2.3±0.6	5.6±0.5	5.0±1.9	16.4±2.8
	C	5.3±2.7	5.0±2.1	5.0±2.4	6.2±2.6
	PL	15.1±0.4	18.2±1.7	17.0±0.6	17.3±1.3
	Pr	7.5±0.1	7.3±1.2	6.6±0.5	7.3±0.9
Normal HDL	TG	2.8±0.5	3.1±0.6	2.5±1.0	2.5±0.5
	CE	17.0±0.8	18.2±1.4	19.5±0.7	19.6±0.1
	C	5.7±0.1	5.0±0.2	4.8±0.6	4.8±0.6
	PL	36.4±0.2	35.2±1.9	35.7±0.1	34.5±0.6
	Pr	39.2±1.0	38.4±0.4	37.5±0.3	38.6±0.9
Leukemic HDL	TG	2.4±0.5	2.8±0.6	1.5±0.3	2.8±1.5
	CE	19.0±0.2	17.9±1.0	18.6±4.5	17.6±0.7
	C	6.6±0.4	6.6±0.3	7.8±0.1	8.2±0.6
	PL	29.4±1.3	32.8±0.9	33.9±2.5	33.0±1.2
	Pr	42.0±2.0	39.9±2.1	38.3±1.7	39.0±1.0

TABLE III

MAJOR FATTY ACIDS OF VLDL-TRIACYLGLYCEROL AND HDL-PHOSPHOLIPIDS OF NORMAL AND LEUKEMIC BLOOD PLASMA, AS A FUNCTION OF DIETARY LIPIDS

Data are wt% of total fatty acids, mean values ± S.E. of three different experiments.

		Corn oil diet	Fat-poor diet	Palm oil diet	Palm oil + cholesterol diet
VLDL-triacylglycerol					
Normal	16:0	17.4±0.8	20.7±0.1	27.4±1.1	22.8±2.0
	18:1	19.9±1.6	29.0±0.4	40.7±0.7	40.3±0.1
	18:2	51.2±4.1	34.2±3.2	19.7±0.3	22.2±1.2
Leukemic	16:0	20.6±3.1	22.2±0.4	29.8±1.0	33.5±1.8
	18:1	21.0±1.6	30.6±1.7	43.1±1.1	40.7±1.3
	18:2	46.4±0.1	35.3±2.3	18.5±1.1	16.4±1.2
HDL-phospholipids					
Normal	16:0	27.4±1.3	31.2±0.1	32.0±2.6	31.8±1.9
	18:0	17.6±1.0	15.5±1.1	14.0±0.7	13.0±1.0
	18:1	6.9±0.2	10.2±1.2	13.5±0.3	15.5±0.7
	18:2	24.8±0.3	22.6±0.2	18.5±2.3	21.2±0.4
Leukemic	16:0	28.2±0.3	29.2±2.8	35.6±2.3	37.6±0.4
	18:0	24.4±0.6	22.0±3.0	15.8±2.4	16.2±2.4
	18:1	5.0±0.1	6.2±0.4	13.8±1.8	13.3±0.8
	18:2	22.0±0.7	24.2±2.4	18.0±2.0	19.6±0.2

TABLE IV

FATTY ACID COMPOSITION OF THE TOTAL PHOSPHOLIPIDS OF ISOLATED PLASMA MEMBRANES OF GRSL ASCITES CELLS AS A FUNCTION OF DIETARY LIPIDS

Data given in wt% of total fatty acids, mean values ± S.E. of three different experiments.

