Reduced cellular cholesterol content in peroxisome-deficient fibroblasts is associated with impaired uptake of the patient's low density lipoprotein and with reduced cholesterol synthesis

Hanna Mandel,[1] Miriam Getsis,* Mira Rosenblat,* Moshe Berant, and Michael Aviram*

Department of Pediatrics, Rambam Medical Center, Technion-Faculty of Medicine, Haifa, 35254, Israel; and Lipid Research Laboratory,* Rappaport Family Institute for Research in Medical Sciences, Technion-Faculty of Medicine, Haifa, Israel

Abstract Mammalian cells acquire cellular cholesterol by de novo synthesis as well as by uptake of low density lipoprotein (LDL). Peroxisomes contain enzymes involved in the synthesis of cholesterol, and peroxisome-deficient (PD) patients have been shown to have hypocholesterolemia and abnormal LDL. We therefore decided to study whether cholesterol synthesis and cellular uptake of LDL are impaired in cultured PD fibroblasts. The present study demonstrates a significantly lower cellular cholesterol mass in fibroblasts from three PD patients, as compared to control cells (41–59% of controls). The rate of cholesterol synthesis was also reduced in all three PD cell lines, being 16–20% of the control values. LDL binding and degradation by fibroblasts were 3- to 5-fold higher in the PD cells as compared to control cells. Similarly, enrichment of normal fibroblasts with tetracosanoic acid (C-24:0), a situation that could mimic the in vivo accumulation of very long chain fatty acid (VLCFA) in PD cells, caused LDL binding and degradation to be 4-fold higher than in non-treated cells. On the other hand, the uptake of LDL derived from PD patients by normal fibroblasts was markedly reduced (by up to 67%) in comparison to the cellular uptake of normal LDL. Similar results were obtained in PD cells. This study demonstrates a lower cellular cholesterol content and reduced cholesterol synthesis rate in PD cell lines. In addition, we demonstrate that regulation of the uptake of normal LDL by cellular LDL receptors is operative in PD cells, whereas LDL derived from PD patients is not recognized normally by the LDL receptor. Thus, the metabolism of cholesterol within PD cells may be hampered by the combined effect of deficient de novo formation due to peroxisomal deficiency and of the deviant interaction of the LDL receptor with abnormal lipoprotein. A deficiency of cholesterol is likely to contribute to the devastating disturbances observed in patients affected with one of the various peroxisomopathies.—**Mandel, H., M. Getsis, M. Rosenblat, M. Berant, and M. Aviram.** Reduced cellular cholesterol content in peroxisome-deficient fibroblasts is associated with impaired uptake of the patient's low density lipoprotein and with reduced cholesterol synthesis. *J. Lipid Res.* 1995. **36:** 1385–1391.

Supplementary key words infantile Refsum disease • Zellweger syndrome • very-long-chain fatty acid • peroxisomes

Peroxisomes are involved in anabolic and catabolic pathways associated with lipid metabolism. These functions include the biosynthesis of cholesterol, plasmalogens, and bile acids, as well as the oxidation of very-long-chain fatty acids (VLCFA), branched-chain fatty acids, dicarboxylic acids, polyunsaturated fatty acids, L-pipecolic acid, and phytanic acid (1–4). Although cholesterol synthesis in mammalian cells has been assumed to be located entirely in the cytosol and endoplasmic reticulum (ER), recent studies demonstrate that rat liver peroxisomes contain enzymes of the cholesterol synthesis pathway (5). Peroxisomes contain acetoacetyl-CoA thiolase (6, 7), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (8, 9), and farnesyl diphosphate synthase (10). Recently, it has been reported that mevalonate kinase appears to be predominantly localized in peroxisomes and is deficient in patients affected by a peroxisome deficiency disorder (e.g., Zellweger syndrome) (11). Peroxisomes also contain the largest concentration of cellular sterol carrier protein 2, a protein thought to facilitate intracellular sterol transport (12, 13), as well as significant levels of apolipoprotein E, a major constituent of several classes of plasma lipoproteins (14). A previous study demonstrated that the cholesterol synthesis rate is significantly reduced in cells from peroxisome-deficient (PD) patients (15), while clinical studies have demon-

Abbreviations: LDL, low density lipoprotein; PD, peroxisome deficiency; PD-LDL, LDL derived from peroxisome-deficient patients; PD-cells, cells from peroxisome-deficient patients; FCS, fetal calf serum; VLCFA, very-long-chain fatty acids; UC, unesterified cholesterol; CE, cholesteryl ester; TLC, thin-layer chromatography; IRD, infantile Refsum disease.

[1]To whom correspondence should be addressed.

strated that patients suffering from PD disorders have abnormally low plasma cholesterol concentrations (16–20). These combined findings support the likelihood that peroxisomes play an important role in the cholesterol biosynthetic pathways.

