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Decreased membrane fluidity and unsaturated fatty acids in Niemann–Pick disease type C fibroblasts

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Abstract

Niemann–Pick disease type C (NP-C) is an autosomal recessive disorder characterized by the sequestration and trapping of endocytosed cholesterol in lysosomes. The *NPC1* gene on chromosome 18 was recently identified but its physiological function remains unknown. We have studied the lipid compositions of cultured human NP-C fibroblasts and mouse SPM-3T3 cell line derived from the C57BL/KsJ NP-C model mouse, which belongs to the same complementation group. Fibroblasts derived from apparently normal age-matched individuals and a subline of SPM-3T3 cells which restores cholesterol metabolism by transfer of human chromosome 18 were used as controls. Levels of free cholesterol in whole cell homogenates increased about 1.5-fold in human NP-C fibroblasts and mouse SPM-3T3 cells, while in the plasma membrane, cholesterol content did not significantly change in NP-C fibroblasts but rather decreased in SPM-3T3 cells. The total phospholipid content did not significantly change; however, among phospholipid head groups, increases in sphingomyelin and decreases in other classes were observed in human NP-C fibroblasts and mouse SPM-3T3 cells. The ratios of saturated fatty acids to unsaturated fatty acids increased in both human and mouse cells. The increase was also confirmed in the plasma membrane fraction of SPM-3T3 cells. Membrane fluidity was examined using a 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescent probe. The DPH anisotropy values were markedly increased in NP-C fibroblasts and in SPM-3T3 cells. The results suggest that a NP-C mutation causes complex alterations in cellular lipid contents and biophysical properties of the membrane.

Keywords: Niemann–Pick disease type C; Cholesterol; Transport; Fatty acid; Membrane fluidity

1. Introduction

Niemann–Pick disease type C (NP-C) is a genetic disorder characterized by progressive neurologic deterioration, hepatosplenomegaly and foamy bone marrow cells. Cultured skin fibroblast from patients with NP-C show a lysosomal accumulation of unesterified cholesterol and failure of low-density lipopro-

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tein (LDL) to stimulate cholesterol esterification. The defect has been considered in a metabolic process that affects the relocation of cholesterol from lysosomes to other cellular sites [for reviews, see Refs. [1,2]]. Two complementation groups have been identified [3,4]. The gene for one major complementation group has been assigned to human chromosome 18 [4,5] and the gene was recently identified [6] but the gene for the minor second group has not been assigned. Two mutant mouse models (BALB/c and C57BL/KsJ strains) for NP-C have been found and maintained [7,8]. Both strains are genetically authentic models for the first major complementation group of NP-C, showing the same biochemical abnormalities to NP-C [9], cross-mating between the two strains [10], transferring human chromosome 18 to a cell line derived from the C57BL/KsJ model mouse [11], by complementation analysis in cell hybrids between NP-C fibroblasts and the cell line [12], and by recent identification of the mutation in *NPC1* gene [13].

Recently, we found that NP-C fibroblasts show an abnormal metabolism of ganglioside GM2 and the abnormality is not corrected in a culture condition that eliminates the accumulation of cholesterol [14]. The findings suggest that a defect in the *NPC1* gene may involve intracellular transport of both cholesterol and gangliosides. In addition, we have noticed that NP-C cells show a resistance to cytotoxic effect of a polyene antibiotic, filipin, which binds to membrane sterols and presumably damages the membrane [15]. Since a common abnormality in filipin-resistant mammalian mutant cells is a reduction of membrane sterols [16], we have speculated that cholesterol contents in the plasma membrane may be decreased or that the contents of other lipid classes that constitute the plasma membrane may be altered in NP-C fibroblasts. In this paper, we have described that NP-C fibroblasts derived from the first major complementation group and a cell line from the C57BL/KsJ model mouse have a decreased content of unsaturated fatty acid and have decreased membrane fluidity.

