Human plasma LDL cryopreserved with sucrose maintains in vivo kinetics indistinguishable from freshly isolated human LDL in cynomolgus monkeys

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Abstract We previously reported that freeze-thawing of LDL causes marked alterations in its structure and in vitro biological behavior, and that such changes can be completely abolished by the addition of sucrose to the LDL solution prior to freezing. (Rumsey, S. C. et al., *J. Lipid Res.* 1992. **33:** 1551–1561). We now questioned whether the cryopreservative action of sucrose would be equally effective in maintaining the in vivo metabolic characteristics of LDL. Two dual-label LDL turnover studies were performed in cynomolgus monkeys (n = 8) comparing freshly isolated human LDL with human LDL that was frozen in sucrose (10% w/v) for a short (20 h) or long period (6 months). The same sucrose-cryopreserved LDL was used for both the short- and long-term studies; different fresh LDL preparations were used in each study. Absorption spectrophotometry, gel filtration, and electron microscopy of LDL samples frozen with sucrose showed no evidence of physical alterations or aggregation, and there was no evidence of very rapid clearance of cryopreserved LDL from monkey plasma after injection. Fractional catabolic rates (FCR) of fresh and frozen LDL were very similar in either the short-term or long-term experiments: 2.09 ± 0.86 versus 2.16 ± 0.88, short-term and 3.03 ± 2.28 versus 3.08 ± 2.29, long-term (pools per day; mean ± SD). The difference between FCR of fresh and frozen LDL for each animal averaged −0.076 ± 0.074 and 0.01 ± 0.22 (mean ± SD), for short-term and long-term freezing, respectively. Squared correlation coefficients (R^2) of FCR between fresh and frozen LDL were 0.994 and 0.991, respectively, for the two periods. Thus, cryopreservation of human LDL with sucrose maintains LDL clearance properties virtually identical to that of freshly isolated LDL preparations.—**Rumsey, S. C., A. F. Stucchi, R. J. Nicolosi, H. N. Ginsberg, R. Ramakrishnan, and R. J. Deckelbaum.** Human plasma LDL cryopreserved with sucrose maintains in vivo kinetics indistinguishable from freshly isolated human LDL in cynomolgus monkeys. *J. Lipid Res.* 1994. **35:** 1592–1598.

Supplementary key words low density lipoprotein • cryopreservation • LDL fractional catabolic rate

A significant problem in the study of low density lipoprotein (LDL) structure and function is the inability to store LDL preparations, unmodified, for long periods of time. LDL undergoes degradative and oxidative damage over the course of a few days to weeks when stored at 4°C (1–4). Although precautions can be taken to minimize alterations in LDL (5), unpredictable modifications can limit the accuracy of comparative studies of LDL performed over long time periods (e.g., in drug or diet trials).

It has been known for many years that plasma lipoproteins are damaged by freeze-thawing. Although there were early suggestions that sucrose might be used as a cryopreservative for β-lipoproteins (6), no subsequent studies tested whether this would truly prevent physical or biological changes in LDL. In a recent publication (7) we demonstrated that sucrose, when used as a cryopreservative, maintains LDL with normal physical and in vitro biological characteristics for at least 18 months. No alterations between frozen and fresh LDL were detected using spectrophotometry, gel filtration, electron microscopy, monoclonal antibody binding to apoB, and circular dichroism. As well, in cultured fibroblasts, LDL binding at 4°C and degradation via the LDL receptor at 37°C were also unchanged. The question remained, however, whether cryopreserved LDL would retain normal kinetic characteristics in vivo.

Cryopreservation of LDL samples for long periods would be extremely useful for many long-term studies of LDL metabolism. One possible application would be in comparing in vivo LDL fractional clearance rates before and after a specific treatment intervention. In this study

Abbreviations: LDL, low density lipoproteins; PBS, phosphate-buffered saline; BSA, bovine serum albumin; EDTA, ethylenediamine tetraacetate; TCA, trichloroacetic acid; FCR, fractional catabolic rate.

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we extend our previous investigation of the ability of sucrose to prevent freezing-induced damage of human LDL by examining whether cryopreservation with sucrose affects the in vivo fractional clearance rate of LDL after short-term (20 h) or long-term (6 months) freezing.