Mammalian cells ensure adequate levels of cholesterol in cellular membranes by de novo cholesterol synthesis as well as by the uptake of cholesterol from plasma LDL (21, 22). The cellular concentration of cholesterol is regulated by a feedback control system, whereby a rise in cellular cholesterol content suppresses the activity of HMG-CoA reductase, and thus turns off the synthesis of intracellular cholesterol. In addition, a rise in cellular cholesterol down-regulates the synthesis of LDL receptors and thus prevents a further entry of LDL-derived cholesterol into the cells (21, 22). The regulation of cellular cholesterol biosynthesis has been shown to be operative in PD cells, as in normal cells, namely via a feedback control of the HMG-CoA reductase activity (15, 23). However, we have recently demonstrated a reduced uptake of LDL derived from a PD patient (PD-LDL) by normal macrophages (19), suggesting an impaired regulation of cholesterol uptake from plasma LDL in PD patients.

In the present study we investigated several aspects of cellular cholesterol homeostasis in peroxisome-deficient (PD) fibroblasts, by analysis of the cellular cholesterol mass, the rate of cholesterol biosynthesis, and LDL binding and degradation rates. This study demonstrates that PD cells have low cellular cholesterol content and reduced cholesterol biosynthesis rate. Furthermore, although the feedback regulation of the cellular uptake of LDL is potentially operative in PD cells, it is hampered when the cells interact with PD-LDL and not normal LDL. Hence, the metabolism of cholesterol within PD cells may be altered by the combined effect of a reduced de novo cholesterol synthesis due to the deficiency of peroxisomes, and also by an impaired interaction of the cells with abnormal lipoprotein.

EXPERIMENTAL PROCEDURES

Cells

Cultured fibroblasts were obtained from skin biopsies from three patients with documented peroxisomal deficiency (PD) diseases and from five normal individuals. The diagnosis of PD disease was based on clinical features, elevated levels of VLCFA in plasma and in fibroblasts, decreased levels of plasmalogens, and deficiency of dihydroxyacetone phosphate acyltransferase (DHAPAT) in fibroblasts (1–4). Cells were cultured in Dulbecco's modified Eagle's (DME) medium supplemented with 5% fetal calf serum (FCS). Cultures were maintained in a humidity-controlled incubator with an atmosphere of 5% CO_2, 95% air at 37°C. The fibroblast cells were studied between passages 5 and 20.

Lipoproteins

LDL was prepared from human plasma derived from fasted normolipidemic volunteers and from three PD patients. LDL was prepared by discontinuous density gradient ultracentrifugation as described previously (24). The LDL was washed at a density of 1.063 g/ml and dialyzed against 150 mM NaCl, 1 mM EDTA (pH 7.4) under nitrogen in the dark, at 4°C. LDL was then sterilized by filtration (0.22 nm) and used within 2 weeks. LDL was iodinated by the method of McFarlane as modified for lipoproteins (25).

Metabolism of lipoprotein by cells

LDL degradation. LDL degradation was measured after incubation of ^{125}I-labeled LDL (180–300 cpm/ng of protein) with cells for 5 h at 37°C. The hydrolysis of LDL protein was assayed in the incubation medium by measurement of trichloroacetic acid-soluble, noniodide radioactivity (26). Cell-free LDL degradation was minimal and was subtracted from total degradation. The cell monolayer was washed three times with phosphate-buffered saline (PBS) and extracted by a 20-h incubation at room temperature with 0.1 N NaOH for determination of cellular protein content (27).

LDL binding. High-affinity binding of LDL to cells was studied by incubation of ^{125}I-labeled LDL (2.5–15 μg of protein) for 4 h at 4°C. After extensive washing (× 4) with PBS, cells were extracted by incubation with 0.1 N NaOH for 20 h at room temperature and the bound radiolabeled LDL was counted (28).

Cholesterol esterification

LDL cholesterol uptake by cells was estimated by measurement of the stimulation of [^{3}H]oleate incorporation into cholesteryl ester (CE) (29). The cells were incubated for 18 h with the lipoproteins, followed by medium removal and a further incubation of the cells with radiolabeled oleate (0.2 mM, 10 μCi/ml [^{3}H]oleate complexed with 0.07 mM fatty acid-free albumin) for 2 h at 37°C. The cells were then washed twice with PBS at 4°C and incubated for 30 min with 1 ml hexane–isopropyl alcohol 3:2 (v/v) in a 35-mm dish at room temperature in order to extract cellular lipids. After two more washes with these solvents, the pooled lipid extract was dried under nitrogen and resolubilized in chloroform. The labeled CE was isolated by thin-layer chromatography (TLC) on silica gel plates developed in hexane–diethyl ether–acetic acid 130:40:1.5 (v/v/v).

Cholesterol mass

Cells were washed (× 3) with cold PBS followed by lipid extraction with hexane–isopropyl alcohol 3:2 (v/v). Cellular cholesterol [unesterified cholesterol (UC) and cholesteryl ester (CE)] were separated from the cellular lipid extract by TLC on silica gel plates using hexane–diethyl ether–acetic acid 130:40:1.5 (v/v/v). The TLC spots corresponding to UC and CE were scraped and analyzed for their cholesterol mass using the ferric chloride assay (30).