2. Materials and methods

2.1. Cells and cell culture

Three fibroblasts strains (UCH, TAN, and YON) were established from Japanese NP-C children and one strain (93059) was established from a Caucasian child. In some experiments, two other strains (GM0110A, GM3123) purchased from the Human Genetic Mutant Cell Depository, Coriell Institute for Medical Research (Camden, USA) and one fibroblast strain (KAI) derived from a 38-year old Japanese NP-C patient were included. All these human NP-C strains were found to belong to the first major complementation group of NP-C [14]. As control fibroblasts, four strains derived from apparently normal Japanese children (aged 3–4 years old) and, in some experiments, three strains derived from apparently normal Japanese adults (aged 42–55 years old) were used. A mouse SPM-3T3 cell line was established according to the 3T3 transfer schedule from a homozygous embryo of C57BL/KsJ model mouse of NP-C [9] and a SPMneo18 cell line, a subline of SPM-3T3 cells which restored cholesterol metabolism by transfer of human chromosome 18 [11], was used as control. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Because fragments of human chromosome 18 introduced in SPMneo18 cells is randomly excluded, the SPMneo18 cells were cultured in the presence of G418 (Geneticin, Gibco, NY, USA) and, at intervals, the cholesterol-non-accumulating phenotype was examined by filipin staining [9]. The cell populations containing less than 5% cholesterol-accumulating cells were used for lipid analysis.

2.2. Plasma membrane isolation

Unless otherwise specified, confluent-resting cells in thirty 100-mm dishes were used for each plasma membrane isolation. When the cells attained confluence, the medium was replaced. Cells on the fourth day after replacement were defined as confluent-resting. In some experiments, the plasma membrane of proliferating cells was isolated from sparsely proliferating cells in seventy 100-mm dishes. The plasma membrane was purified according to the procedures of Bauvois [17]. Briefly, washed cells were suspended in sucrose homogenization medium, followed by tight-fitting Dounce homogenization. The homogenate was centrifuged to remove the nuclear pellet and the supernatant was recentrifuged. Plasma membrane fraction was separated from the resulting

pellet using sucrose gradient centrifugation. The membrane fraction was pelleted at 30,000 × *g* for 30 min after dilution with 10 mM Tris/HCl (pH 7.4) containing 0.154 M NaCl and 0.2 mM $MgCl_2$. The final pellet was stored at −80°C prior to analyses. All steps were carried out at 4°C. Marker enzymes were measured to establish purity of the plasma membrane fraction. The specific activity of 5′-nucleosidase was measured by the method of Heppel and Hilmore [18], and Mg^{2+}-ATPase was measured under conditions described by Lotersztajn et al. [19] with slight modifications except that phosphate release was determined according to Prpic et al. [20]. The activity of 5′-nucleosidase and Mg^{2+}-ATPase were increased by approximately 5- to 7-fold and about 3- to 5-fold, respectively, in purified plasma membranes as compared with those in the crude homogenates. Protein concentrations were determined by the Bio-Rad Protein Assay Kit I based on the Bradford method [21], using lyophilized bovine γ-globulin as a standard.

2.3. Lipid extraction

Whole cellular lipids were extracted from cells cultured in three 100-mm dishes (confluent-resting cultures) or ten 100-mm dishes (proliferating cultures) or from plasma membrane fractions purified as above. Lipid extracts were prepared by homogenization of cell pellets in 50 vol. of chloroform–methanol (2:1, v/v) [22]. After brief centrifugation, the solvent extracts were recovered and the deposit was re-extracted as above. The pooled extracts were partitioned against 0.2 vol. of H_2O and the lower phase was recovered. The extract was dried under N_2 and dissolved in chloroform.