	Corn oil diet	Fat-poor diet	Palm oil diet	Palm oil + cholesterol diet
16:0	19.8 ± 0.1	17.8 ± 0.6	18.2 ± 1.7	19.4 ± 1.2
18:0	21.7 ± 0.7	18.2 ± 2.4	22.5 ± 0.3	18.9 ± 3.0
18:1	12.1 ± 0.8	15.2 ± 1.2	23.1 ± 4.3	19.9 ± 0.7
18:2	22.1 ± 4.4	23.6 ± 1.0	15.2 ± 1.4	17.5 ± 2.0
20:4	15.9 ± 0.9	14.5 ± 2.0	13.9 ± 1.9	13.3 ± 0.2
22:6	2.6 ± 0.4	1.3 ± 0.4	1.8 ± 0.5	1.4 ± 0.4

ascitic GRSL cell membranes were considerably lower due to their low cholesterol/phospholipid ratio. The dietary lipids did not induce any differences in these polarization values, except for a significant increase in the membranes from cholesterol-fed animals. These GRSL ascites cell membranes showed also an increased cholesterol/phospholipid ratio, when compared with the corresponding membranes from all other diet groups. However, polarization value and cholesterol content of ascites cell membranes from cholesterol-fed mice did not reach the 'normal' levels of splenic cell membranes. The finding that only by the cholesterol-rich diet the fluidity of the plasma membrane of a certain cell type (ascites tumor cell) can be modulated indicates that diet-induced variations in fatty acid composition, at least in the present study, are less important for membrane fluidity. The major importance of cholesterol as a determinant of GRSL cell membrane fluidity is clearly illustrated in Fig. 2 where the DPH-fluorescence polarization is plotted against the cholesterol/phospholipid molar ratio for individual membrane preparations. Linear relationships appeared for GRSL cells from both the ascites and the spleen, irrespective of the fatty acid composition. The lower level and the higher slope for the ascites cell membranes are due to their low

TABLE V

DPH-FLUORESCENCE POLARIZATION (LIPID STRUCTURAL ORDER) AND CHOLESTEROL/PHOSPHOLIPID MOLAR RATIO IN ISOLATED PLASMA MEMBRANES OF LYMPHOID CELLS FROM NORMAL AND LEUKEMIC MICE

Mean values are given ± S.E. of three different experiments.

	Corn oil diet	Fat-poor diet	Palm oil diet	Palm oil + cholesterol diet
DPH-fluorescence polarization (25 °C)				
Normal splenocytes	0.307 ± 0.001	0.306 ± 0.001	0.304 ± 0.001	0.304 ± 0.001
Splenic GRSL cells	0.294 ± 0.002	0.294 ± 0.006	0.292 ± 0.004	0.295 ± 0.001
Ascitic GRSL cells	0.257 ± 0.002	0.254 ± 0.007	0.259 ± 0.007	0.268 ± 0.001
Cholesterol/phospholipid (mol/mol)				
Normal splenocytes	0.50 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.50 ± 0.01
Splenic GRSL cells	0.51 ± 0.05	0.54 ± 0.07	0.50 ± 0.02	0.51 ± 0.02
Ascitic GRSL cells	0.29 ± 0.02	0.27 ± 0.05	0.31 ± 0.03	0.41 ± 0.02

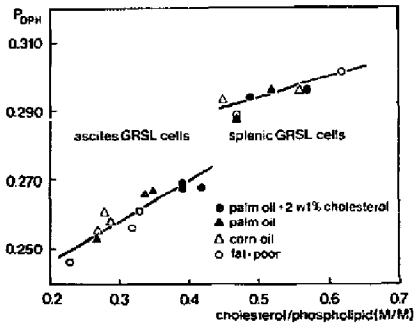

Fig. 2. Linear relationships between steady-state DPH-fluorescence polarization (P_{DPH}, at 25 °C) and the cholesterol/phospholipid (C/PL) molar ratio in plasma membranes isolated from GRSL tumor cells of different locations (ascites and spleen). Different symbols denote different diets, as indicated. $P_{DPH} = 0.222 + 0.120$ (C/PL) (correlation coefficient = 0.80) for ascites cell membranes, whereas $P_{DPH} = 0.262 + 0.062$ (C/PL) (corr. coeff. = 0.72) for the splenic GRSL cell membranes.

sphingomyelin content [19] (0.9% of the total phospholipids) when compared with the splenic cell membranes (16%) [18].