Cholesterol synthesis

Cellular cholesterol synthesis was measured by analysis of the incorporation of labeled acetate (1 μCi/ml [^{3}H]acetate 48 mCi/mmol, Amersham Radiochemical Center, Amersham, Bucks, UK) into unesterified cholesterol (UC) after incubation of cells with the [^{3}H]acetate for 20 h. ^{14}C-labeled cholesterol was added to the lipid extract as an internal standard in order to control for the recovery of cellular cholesterol. At the end of the incubation, and after cellular lipid extraction and TLC, the radioactivity in the spot related to UC was determined. The radioactivity in the cholesteryl ester (CE) spot under the experimental procedure was less than 5% of that found in the UC spot. These studies were repeated 3 times.

Very-long-chain fatty acids (VLCFA)

Cellular and plasma content of the VLCFA including that of tetracosanoic acid (C-24:0) were determined by gas chromatography (31). Enrichment of normal fibroblasts with C-24:0 was obtained by cell incubation with C-24:0 complexed with fatty acid-free albumin (0.2 mM and 0.07 mM, respectively) for 20 h at 37°C. Cellular C-24:0 content was measured by gas chromatography (31).

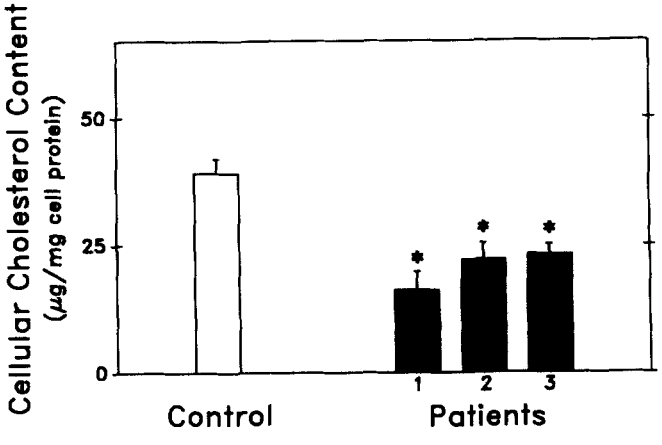

Fig. 1. Cholesterol mass in peroxisome deficient (PD) and in control fibroblasts. Human skin fibroblasts (HSF) from PD patients or from normal control subjects were grown in DMEM supplemented with 5% FCS. Cells were washed with PBS (× 3), followed by lipid extraction and analysis of the cellular cholesterol content. Results are given as the mean ± SD (*P < 0.01 vs. control, n = 3).

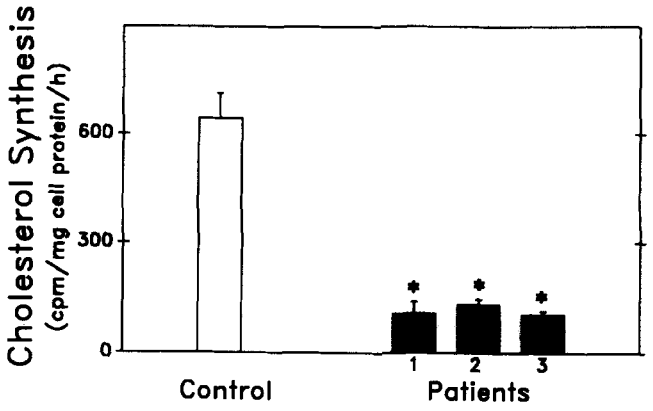

Fig. 2. Cholesterol synthesis rate in fibroblasts from PD patients and in normal cells. Human skin fibroblasts (HSF) from PD patients and from normal control subjects were grown in DMEM supplemented with 5% FCS. Cells were incubated with 1 μCi/ml of [^{3}H]acetate for 20 h at 37°C. Cells were then washed with PBS (× 3), lipids were extracted, and TLC analysis of the radioactivity in the unesterified cholesterol (UC) spot was determined. Results are given as mean ± SD (*P < 0.01 vs. control, n = 3).

RESULTS

Cholesterol mass in cultured skin fibroblasts from three peroxisome-deficient (PD) patients (grown to confluency in DME medium supplemented with 5% FCS) was found to be reduced in comparison to control cells by about 50% (**Fig. 1**). Because fibroblast cholesterol content is dependent on the rate of cholesterol synthesis, we analyzed the formation of radiolabeled cholesterol after incubation of fibroblasts with [^{3}H]acetate for 20 h at 37°C. **Figure 2** shows that in all three fibroblast cell lines derived from PD patients, the rate of cholesterol synthesis was only 16–20% of that observed in normal fibroblasts.

There was no difference in the radioactive cholesterol found in the media of control versus patient cells, suggesting that the reduced cholesterol synthesis rate is not due to cholesterol efflux out of the cells.

Because the cholesterol content of fibroblasts is also determined by the extent of LDL uptake by the cells, and up-regulation of LDL receptors is to be expected to occur in cholesterol-deficient cells, we evaluated cellular binding and degradation of LDL from normal subjects, in fibroblasts from PD patients, and from healthy individuals. The cells were incubated with increasing concentrations of ^{125}I-labeled LDL for 4 h at 4°C prior to analysis of lipoprotein binding (**Fig. 3A**), or for 5 h at 37°C for determination of LDL degradation (Fig. 3B). At LDL concentrations of 2–15 μg of protein/ml, the PD cells revealed a 3.4- to 5.1-fold increased binding in comparison to control normal cells (Fig. 3A). Similarly, on using LDL at 10–50 μg protein/ml, the degradation of LDL in PD cells was increased 2.7- to 3.1-fold (Fig. 3B) as compared to control cells grown under the same conditions.