2.4. Determination of cholesterol and phospholipids

Total cholesterol content of the lipid fraction was assayed by an enzymatic method using a cholesterol determination kit (Wako Pure Chemical Industries, Osaka). For this purpose, the total lipid fraction, suspended in 20 μl of isopropanol, was incubated for 5 min at 37°C in 3 ml of assay mixture containing 4.8 units cholesterol esterase, 0.93 units cholesterol oxidase, 15.6 units peroxidase, 2.85 pmol DAOS, 0.6 pmol 4-aminoantipyrine and 13 units ascorbic oxidase. The absorbance at 600 nm was measured. To assay free cholesterol, the total lipid fraction was incubated in the same mixture but without cholesterol esterase. The level of phosphorus in the lipid extracts was determined according to the method described by Ames [23]. Phospholipid values were calculated by multiplying the average value of phosphorus by a factor of 25 to yield the level of phospholipid in milligrams.

2.5. Separation and quantification of phospholipids

The phospholipid classes were separated by two-dimensional thin-layer chromatography on a glass HPTLC plate (Art. 5641, Merk. Darmstadt, Germany), in a solvent system of chloroform–methanol–15.1 N ammonia water 65:35:5.5 (v/v) in the first dimension, and chloroform–acetone–methanol–acetic acid–water 3:4:1:1:0.5 (v/v) in the second development [24]. The spots were visualized by spraying with 0.2 M cupric acetate in 8% phosphoric acid and heating at 210°C for 10 min. The density of spots visualized by cupric acetate–phosphoric acid regent was quantitatively determined at 500 nm with a TLC densitometer (CS-9000, Shimadzu, Kyoto) [25]. The standards for quantitative determination by TLC-densitometer were phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SM).

2.6. Fatty acid analysis

Fatty acid analysis was done by the method of MacGee and Allen [26]. Fatty acids were extracted with *n*-hexane after saponification of total lipids from the homogenized cells and plasma membrane and neutralization. Then trimethyl-α,α,α-trifluoro-*m*-tolyl ammonium hydroxide was added to take up fatty acids from the hexane phase, followed by methylation on the capillary column (Omegawax 320 0.32 mm, 30 m, Supelco) of a gas chromatography (GC-14A, Shimadzu). The initial temperature was 150°C and the temperature was raised 10°C per min to a final temperature of 270°C. The peak area was calculated with Chromatopack (C-R5A, Shimadzu) [27].

2.7. Membrane fluidity

Membrane fluidity was monitored according to the method of Kawato et al. [28]. In brief, a lipophilic probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) was

added to the homogenized cells and embedded in the membrane by incubation at 37°C for 20 min. Fluorescence intensity was measured with a fluorescence spectrophotometer (FP-2070, JASCO). The excitation wavelength was 362 nm, and the emission light (426 nm) was polarized vertically (I_v) and horizontally (I_h) by a polarizing filter. Steady-state emission anisotropy (r) was calculated as follows.

$$r = (I_v - I_h)/(I_v + 2I_h)$$

2.8. Statistical analysis

Statistical analysis was performed using Student's *t*-test.

3. Results

3.1. Lipid analysis

Cellular concentrations of cholesterol and phospholipids in mouse SPM-3T3 cells are shown in Fig. 1. Because the level of cellular cholesterol contents may be different between proliferating and resting states, as demonstrated in our preliminary observation [9], the levels of cellular cholesterol content were examined at different proliferating states. Free cholesterol, esterified cholesterol and phospholipid were higher in cells at the proliferating state (Fig. 1A) than in those at the resting state (Fig. 1C) in both SPM-3T3 and control (SPMneo18) cell lines. The most prominent difference was the content of esterified cholesterol, whose content in the resting cells were approximately 1/3–1/6 of those in the proliferating cells. The contents of cholesterol and phospholipids in the plasma membrane were also higher in the proliferating cells (Fig. 1B) than in the resting cells (Fig. 1D).