METHODS

Low density lipoproteins

Blood from normolipidemic human subjects was collected into Vacutainer (Becton Dickinson, Rutherford, NJ) tubes containing EDTA and the plasma was separated by low speed centrifugation. Disodium EDTA (1.2 g/l), NaN_3 (0.1 g/l), and aprotinin (10,000 U/l) were added to the plasma (8) and LDL was isolated by sequential ultracentrifugation (1.019 g/ml < d < 1.063 g/ml) (9). LDL preparations were dialyzed at 4°C against saline (150 mM NaCl, 0.24 mM EDTA, pH 7.4) after ultracentrifugation to remove sodium bromide prior to iodination, sucrose addition, freezing, and all other experimental procedures.

Sucrose cryopreservation of LDL

Sucrose was added to LDL solutions by direct addition of a stock sucrose solution (50% sucrose, 150 mM NaCl, 0.24 mM EDTA, pH 7.4). LDL solutions (1–1.5 ml aliquots, 7 mg LDL protein/ml) containing sucrose were frozen to −70°C in 1.5-ml plastic microcentrifuge tubes by placement in a Fisher 212Z upright −70°C freezer. Samples remained frozen for a minimum of 20 h and a maximum of 6 months. Freezing rate was not controlled but was measured using a temperature probe and found to be approximately 4°C per min. LDL was thawed by immersion in a 37°C water bath for 3 min. LDL was iodinated after freeze-thawing. Prior to iodination, sucrose was removed by dialysis at 4°C against saline (150 mM NaCl, 0.24 mM EDTA, pH 7.4, 2000 × sample volume, changed 3 times over a 24-h period) (7).

LDL radioiodination

LDL was radioiodinated using Bilheimer's modification of McFarlane's procedure (10) using sodium ^{125}I or ^{131}I (Amersham, Arlington Heights, IL). The specific activity of ^{125}I-labeled LDL and ^{131}I-labeled LDL ranged between 200 and 400 cpm/ng of LDL protein. Over 99% of the radioactivity was precipitable after a 60-min incubation with 10% (v/v) trichloroacetic acid at 4°C. As in previous studies (7), no differences were seen in the efficiency of iodination between fresh and sucrose cryopreserved LDL samples.

Physical analyses

Spectrophotometric measurements were performed on a Beckman DU-50 Spectrophotometer in Fisher polystyrene semi-micro cuvets over a range of wavelengths from 325 to 900 nm. Gel filtration was performed on a Pharmacia column (40 cm × 1.6 cm) containing Sepharose CL2B with a flow rate of 0.8 ml/min. The eluent consisted of saline (150 mM NaCl, 0.24 mM EDTA, 0.02% NaN_3). Samples were applied to the column in 500-μl aliquots and collected into 1.8-ml fractions. Prior to column application, all LDL preparations were adjusted to an LDL protein concentration of 2 mg/ml.

Electron microscopy of LDL particles was performed on a JEOL 1200 EX (Peabody, MA), with negative staining using a modification of a previously described method (11). Briefly, LDL (1 mg/ml) was dialyzed against saline buffer (described above) and 1 drop of sample was placed on a 200-mesh Formvar–carbon-coated grid (Ladd Scientific Equipment, Burlington, VT). Excess sample was removed with filter paper; one drop of 2% sodium phosphotungstic acid (pH 7.2) (Ladd Research, Burlington, VT) was placed on the grid, and the preparation was air-dried for 30 min. Particle size histograms were generated by measuring the diameter of 100 free-standing particles in enlarged photographs using a Peak Scale Lupe (Electron Microscopy Sciences, Fort Washington, PA).

Chemical analysis

LDL protein was measured using the methods of Lowry et al. (12). LDL triglycerides, total and free cholesterol were analyzed using Boehringer Mannheim enzymatic kits #877557, 290319, and 310328, respectively. LDL phospholipids were analyzed using the Bartlett phosphorus assay (13). Monkey total cholesterol, HDL cholesterol, and triglycerides were measured before each turnover study as described previously (14). Plasma lipids represent the means of three blood samples.

Animals and diets

Eight adult female cynomolgus monkeys (*Macaca fascicularis*) ranging in age from 4 to 8 years were fed isocaloric diets containing 36% of calories as fat, 52% carbohydrate, 12% protein, and 0.1% cholesterol with a polyunsaturated to saturated fat ratio of 0.45. Fat in the diet was derived from a blend of corn, coconut, and olive oils with crystalline cholesterol dissolved into the oils prior to mixing into diets. Animals were maintained on the diet for at least 3 months prior to each turnover period.