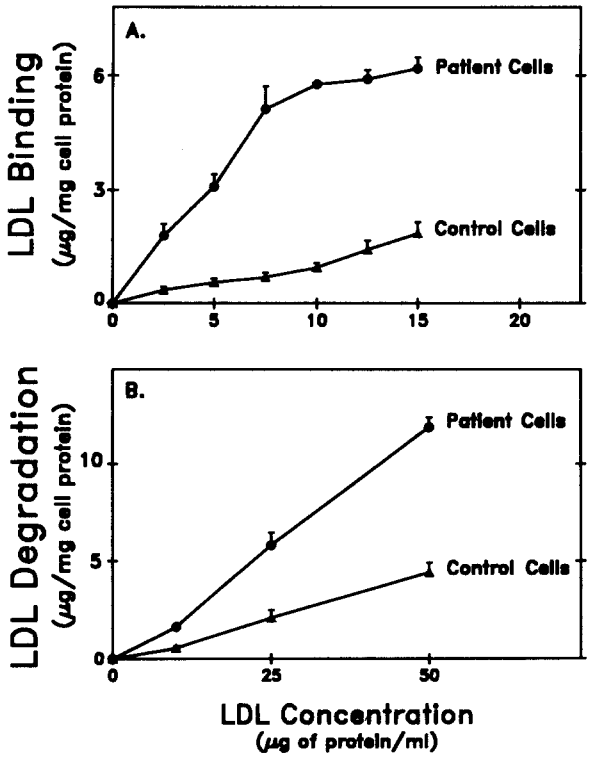

Fig. 3. Cellular binding (A) and degradation (B) of ^{125}I-labeled LDL by peroxisome-deficient (PD) or by normal control fibroblasts. Cells were incubated with increasing concentrations of ^{125}I-labeled LDL for 4 h at 4°C before the analysis of lipoprotein binding (A), or for 5 h at 37°C prior to determination of LDL degradation rate by the fibroblasts (B). Results represent mean ± SD (n = 3).

Due to the peroxisomal β-oxidation defect in peroxisomal disorders, very-long-chain fatty acids (VLCFA), including tetracosanoic acid (C-24:0), accumulate in plasma and in tissues. Preincubation of normal fibroblasts with 100 μg/ml of C-24:0 complexed to albumin resulted in a 2- to 3-fold elevation in the cellular content of VLCFA as determined by gas chromatography (data not shown). We therefore questioned whether C-24:0 could affect cellular binding and degradation of LDL by fibroblasts. For binding analysis, cells were incubated for 20 h at 37°C with 100 μg/ml of C-24:0, followed by cell wash and a further incubation for 4 h at 4°C with increasing concentrations of LDL. Preincubation of the control fibroblasts with C-24:0 induced a 5.2- to 8.0-fold increase in LDL binding on using 2.5–15 μg of ^{125}I-labeled LDL protein/ml, in comparison to cells that were not treated with the VLCFA (**Fig. 4A**). Similarly, on using 5–50 μg of ^{125}I-labeled LDL protein/ml, normal fibroblasts that were preincubated with VLCFA showed a 2.8- to 3.9-fold increase in cellular degradation of normal LDL in comparison to non-treated fibroblasts (Fig. 4B; n = 3).

Our results thus demonstrate that the reduced cholesterol mass in PD fibroblasts can be attributed to reduced cellular cholesterol synthesis. On the other hand, our observation of an increased rate of LDL uptake by PD cells is not easy to reconcile with the low cholesterol mass observed in PD cells. As the LDL uptake studies in the present study were performed using normal LDL, whereas in a previous study we demonstrated a reduced cholesterol esterification rate of LDL derived from a PD patient (19), we questioned whether the use of LDL derived from PD patients would demonstrate alteration in the cellular uptake and metabolism of this abnormal LDL. Cholesterol, phospholipid, and triglyceride content of the patients' LDL were 0.78 ± 0.08, 0.80 ± 0.01, and 0.12 ± 0.03 mg/mg LDL protein, respectively, in comparison to 1.40 ± 0.33, 0.51 ± 0.07, and 0.24 ± 0.04 mg/mg LDL protein in control LDL, respectively (n = 4). LDL concentration studies were thus carried out by incubating normal cells with either normal LDL or with PD-LDL (**Fig. 5**), followed by analysis of LDL degradation rate (Fig. 5A) and LDL-induced stimulation of cholesterol esterification rate (Fig. 5B) in control fibroblasts. At all