The contents of free cholesterol in the whole cell homogenate from SPM-3T3 cells were significantly higher than those in control cells. The cholesterol/phospholipid ratios were 2- to 3-fold higher in SPM-3T3 cells than in controls in whole cell homogenates either at a proliferating state or resting state. This finding well agrees with the findings that free cholesterol accumulates in the liver and spleen of the model mice [1,2,7,8]. On the other hand, levels of cholesterol and cholesterol/phospholipid ratio in the plasma membrane of SPM-3T3 cells were lower than those of control cells both in proliferating and resting cultures. We have speculated

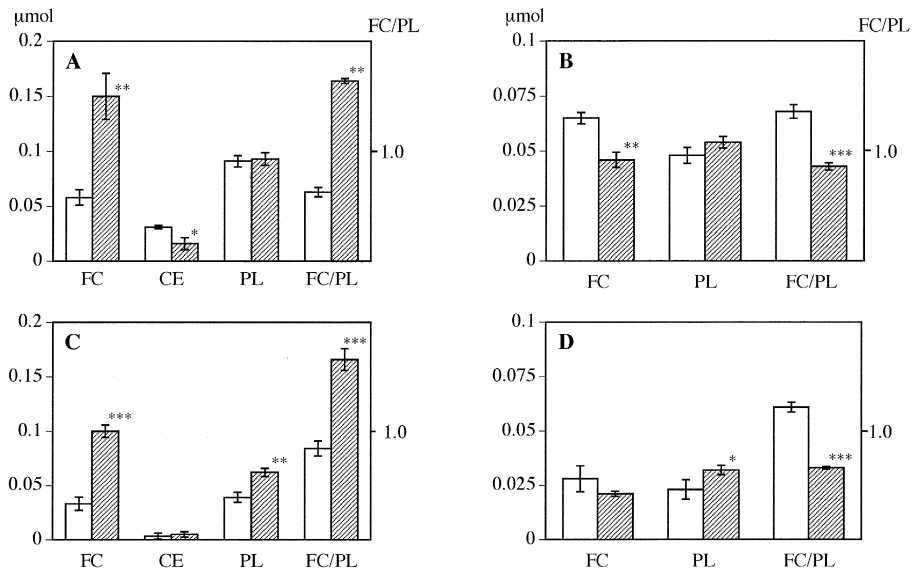

Fig. 1. Concentration of lipids in whole cell homogenates (A, C) and in plasma membrane-enriched fractions (B, D) from proliferating (A, B) and resting (C, D) mouse cell lines. Values are μmol/10^6 cells for free cholesterol (FC), cholesteryl ester (CE) and phospholipids (PL), and molar ratios for FC/PL. Open columns are values for control (SPMneo18) cells and shaded columns are values for SPM-3T3 cells. Error bar is mean and standard deviation of triplicated samples. $*\ P < 0.05$, $**\ P < 0.005$, $***\ P < 0.001$.