The animals used in these studies were maintained in accordance with the guidelines of the Committee on Animal Care and Use of the University of Massachusetts-Lowell Research Foundation and the guidelines prepared

by the Committee on Care in Use of Lab Animals of the Institute of Lab Animal Resources, National Research Council (DHEW publication No. 85-23, revised 1985).

Study protocol (Fig. 1)

Human LDL obtained from a normolipidemic donor (LDL-1) was frozen either for a short period of time (20 h) or for a long period of time (6 months) in the presence of 10% sucrose (w/v) as described above. The cryopreserved LDL-1, frozen for 20 h, was compared with the same fresh LDL (LDL-1 maintained at 4°C); and LDL-1 frozen for 6 months was compared with a different fresh LDL (LDL-2) obtained from another normolipidemic donor.

Fresh and cryopreserved LDL were labeled with ^{125}I or ^{131}I for simultaneous injection into cynomolgus monkeys. In each experiment, four monkeys received ^{125}I-labeled fresh LDL and ^{131}I-labeled frozen LDL, and four monkeys received ^{125}I-labeled frozen LDL and ^{131}I-labeled fresh LDL in order to rule out any difference in ^{125}I or ^{131}I labeling.

After a 16-h fast, animals were anesthetized with 5–7 mg/kg ketamine (Vetalar Aveco, Fort Dodge, IA) and after the simultaneous injection of 20 μCi of ^{125}I-labeled LDL and 45 μCi ^{131}I-labeled LDL into the saphenous vein, 2-ml blood samples were collected from the opposite femoral vein at 10 min, 1, 3, 4.5, 6, 24, 48, and 72 h. An aliquot of plasma was used to measure total radioactivity at each time point, and then the radioactivity in LDL apoB was selectively precipitated from whole plasma with isopropanol (15).

The fractional catabolic rate (FCR) of LDL apoB was subsequently determined using the counts from the washed, precipitated apoB pellet. FCR in pools/day were determined by fitting the data with a two-pool model as described previously (16). Initial volumes of distribution were calculated by dividing the dose injected by the initial plasma radioactivity (calculated by extrapolating the model fitted curve back to zero time). This was done separately for each tracer in each animal in order to determine whether either freezing or radiolabeling resulted in unexpectedly rapid LDL clearance.

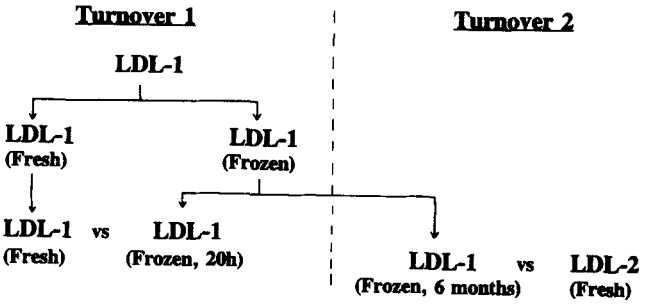

Fig. 1. Study protocol. The in vivo kinetics of human LDL-1 cryopreserved (−70°C) in 10% sucrose either for a short period (20 h) or for a long period (6 months) were compared with those of freshly obtained LDL-1 and freshly obtained LDL-2, respectively. Turnover 1: comparison of fresh LDL-1 with LDL-1 frozen 20 h. Turnover 2: comparison of fresh LDL-2 with LDL-1 frozen for 6 months. Fresh and frozen LDL were radiolabeled with ^{125}I or ^{131}I and injected simultaneously into eight cynomolgus monkeys. LDL fractional catabolic rates were determined as described in Methods.

Thyroidal uptake of radiolabeled iodine was inhibited by the oral administration of approximately 1 ml of 1% potassium iodide solution for 7 days prior to the injection of label and for the duration of the study.

As has been previously reported (14), ketamine can induce anorexia and therefore those animals that did not resume eating within 12–24 h were given an enteral bolus of their regular diet in order to minimize any effects of extended fasting on steady-state kinetics.

Statistical analyses of differences in FCR were performed using paired *t*-tests.