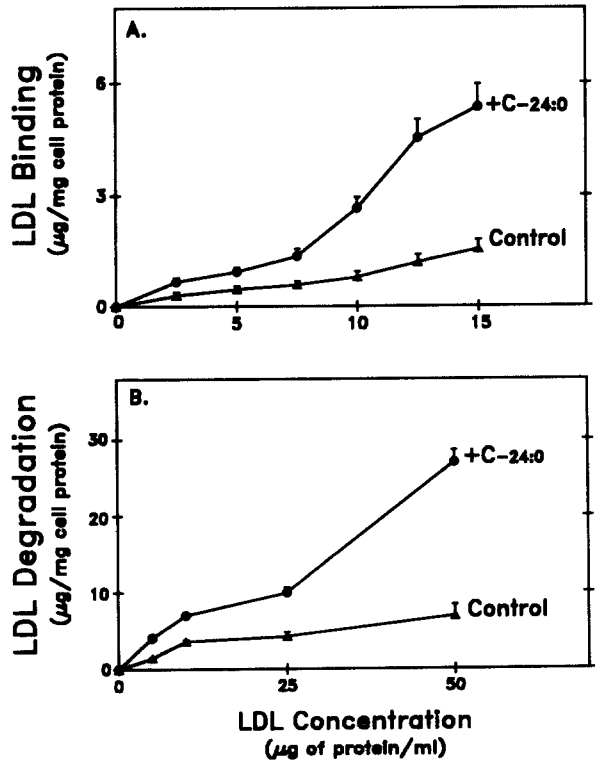

Fig. 4. Binding (A) and degradation (B) of normal LDL by normal fibroblasts that were enriched with very-long-chain fatty acid. Normal fibroblasts that were preincubated with no addition (▲, control) or with addition of 100 μg/ml of C-24:0 (●, +C-24:0) for 20 h at 37°C, followed by cell wash, were incubated with increasing concentrations of ^{125}I-labeled LDL. At the end of the incubation, LDL binding (incubated for 4 h at 4°C) (A), or LDL degradation (incubated for 5 h at 37°C) was determined. Results are given as the mean ± SD (n = 3).

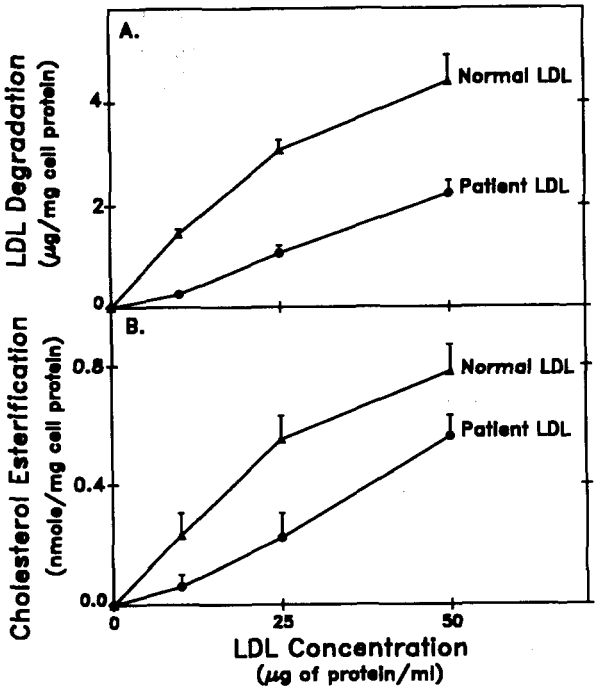

Fig. 5. LDL degradation (A) and LDL-induced stimulation of cellular cholesterol esterification (B) by normal fibroblasts. Increasing concentrations of LDL derived from either a PD patient (●) or from a normal subject (▲) were incubated with normal fibroblasts for 5 h at 37°C prior to analysis of cellular degradation of ^{125}I-labeled LDL (A), or of cholesterol esterification rate in the fibroblasts (B). Results are given as mean ± SD (n = 3).

LDL concentrations studied (10–50 μg protein/ml), there was a significant reduction of both cellular lipoprotein degradation (Fig. 5A) and of cholesterol esterification rates (Fig. 5B) (by 82–65% and 74–29%, respectively) of the PD-LDL in comparison to normal LDL (Figs. 5A and 5B). On using fibroblasts from PD patients (PD-cells), the degradation rate of PD-LDL was also lower by 74–67% on using 10–50 μg protein/ml, as compared with the LDL degradation rate observed with normal LDL (**Table 1**).

DISCUSSION

The present study demonstrates a low cholesterol content and a reduced de novo cholesterol biosynthesis in fibroblasts from PD patients in comparison to control cells. In addition it shows, for the first time, that although the regulation of the uptake of normal LDL via the LDL-receptors by PD cells is potentially operative (as in normal cells), LDL derived from PD patients is not recognized normally by the fibroblasts LDL-receptor. This novel observation of a reduced fibroblast uptake of the PD-LDL could explain, at least partially, the low cholesterol mass in fibroblasts from PD patients, in addition to the lowered capacity of these cells to synthesize cholesterol. The relative importance of peroxisomes in cellular cholesterol biosynthesis in human cells has been suggested (5, 15), and was reconfirmed in the present study. Whereas several reports have indicated that the normal mechanism for regulation of de novo cholesterol biosynthesis is operative in PD cells (15, 23), the cholesterol mass was shown to be markedly reduced in this study, suggesting a limited capacity of PD cells to compensate for their reduced cholesterol biosynthesis rate. Peroxisomes contain enzymes that are involved in cholesterol biosynthesis (5–11), including the rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase (8, 9). It has been shown that peroxisomal HMG-CoA reductase activity has a distinctly different diurnal cycle from that of the microsomal reductase (32). In addition, cholestyramine treatment induced about a 6-fold increase in the specific activity of the peroxisomal HMG-CoA reductase, whereas the specific activity of the microsomal reductase increased only 2-fold (7). These observations suggest an independent regulation of microsomal and peroxisomal enzymes. The possible significance for the presence of a second cholesterol biosynthesis system remains unclear. However, the reduced cholesterol content in PD cells, as shown in this study, might reflect an important covert contributory role for the peroxisomal cholesterol metabolic pathway, in the overall de novo cellular cholesterol biosynthesis capacity.