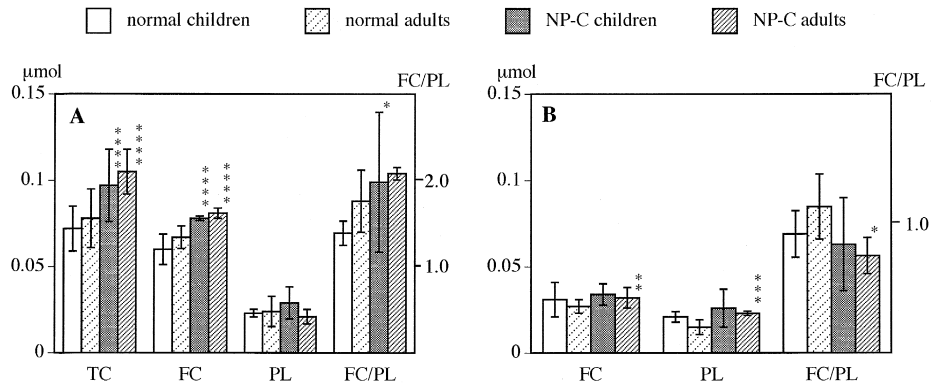

Fig. 2. Concentrations of lipids in whole cell homogenates (A) and in plasma membrane-enriched fragments (B) of cultured human fibroblasts from four childhood NP-C patients, one adult NP-C patient, four normal childhood controls, and three normal adult controls. Values are μmol/10^6 cells for free cholesterol (FC), cholesteryl ester (CE) and phospholipids (PL) and molar ratios for FC/PL. Error bar is mean and standard deviation of five independent samples. $*$ $P < 0{,}05$, $**$ $P < 0.01$, $***$ $P < 0.005$, $****$ $P < 0.001$.

that the decreased plasma membrane cholesterol may be associated with filipin-resistance in NP-C fibroblasts [15]. Levels of phospholipids in proliferating cells were not significantly different between SPM-3T3 and control cells but those in resting cells were higher in SPM-3T3 cells than in control cells.

Next, we studied cholesterol and phospholipid contents in whole cell homogenates and enriched plasma membrane fractions of human NP-C fibroblasts at the resting state. Since we noticed that contents of cholesterol in fibroblasts vary by cellular aging in vitro and in vivo, we used cell strains from age-matched controls and with similar proliferating potentials. As shown in Fig. 2, contents of total and free cholesterol in the whole cell homogenates of resting NP-C fibroblasts were higher than the controls. However, free cholesterol contents in the plasma membrane fractions were not significantly altered, except for adult NP-C cells. The contents of phospholipids in whole cells and in plasma membrane fractions did not change between controls and NPC fibroblasts, except for adult NP-C cells.

3.2. Phospholipid classes

Thin-layer chromatogram of mouse SPM-3T3 cells showed a marked increase in lysophosphatidylcholine (LPC) (spraying with Ninhydrin regent was negative but Dittmer regent was positive, data not shown). The LPC contents of resting SPM-3T3 cells was elevated about 2.5-fold, as compared to control cells. The sphingomyelin (SM) in SPM-3T3 cells was elevated about 1.5 folds when compared with that in control cells. While the contents of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol + phosphatidylserine (PI + PS) were lower in SPM-3T3 cells than in control cells (Table 1). Eventually, the sum of these phospholipid classes were not significantly altered between SPM-3T3 (65.7 μg/mg dry weight) and control cells (66.1 μg/mg dry weight). The increased level of LPC was accounted for by increased levels of total phospholipids in resting SPM-3T3 cells (Fig. 1).

In human NP-C fibroblasts, the content of SM was significantly increased, while that of PC, PE and PI + PS was decreased (Table 1). The sum of these phospholipid classes between NP-C (28.95 μg/mg dry weight) and control cells (30.46 μg/mg dry weight) were not significantly changed. Abnormal increases of LPC were not detected in NP-C fibroblasts. Interestingly, PE/PC ratios were significantly higher in most NP-C fibroblasts examined.

3.3. Fatty acid composition

The ratio of the sum of saturated fatty acids (C14–C26) to that of unsaturated fatty acids was 0.55 ± 0.04 in mouse SPM-3T3 cells and 0.44 ± 0.01 in control cells (Table 2). The increased ratio in SPM-3T3 cells is associated with increased C18:0 and decreased C16:1. The composition of C18:0 in SPM-3T3 was

Table 1
Contents of individual phospholipid classes in childhood NP-C fibroblasts and mouse cells

Phospholipid class	Control children ($n = 4$)	NP-C ($n = 4$)	Control (SPMneo18)	SPM-3T3
PC	14.63 ± 2.84	10.87 ± 0.83[a]	27.38 ± 0.02	25.65 ± 0.04[c]
PE	6.50 ± 0.60	5.11 ± 0.31[a]	17.77 ± 0.13	16.17 ± 0.53[c]
PI + PS	5.59 ± 1.72	4.10 ± 0.60[b]	12.99 ± 0.17	12.37 ± 0.04[c]
SM	3.74 ± 0.41	8.87 ± 0.64[a]	7.96 ± 0.02	11.51 ± 0.04[c]
PE/PC	0.37 ± 0.02	0.47 ± 0.06[b]	0.65 ± 0.01	0.64 ± 0.01