Discussion

The present study shows that the plasma lipoprotein density profiles (measured by the cholesterol content) of healthy GR/A mice are almost irresponsive to dietary lipids. Even a cholesterol-rich diet, which has been found to give rise to an increase of abnormal lipoproteins in a number of rodent species including other mouse strains [33–35], did not induce alterations in the lipoprotein profile, but only effectuated an increased cholesterol content in VLDL. Studies by others [35–38] have shown that responses to dietary lipids indeed may differ widely between various mouse strains. Also in man the existence of hypo- and hyperresponders to dietary cholesterol has been reported [39].

Tumor bearing GR/A mice also showed identical lipoprotein profiles and lipoprotein compositions in three out of the four diet groups, but the cholesterol-rich diet effectuated the increase of lipoproteins in the VLDL and LDL regions of blood and ascites plasma. The abnormal LDL have not been further characterized in the present study, but may be similar to the cholesterol-induced lipoproteins described by others [33–35]. The VLDL that was increased in cholesterol-fed tumor-bearing mice showed a similar composition as the VLDL of healthy cholesterol-fed mice (Table II), but in their triacylglycerol the ratio of 18 : 2/16 : 0 was decreased (Table III). Apparently, the disturbance of lipoprotein metabolism in tumor-bearing mice [21–23] has become even more complicated by the cholesterol loading, and the homeostatic capacity of cholesterol turnover seems to be largely exceeded in these mice.

Effects of the dietary fatty acids were found in the lipoprotiens of all diet groups, but in the course of the processing and utilization of the fatty acids, the original composition as found in the diets was rapidly lost: while VLDL-triacylglycerols still strongly resemble the dietary lipids, plasma phospholipids (measured as HDL-phospholipids) showed considerably less pronounced differences. Compositional differences in the plasma membrane phospholipids were in turn even smaller than in the lipoprotein phospholipids.

Mathur and Spector [38] have previously investigated the effect of dietary fat (coconut oil or sunflower oil) on the Ehrlich ascites tumor fluid lipoproteins in CBA mice. The sunflower oil-enriched diet in this system resulted in an increase in the 18 : 2 levels in the triacylglycerols and the phospholipids of both VLDL and HDL, to a much larger extent than was presently found in the (blood) plasma lipoproteins of the GR/A mice. One should be cautious, however, to compare compositions of ascites plasma lipoproteins with those of blood plasma lipoproteins directly, since the lipid content and fatty acid composition of lipid classes between given types of lipoproteins from these two sources within the same animal may differ significantly [23,38].

In a number of studies (reviewed in Refs. 2 and 17) the fatty acid composition of the phospholipids in a variety of tissues or cells could be modified to a certain extent by dietary lipids. Less extensive data are available for isolated plasma membranes, and in only a limited number of

studies these compositional data were related to physical parameters, such as membrane fluidity measured by DPH-fluorescence polarization: Brasitus et al. [40] have determined the lipid composition and fluidity of a number of intestinal membranes after feeding rats diets enriched in unsaturated (corn oil) or saturated (butter fat) triacylglycerols. The corn oil diet (enriched in 18:2) increased the overall unsaturation of the acyl chains and the lipid fluidity (decreased DPH-fluorescence polarization) of the membranes. Concomitantly, the cholesterol/phospholipid molar ratio was increased in the microvillus but not in the basolateral membranes. Apparently, rat enterocytes possess regulatory mechanisms which modulate the cholesterol content of the microvillus membranes, so a to mitigate changes in lipid fluidity. Burns, Spector and their coworkers have fed mice diets containing either coconut oil (saturated fat) or sunflowerseed oil (polyunsaturated fat) to modify the fatty acids in the plasma membrane of normal liver [41], of L1210 leukemic lymphoblasts [42], and of the Ehrlich ascites tumor cells [43,44]. The main differences were found in the 18:1, 18:2 and 20:4 in the former cases [41,42] or in 18:0, 18:1 and 18:2 in the latter cell membranes [16,43,44], the sunflowerseed oil-containing diet giving rise to a significantly increased ratio of 18:2/18:1. In our present study we also found an increase in the 18:2/18:1 ratio in the various plasma membrane preparations obtained from corn oil-fed mice as compared to palm oil-fed mice, but not as pronounced as in the american studies [41–44]. The diet-induced differences in the fatty acid composition of L1210 and Ehrlich carcinoma cell membranes were sufficient to yield small differences in the order parameter as measured by electron spin resonance using nitroxystearate spin probes [16]. In the present study, the differences in the fatty acids were too small to affect the DPH-fluorescence polarization in normal lymphocyte or GRSL tumor cell membranes in the spleen. However, the cholesterol-containing diet gave rise to an increased DPH-fluorescence polarization (decreased fluidity) in GRSL ascites cell membranes, which can be attributed to an increase in the cholesterol/phospholipid molar ratio (Table V, Fig. 2) [19]. The significant effect of this diet on the ascites cell membranes, rather than on the spleen cell membranes, supports our proposition [23] that the availability of lipoprotein cholesterol to the GRSL ascites cells is a limiting factor for these cells to obtain a higher cholesterol/phospholipid molar ratio. However, even by feeding the cholesterol-rich diet, this molar ratio and the DPH-fluorescence polarization of the GRSL ascites plasma membranes did not reach the 'normal' values found in the spleen cells (Table V, Fig. 2).