Mammalian cells acquire exogenous cholesterol mainly through receptor-mediated endocytosis of LDL via the LDL receptors; this cellular uptake of LDL is regulated by cellular cholesterol content (21, 22). This regulatory system, as opposed to the regulation of cholesterol synthesis, has not been previously investigated in PD cells. In the present study, the cellular uptake of normal LDL was shown to be enhanced in PD cells, in comparison to normal cells. As we demonstrated a reduced cholesterol mass in the PD cells, this novel finding of an increased LDL uptake capacity by PD cells suggests that the regulation of the LDL receptor pathway by cellular cholesterol content holds true also for PD cells. Cellular accumulation of VLCFA in cells from PD patients is assumed to be responsible, at least in part, for the underlying pathophysiology

TABLE 1. Low density lipoprotein degradation by peroxisome-deficient (PD) fibroblasts

	LDL Degradation (at LDL Concentrations)		
	(10 μg/ml)	(25 μg/ml)	(50 μg/ml)
	μg LDL protein/mg cell protein		
PD-LDL	0.7 ± 0.3	2.1 ± 0.9	5.1 ± 1.5
C-LDL	1.5 ± 0.3	5.4 ± 0.5	10.4 ± 1.1

Increasing concentrations of ^{125}I-labeled PD-LDL or control (C)-LDL were incubated with PD-fibroblasts for 5 h at 37°C prior to the analysis of cellular LDL degradation. Results are given as mean ± SD (n = 3).

of peroxisomal disorders. In order to investigate whether cellular accumulation of VLCFA could account for an enhanced degradation of normal LDL by PD cells, we added a VLCFA (tetracosanoic acid, C-24:0) to normal fibroblasts (in order to mimic the condition that exists in PD cells), and analyzed LDL binding and degradation rates in the VLCFA-enriched fibroblasts. A 2.5-fold increase in the cellular C-24:0 content was associated with about a 4-fold increase in LDL binding and degradation by the VLCFA-enriched cells, suggesting that the accumulation of VLCFA in PD cells might affect membrane fluidity (by interacting with polar phospholipids in plasma membranes) and thus increase LDL uptake by these cells. In addition, the reduced plasmalogen content and the increased content of phosphatidylethanolamine and long chain fatty acyl groups in the plasma membrane of PD cells (1) could also affect LDL receptor activity via the induction of conformational changes in the region of the LDL receptor binding domains.

Hypocholesterolemia and abnormal lipoproteins have been frequently found in infantile Refsum disease (IRD) (16–20). The hypocholesterolemia could result in part from gastrointestinal malabsorption associated with liver disease and abnormal bile acid metabolites. In a previous study, we have shown compositional abnormalities in LDL derived from an IRD patient, with markedly reduced ratios of the lipid components of LDL to protein (19). The ratio of LDL cholesterol to protein in the PD patient lipoprotein was reduced by 40% in comparison to normal LDL, with 45% and 32% reduction in the LDL cholesteryl ester and unesterified cholesterol to protein ratios, respectively (19). We also demonstrated in that study a reduced uptake of the patient's LDL by normal macrophages as analyzed by cholesterol esterification assay (19).

The present study demonstrates an enhanced uptake and degradation of normal LDL by PD cells, which does not conform with the low cholesterol mass of PD cells. All previous in vitro studies that were performed on PD cells used as a source of lipoproteins either calf serum (15, 23) or LDL that was derived from normal individuals (23). When we used LDL that was derived from five IRD patients, we could demonstrate that this abnormal LDL was taken up at a reduced rate by normal control cells as well as by PD cells. These results suggest that the abnormal PD-LDL hampers the regulation of the LDL receptor activity by cellular cholesterol content, leading to reduced cellular cholesterol content.

Modified lipoproteins have been shown to affect the rate of LDL uptake by cells (33–38). The altered composition of the PD-LDL with its low cholesterol content may have induced changes in the conformation of the apoB-100 on the surface of this LDL, and thus could impair its interaction with the cell-surface LDL receptors.

We conclude that at least two processes contribute to the disturbances of cellular cholesterol homeostasis in peroxisome-deficient cells, i.e., a reduced uptake of the abnormal PD-LDL and an impairment in de novo cholesterol biosynthesis. The results of this study provide further evidence for the significant involvement of peroxisomes in cholesterol synthesis as well as in lipoprotein metabolism.