The values are expressed as μg/mg dry weight of total phosphorus and expressed as the mean $\pm$ S.D. of duplicated measurements of two independent experiments.
Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.
[ab]Represent significant levels of $P < 0.001$ and $P < 0.05$, respectively, from each level of normal controls.
[c]Represents significant levels of $P < 0.005$ from each level of control.

1.4-fold in control cells and that of C16:1 was 0.2-fold in control cells. In the plasma membrane, C16:0 was elevated, C16:1 decreased, C18:0 remained unchanged and the ratio of saturated/unsaturated fatty acids was increased.

In human NP-C fibroblasts, increased ratios of saturated/unsaturated fatty acids (C14–C26) with an increase of C18:0 and with reductions of C16:1 and C18:1 was observed (Table 3). Decreases in the content of unsaturated fatty acids with a marked reduction in C16:1 were common findings both in

Table 2
Fatty acid compositions (% of total fatty acids) in whole cell and plasma membrane of the mouse cell lines

	Whole cell		Plasma membrane	
	Control	SPM-3T3	Control	SPM-3T3
C14:0	0.80	0.52	0.31	1.07
C16:0	11.48	11.72	8.88	18.80
C16:1	8.55	1.70	13.00	6.50
C18:0	13.23	18.47	13.48	13.25
C18:1	28.22	29.43	24.29	28.04
C18:2	1.52	1.40	0.36	1.32
C20:0	1.11	0.86	0.97	1.28
C20:4	6.76	7.93	8.94	6.57
C22:0	0.62	ND	0.79	0.42
C22:6	3.36	2.98	3.27	2.73
C24:0	3.22	3.91	3.01	2.52
C26:0	ND	ND	0.16	0.11
Others	21.54	21.01	20.37	17.65
Sum of unsaturated FFA	69.55	64.52[a]	72.39	62.50
Sum of saturated FFA	30.46	35.48[a]	27.61	37.50
Saturated/unsaturated	0.44	0.55[a]	0.38	0.6

Fatty acid composition in the total lipid extracts was examined by GLC as described in Section 2. Values are % of total fatty acids and are expressed as the mean of duplicated readings of two independent experiments in whole cells and of one experiment in the plasma membrane fraction. S.D. values of the means of whole cells were 1–15%.
[a]Represents significant differences of $P < 0.005$ from the level of control.

Table 3
Fatty acid compositions (% of total fatty acids) in whole cell homogenates of childhood NP-C fibroblasts

	Control ($n = 4$)	NP-C ($n = 4$)
C14:0	1.26 ± 0.12	0.72 ± 0.19
C16:0	18.47 ± 0.75	16.75 ± 7.67
C16:1	5.15 ± 1.56	1.97 ± 0.41
C18:0	14.79 ± 0.90	20.91 ± 4.00
C18:1	28.70 ± 2.31	22.75 ± 3.04
C18:2	4.68 ± 3.32	10.32 ± 1.13
C18:3	0.20 ± 0.15	0.34 ± 0.10
C20:0	0.18 ± 0.16	0.34 ± 0.04
C20:4	9.00 ± 0.98	11.45 ± 1.04
C22:0	0.36 ± 0.96	0.36 ± 0.12
C22:6	2.35 ± 2.40	0.88 ± 0.27
C24:0	2.38 ± 0.12	1.71 ± 0.41
C26:0	0.09 ± 0.06	0.33 ± 0.39
Others	13.05 ± 2.11	11.66 ± 1.40
Sum of unsaturated FFA	64.00 ± 0.96	58.96 ± 4.02[a]
Sum of saturated FFA	36.00 ± 0.96	41.04 ± 4.02[a]
Saturated/unsaturated	0.56 ± 0.08	0.70 ± 0.13[a]

Values are % of total fatty acids and are expressed as the mean of duplicated measurements of two independent experiments in whole cell homogenates.
[a]Represents significant differences of $P < 0.005$ from the level of normal children.

human NP-C fibroblasts and the cell line derived from the model mouse.