In this ascites tumor system we have investigated the possibility that the degree of membrane fluidity would influence the expression of cell surface antigens [1,4,45]. Using an antibody and complement-dependent cytotoxicity test [18] and the Fluorescence Activated Cell Sorter [45] we were unable to detect consistent alterations in the expression of five previously described surface antigens [18,45] (among which the tumor-specific antigen MLr) as a consequence of the cholesterol-rich diet (results not shown).

In conclusion, the GR/A mouse strain used in the present study is a low-responder to dietary lipids including cholesterol. As a result, plasma lipoproteins and splenic lymphoid cell membranes show only minor compositional differences induced by the diets, too small to affect the overall membrane fluidity. However, GRSL ascites tumor cells are susceptible to a cholesterol-rich diet, in that their plasma membrane cholesterol/phospholipid molar ratio and the structural order of membrane lipids are increased by this diet. The exquisite homeostatic control of cholesterol and cell membrane fluidity operating in these mice, has apparently become partially lost in their ascites compartment. Diet-induced cholesterol loading in GRSL ascites cell membranes does not seem to affect membrane antigen expression, and if tumor–host interaction would be affected by the diet in some other way, this apparently does not affect the rate of tumor growth.

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References

1 Van Blitterswijk, W.J. (1985) in Membrane Fluidity in Biology, Vol. 3 (Aloia, R.C. and Boggs, J., eds.), pp. 85–159, Academic Press, New York.
2 Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys Acta 779, 89–137.
3 Spector, A.A. and Yorek, M.A. (1985) J. Lipid Res. 26, 1015–1035.
4 Shinitzky, M. (1984) in Physiology of Membrane Fluidity, Vol. 1 (Shinitzky, M., ed.), pp. 1–51, CRC Press, Boca Raton.
5 Gould, R.J. and Ginsberg, B.H. (1985) in Membrane Fluidity in Biology, Vol. 3, (Aloia, R.C. and Boggs, J., eds.), pp. 257–280, Academic Press, New York.
6 Muller, C.P. and Krueger, G.R.F. (1986) Anticancer Res. 6, 1181–1194.
7 Farias, P.N. (1987) Biochim. Biophys. Acta 906, 459–468.
8 Needham, L., Dodd, N.J.F. and Houslay, M.D. (1987) Biochim. Biophys. Acta 899, 44–50.
9 Friedlander, G., Le Grimellec, C., Giocondi, M.C. and Amiel, C. (1987) Biochim. Biophys. Acta 903, 341–348.
10 McMurchie, E.J., Patten, G.S., Charnock, J.S. and McLennan, P.L. (1987) Biochim. Biophys. Acta 898, 137–153.
11 McMurchie, E.J., Patten, G.S., McLennan, P.L., Charnock, J.S. and Nestel, P.J. (1988) Biochim. Biophys. Acta 937, 347–358.
12 Shinitzky, M., Skornick, Y. and Haran-Ghera, N. (1979) Proc. Natl. Acad. Sci. USA 76, 5313–5316.
13 Skornick, Y., Kurman, C.C. and Sindelar, W.F. (1984) Cancer Res. 44, 946–948.