Cholesterol is an essential metabolite that determines the integrity as well as the fluidity of cellular membranes (39, 40). Hence, derangements of cellular cholesterol content may cause widespread abnormalities in many cells. Hypocholesterolemia induced by hypocholesterolemic agents has been shown to impair myelinogenesis and to induce degenerative changes in the central nervous system in rats (41). The abnormal metabolism of cholesterol in PD cells could thus play a role in the pathophysiology of the devastating manifestations observed in peroxisome deficiency disorders.

The authors are indebted to Dr. Ruud B. H. Schutgens and Dr. Ronald J. A. Wanders from the Department of Pediatrics, University Hospital Amsterdam, who contributed cell lines and valuable comments. This work was supported in part by the Rappaport Family Fund for Research in the Medical Sciences, and the Joseph Elias Fund for Medical Research, B. Rappaport-Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Manuscript received 6 December 1994 and in revised form 9 February 1995.

REFERENCES

1. Lazarow, P. B., and H. M. Moser. 1989. Disorders of peroxisome biogenesis. *In* The Metabolic Basis of Inherited Disease. 6th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle. editors. McGraw-Hill Information Services Company, New York. 1479–1509.
2. Fournier, B., J. A. M. Smeitink, L. Dorland, R. Berger, J. M. Saudubray, and B. T. Poll-The. 1994. Peroxisomal disorders: a review. *J. Inher. Metab. Dis.* **17:** 470–486.
3. Van den Bosch, H., R. B. H. Schutgens, R. J. A. Wanders, and J. M. Tager. 1992. Biochemistry of peroxisomes. *Annu. Rev. Biochem.* **61:** 157–197.
4. Wanders, R. J. A., H. S. A. Heymans, R. B. H. Schutgens, P. G. Barth, H. Den den Bosch, and J. M. Tager. 1989. Peroxisomal disorders in neurology. *J. Neurol. Sci.* **88:** 1–39.
5. Krisans, S. K. 1992. The role of peroxisomes in cholesterol metabolism. *Am. J. Resp. Cell Mol. Biol.* **7:** 358–364.
6. Thompson, S. L., and S. K. Krisans. 1990. Rat liver peroxisomes catalyze the initial step in cholesterol synthesis. *J. Biol. Chem.* **265:** 5731–5735.
7. Hovik, R., B. Brodal, K. Bartlett, and H. Osmundsen. 1991. Metabolism of acetyl-CoA by isolated peroxisomal fractions: formation of acetate and acetoacetyl-CoA. *J. Lipid Res.* **32:** 993–999.
8. Keller, G. A., M. C. Barton, D. J. Shapiro, and S. J. Singer. 1985. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase is present in peroxisomes in normal rat liver cells. *Proc. Natl. Acad. Sci. USA.* **82:** 770–774.
9. Keller, G. A., M. Paziranden, and S. K. Krisans. 1986.