3.4. Membrane fluidity

The anisotropy values were measured with DPH as a fluorescent probe in the whole cells and plasma membrane of cell lines derived from the NP-C model mouse. The anisotropy value for control mouse cells was 0.199 ± 0.004 in the whole cells, while that for SPM-3T3 cells was 0.233 ± 0.003, which was about 15% higher than controls ($P < 0.0001$). In the plasma membrane, the anisotropy value for SPM-3T3 cells was 0.254 ± 0.002, which was also 30% higher than that for control cells (0.204 ± 0.003, $P < 0.0001$). The increased anisotropy values in the whole cells and enriched plasma membrane fraction indicated a decreased membrane fluidity in SPM-3T3 cells. The DPH fluorescent anisotropy values in human NP-C fibroblasts were also significantly higher than those in the controls (Table 4). The findings suggest that the membrane fluidity is reduced in NP-C fibroblasts and in the cell line derived from the model mouse. In the strain derived from an adult type NP-C patient, the anisotropy value was not significantly altered. The cells from this patient showed intracellular accumulation of free cholesterol and a mild defect in low density-lipoprotein induced cholesteryl ester formation [9,14], but did not show GM2 ganglioside accumulation [14]. It is considered that the defect in this patient was mild and, therefore, did not affect the membrane fluidity.

Table 4
Fluorescent anisotropy values in whole cell of NP-C fibroblasts treated with DPH

Control children ($n = 4$)	0.180 ± 0.002
NP-C children ($n = 6$)	0.198 ± 0.009[a]
Control adults ($n = 3$)	0.184 ± 0.005
NP-C adult ($n = 1$)	0.180 ± 0.001

Two milliliters of cell suspension in PBS (0.075 mg protein/ml in whole cell and 0.02 mg protein/ml in plasma membrane) was added to 2 μl of DPH solution (190 mM) in a cuvette and the fluorescent anisotropy was measured after incubation at 37°C for 20 min. The values denote mean ± S.D. of four independent determinations. [a]Represents a significant difference of $P < 0.0001$ from the control level.

4. Discussion

Common abnormalities in human NP-C fibroblasts and mouse SPM-3T3 cells revealed in this study were increased levels of cholesterol and sphingomyelin, decreased levels of PC, PE and PI + PS, increased levels of saturated fatty acids (C14–C26) and saturated/unsaturated fatty acid ratios, and an increased DPH anisotropy in the whole cell homogenate. Among these findings, a decrease in unsaturated fatty acid with reduction of C16:1 and an increased level of DPH anisotropy were also demonstrated in the plasma membrane fraction of SPM-3T3 cells.

In the liver and spleen from patients with NP-C, it has been shown that the concentrations of total cholesterol, sphingomyelin and total phospholipids, increase several folds [29]. In the liver and spleen of C57BL/KsJ model mice, cholesterol and sphingomyelin are known to be increased 3- to 5-fold [30] and 3- to 10-fold [30,31], respectively, with a mild increase in total phospholipids. Our observations in SPM-3T3 cells showed 2- to 3-fold increases in free cholesterol and 1.5 folds increase in sphingomyelin. In human NP-C fibroblasts, the content of sphingomyelin and free cholesterol increased about 2 folds and 1.5 folds, respectively. Total phospholipids in resting human NP-C cells remained unchanged in terms of decreases in PC, PE and PI + PS, and total phospholipids in resting mouse SPM-3T3 cells increased probably by an increase in LPC. Increased LPC in SPM-3T3 cells is an interesting phenomenon but the reason for it remains uncertain. The abnormalities in cholesterol, sphingomyelin and total phospholipid content in cultured cells are in good accordance with those observed in the tissues of affected patients and affected mice.