14 Skornick, Y.G., Rong, G.H., Sindelar, W.F., Richert, L., Klausner, J.M., Rozin, R.R. and Shinitzky, M. (1986) Cancer 58, 650–654.
15 Siegfried, J.A., Kennedy, K.A., Sartorelli, A.C. and Tritton, T.R. (1983) J. Biol. Chem. 258, 339–343.
16 Burns, C.P. and Spector, A.A. (1987) Lipids 22, 178–184.
17 Spector, A.A. and Burns, C.P. (1987) Cancer Res. 47, 4529–4537.
18 Van Blitterswijk, W.J., Hilkmann, H. and Hengeveld, T. (1984) Biochim. Biophys. Acta 778, 521–529.
19 Van Blitterswijk, W.J., van der Meer, B.W. and Hilkmann, H. (1987) Biochemistry 26, 1746–1756.
20 Van Blitterswijk, W.J., De Veer, G., Krol, J.H. and Emmelot, P. (1982) Biochim. Biophys. Acta 688, 495–504.
21 Damen, J., Van Ramshorst, J., Van Hoeven, R.P. and Van Blitterswijk, W.J. (1984) Biochim. Biophys. Acta 793, 287–296.
22 Damen, J., De Widt, J., Hengeveld, T. and Van Blitterswijk, W.J. (1985) Biochim. Biophys. Acta 833, 495–498.
23 Van Blitterswijk, W.J., Damen, J., Hilkmann, H. and De Widt, J. (1985) Biochim. Biophys. Acta 816, 46–56.
24 Redgrave, T.C., Roberts, D.C.K. and West, C.E. (1975) Anal. Biochem. 65, 42–49.
25 Van Blitterswijk, W.J., Emmelot, P., Hilkmann, H.A.M., Hilgers, J. and Feltkamp, C.A. (1979) Int. J. Cancer 23, 62–70.
26 Giegel, J.L., Ham, A.B. and Clena, W. (1975) Clin. Chem. 21, 1575–1581.
27 Morrison, W.R. (1964) Anal. Biochem. 7, 218–224.
28 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
29 Bligh, E.G. and Dyer, W.J. (1959) Canad. J. Biochem. Physiol. 37, 911–917.
30 Kunz, F. (1973) Biochim. Biophys. Acta 296, 331–334.
31 Morrison, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608.
32 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332.
33 Mahley, R.W. and Holcombe, K.S. (1977) J. Lipid Res. 18, 314–324.
34 Shore, V.G., Shore, B. and Hart, R.G. (1974) Biochemistry 13, 1579–1585.
35 Breckenridge, W.C., Roberts, A. and Kuksis, A. (1985) Arteriosclerosis 5, 256–264.
36 Walker, B.L. and Mulvihill, B.J. (1984) Nutrition Res. 4, 601–610.
37 Mulvihill, B.J. and Walker, B.L. (1984) Nutrition Res. 4, 611–619.
38 Mathur, S.N. and Spector, A.A. (1978) J. Lipid Res. 19, 457–466.
39 Katan, M.B., Beynen, A.C., De Vries, J.H.M. and Nobels, A. (1986) Am. J. Epidemiol. 123, 221–234.
40 Brasitus, T.A., Davidson, N.O. and Schachter, D. (1985) Biochim. Biophys. Acta 812, 460–472.
41 Burns, C.P., Rosenberger, J.A. and Luttenegger, D.G. (1983) Ann. Nutr. Metab. 27, 268–277.
42 Burns, C.P., Luttenegger, D.G., Dudley, D.T., Buettner, G.R. and Spector, A.A. (1979) Cancer Res. 39, 1726–1732.
43 Awad, A.B. and Spector, A.A. (1976) Biochim. Biophys. Acta 426, 723–731.
44 Kaduce, T.L., Awad, A.B., Fontenelle, L.J. and Spector, A.A. (1977) J. Biol. Chem. 252, 6624–6630.
45 Benkö, S., Hilkmann, H., Vigh, L. and Van Blitterswijk, W.J. (1987) Biochim. Biophys. Acta 896, 129–135.