3-Hydroxy-3-methylglutaryl coenzyme A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine-treated animals: quantitative biochemical and immunoelectron microscopical analysis. *J. Cell Biol.* **103:** 875–886.
10. Krisans, S. K., J. Ericsson, P. A. Edward, and G. A. Keller. 1994. Farnesyl-diphosphate synthase is localized in peroxisomes. *J. Biol. Chem.* **269:** 14165–14169.
11. Biardi, L., A. Sreedhar, A. Zokaei, N. B. Vartak, B. L. Bozeat, J. E. Shackelford, G. A. Keller, and S. K. Krisans. 1994. Mevalonate kinase is predominantly localized in peroxisomes and is defective in patients with peroxisome deficiency disorders. *J. Biol. Chem.* **269:** 1197–1205.
12. Keller, G. A., T. J. Scallen, D. Clarke, P. A. Maher, S. K. Krisans, and S. J. Singer. 1989. Subcellular localization of sterol carrier protein-2 in rat hepatocytes: its primary localization to peroxisomes. *J. Cell Biol.* **108:** 1353–1361.
13. Suzuki, Y., S. Yamaguchi, T. Orii, M. Tsuneoka, and Y. Tashiro. 1990. Nonspecific lipid transfer protein (sterol carrier protein-2) defective in patients with deficient peroxisomes. *Cell Struc. Func.* **15:** 301–308.
14. Hamilton, R. L., J. S. Wong, L. S. Guo, S. K. Krisans, and R. J. Havel. 1990. Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. *J. Lipid Res.* **31:** 1589–1604.
15. Hodge, V. J., S. J. Gould, S. Subramani, H. W. Moser, and S. K. Krisans. 1991. Normal cholesterol synthesis in human cells requires functional peroxisomes. *Biochem. Biophys. Res. Commun.* **181:** 537–541.
16. Scotto, J. M., M. Hadchouel, and M. Odievre. 1982. Infantile phytanic acid storage disease, a possible variant of Refsum disease: three cases, including ultrastructural studies of the liver. *J. Inher. Metab. Dis.* **5:** 83–90.
17. Poll-The, B. T., J. M. Saudubray, H. A. M. Ogier, M. Odievre, J. M. Scotto, L. Mommens, L. C. P. Govarets, F. Roels, A. Cornelis, R. B. H. Schutgens, R. J. A. Wanders, A. W. Schram, and J. M. Tager. 1989. Infantile Refsum Disease: an inherited peroxisomal disorder. Comparison with Zellweger syndrome and neonatal adrenoleukodystrophy. *Eur. J. Pediatr.* **146:** 477–483.
18. Wanders, R. J. A., E. Boltshause, and B. Steinmann. 1990. Infantile phytanic acid storage disease, a disorder of peroxisomes biogenesis: a case report. *J. Neurol. Sci.* **98:** 1–11.
19. Mandel, H., M. Berant, D. Meiron, A. Aizen, J. Oiknine, J. G. Brook, and M. Aviram. 1992. Plasma lipoproteins and monocyte-macrophages in peroxisome-deficient system: study of a patient with Infantile Refsum disease. *J. Inher. Metab. Dis.* **15:** 774–784.
20. Mandel, H., D. Meiron, R. B. H. Schutgens, R. J. A. Wanders, and M. Berant. 1992. Infantile Refsum disease: gastrointestinal presentation of a peroxisomal disorder. *J. Pediatr. Gastroenterol. Nutr.* **14:** 83–85.
21. Goldstein, J. L., M. S. Brown, R. G. Anderson, D. W. Russel, and W. J. Schneider. 1985. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu. Rev. Cell Biol.* **1:** 1–39.
22. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
23. Van Heusden, G. P. H., J. R. C. M. van Beckhoven, R. Thieringer, C. R. H. Raetz, and K. W. A. Wirtz. 1992. Increased cholesterol synthesis in Chinese hamster ovary cells deficient in peroxisomes. *Biochim. Biophys. Acta.* **1126:** 81–87.
24. Aviram, M. 1983. Plasma lipoprotein separation by discontinuous density gradient ultracentrifugation in hyperlipoproteinemic patients. *Biochem. Med.* **30:** 11–118.
25. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein protein. I. Preliminary in vitro and in vivo observation. *Biochim. Biophys. Acta.* **260:** 212–221.
26. Bierman, E. L., O. Stein,and Y. Stein. 1974. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. *Circ. Res.* **35:** 136–154.
27. Lowry, O., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
28. Innerarity, T. E., R. E. Pitas, and R. H. Mahley. 1986. Lipoprotein receptor interactions. *Methods Enzymol.* **124:** 542–565.
29. Brown, M. S., Y. K. Ho, and J. L. Goldstein. 1980. The cholesteryl ester cycle in macrophage foam cells. *J. Biol. Chem.* **255:** 9344–9352.
30. Chiamori, N., and R. J. Henry. 1959. Study of the ferric chloride method for determination of total cholesterol and cholesterol esters. *Am. J. Clin. Pathol.* **31:** 305–309.
31. Moser, H. W., A. E. Moser, N. Kawamura, J. Murphy, K. Suzuki, H. H. Schaumburg, Y. Kishimoto, and A. Milunsky. 1980. Adrenoleukodystrophy: elevated C26 fatty acid in cultured skin fibroblasts. *Ann. Neurol.* **7:** 542–549.
32. Rusnak, N., and S. K. Krisans. 1987. Diurnal variation of HMG-CoA reductase activity in rat liver peroxisomes. *Biochem. Biophys. Res. Commun.* **148:** 890–895.
33. Aviram, M. 1993. Modified forms of low density lipoprotein and atherosclerosis. *Atherosclerosis.* **98:** 1–9.
34. Aviram, M. 1992. Low density lipoprotein modification by cholesterol oxidase induces enhanced uptake and cholesterol accumulation in cells. *J. Biol. Chem.* **267:** 218–225.
35. Aviram, M., and I. Maor. 1993. Phospholipase D-modified low density lipoprotein is taken up by macrophages at increased rate. *J. Clin. Invest.* **91:** 1942–1952.
36. Aviram, M., E. L. Bierman, and A. Chait. 1988. Modification of low density lipoprotein lipase or hepatic lipase induces enhanced uptake and cholesterol accumulation in cells. *J. Biol. Chem.* **263:** 15416–15422.
37. Aviram, M., S. Lund-Katz, M. C. Phillips, and A. Chait. 1988. The influence of the triglyceride content of low density lipoprotein on the interaction of apolipoprotein B-100 with cells. *J. Biol. Chem.* **263:** 16842–16848.
38. Aviram, M., S. Keidar, M. Rosenblat, and J. G. Brook. 1991. Reduced uptake of cholesterol esterase-modified low density lipoprotein by macrophages. *J. Biol. Chem.* **266:** 11567–11574.
39. Yeagle, P. L. 1985. Cholesterol and the cell membrane. *Biochim. Biophys. Acta.* **822:** 267–287.
40. Copper, R. A., and S. J. Shatill. 1980. Membrane cholesterol—is enough too much? *N. Engl. J. Med.* **302:** 49–51.
41. Suzuki, K., and L. D. DePaul. 1971. Cellular degeneration in developing central nervous system of rats produced by hypocholesterolemic drug AY9944. *Lab. Invest.* **25:** 546–555.