We have initiated this study to characterize the resistance to cytotoxic effects of polyene antibiotics in NP-C fibroblasts [15], expecting a similar lipid change observed in the plasma membrane of filipin-resistant LM cell mutants [16]. Except for a decrease in membrane cholesterol of SPM-3T3 cells, the lipid changes including the content of SM and unsaturated fatty acids, and the PE/PC ratio in NP-C and SPM-3T3 cells were opposite from those observed in the plasma membrane of filipin-resistant LM cell mutants. Although it seems likely that filipin-resistance is associated with changes in the plasma membrane

sterol level, our method has failed to show evidence of decreased plasma membrane cholesterol in human NP-C fibroblasts.

The NP-C gene product is required for exogenous (LDL-derived) cholesterol egress from lysosomes and the inability of cholesterol to leave lysosomes leads to impaired regulation of cholesterol homeostasis. The NP-C mutation has no effect on the transport of endogenously synthesized cholesterol from the endoplasmic reticulum to the plasma membrane [32] but it has been shown that there is impaired esterification of plasma membrane cholesterol following treatment with sphingomyelinase, indicating that the transport of cholesterol from the plasma membrane to the endoplasmic reticulum is deficient [33]. More recently Garver et al. [34] have shown abnormal expressions of caveolin-1 protein or mRNA in heterozygous and homozygous NP-C fibroblasts, suggesting that a subcellular structure containing caveolin-1 is involved in the pathophysiology of NP-C. In addition to cholesterol and sphingomyelin, it has been reported that several lipids are changed in tissues of NP-C patients or the model mice: bis(monoacylglycerol)phosphate, glucosylceramide, lactosylceramide [1,7,35], gangliosides GM2 and GM3 [29,35], sphingoid bases [36] and dolichol [37]. Peroxisomal impairment in NP-C model mice has also been reported [38]. Although the mechanism of complex lipid changes in NP-C tissues or cultured cells is unknown, it seems to be unlikely that lipid compositions and structure of the plasma membrane lipid bilayer are intact in NP-C cells.

Decreased membrane fluidity in NP-C cells is the most important finding in this study. Membrane fluidity is a biophysical property of the membrane quantitatively expressing the mobility and the rate of rotational motion of membrane lipid molecules. Changes in membrane fluidity are known to be linked with the alteration in physiological process of the cell membranes such as carrier-mediated transport, activities of membrane bound enzymes and receptor binding [39]. Cholesterol has a stabilizing effect on the membranes, decreasing fluidity and permeability. In human NP-C and mouse SPM-3T3 cells cholesterol levels in whole cells were increased but the levels in the plasma membrane were not changed but, rather, decreased. It is difficult to attribute the decrease in membrane fluidity to the level of plasma membrane cholesterol. At present decreases in unsaturated fatty acids ratios in NP-C cells and in plasma membrane of SPM-3T3 cells and increases in the PE/PC ratios observed in most NP-C cells seem to be associated with the alteration in membrane fluidity, because both changes have been suggested to decrease the membrane fluidity [40]. Dolichol, levels of which are known to be changed in the NP-C model mouse [41], may be one of factors affecting membrane fluidity in NP-C.

In conclusion, it was suggested that complex lipid changes observed in NP-C tissues and cultured cells may alter the plasma membrane lipid compositions and structure. Decreased membrane fluidity in cultured cells from NP-C patients and from a murine model might be the result of alterations in plasma membrane lipid compositions, changing the cellular functions to a disadvantage. It will be a future task to establish whether the function defective in NP-C directly participates in the homeostasis of membrane lipid compositions or not.

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