RESULTS

Lipid levels

Body weights and plasma lipid values for each of the eight monkeys determined before each study period are

TABLE 1. Body weights and plasma lipid levels of cynomolgus monkeys

	Body Weight		Total Cholesterol		HDL Cholesterol		Triglycerides	
Animal	1[a]	2[b]	1[a]	2[b]	1[a]	2[b]	1[a]	2[b]
	kg		*mg/dl*		*mg/dl*		*mg/dl*	
1	4.14	3.53	430	369	90	110	47	45
2	5.79	5.45	226	253	86	111	202	142
3	2.98	2.86	256	250	94	107	23	41
4	4.79	4.23	267	233	77	68	55	74
5	4.92	4.94	222	180	138	124	60	60
6	7.31	6.63	234	221	78	63	70	78
7	4.89	3.74	188	155	59	78	19	41
8	4.87	5.06	174	162	85	83	31	42
Mean ± SD	4.96 ± 1.2	4.56 ± 1.2	249.6 ± 79.2	227.9 ± 68.7	88.4 ± 22.7	93.0 ± 22.0	63.4 ± 58.9	65.4 ± 34.4

Animal weights and plasma lipids presented are the mean of three determinations measured prior to each turnover study as described in Methods.
[a] Turnover 1: Comparison of fresh LDL-1 with LDL-1 frozen 20 h. [b] Turnover 2: Comparison of fresh LDL-2 with LDL-1 frozen for 6 months.

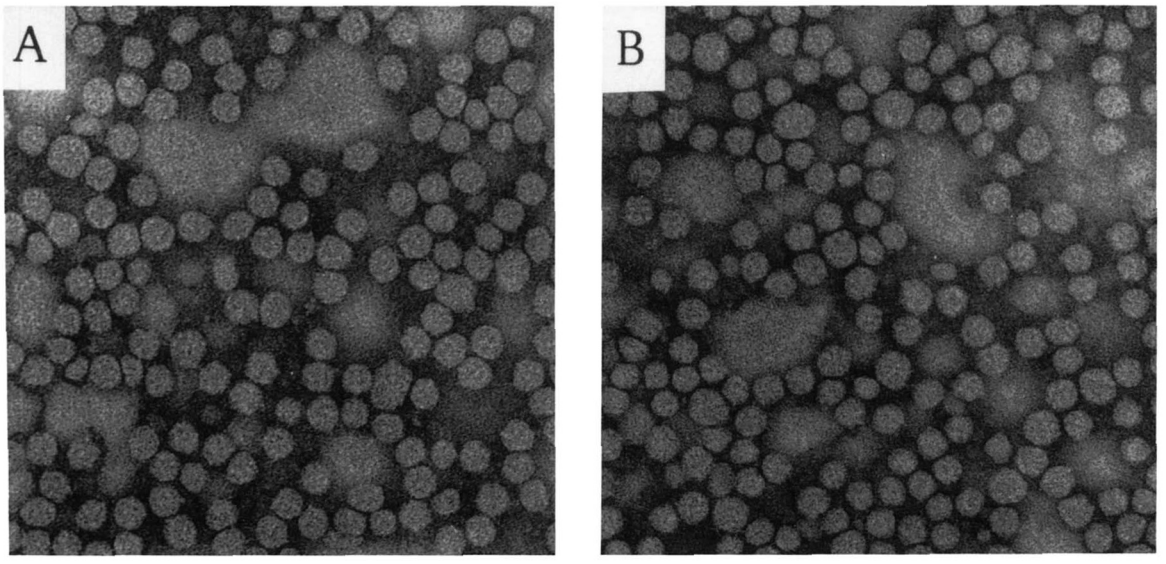

Fig. 2. Electron photomicrographs of negatively stained fresh human LDL-2 (left panel) and LDL-1 cryopreserved in sucrose for 6 months (right panel). Initial instrument magnification was × 80,000. Sucrose was removed from the frozen sample prior to analysis by dialysis against saline. Average particle size of fresh and cryopreserved LDL by measure of particle diameter (n = 100) was 21.1 ± 1.8 nm and 20.6 ± 2.9 nm (mean ± SD), respectively.

given in **Table 1.** The results given are the means of three blood drawings. There were no significant differences in mean levels of total cholesterol, HDL cholesterol, or triglycerides between the two periods.

LDL physical analyses

The chemical compositions of LDL obtained from normolipidemic human donors were similar and within the normal range. LDL-1 contained 21.4% protein, 7.8% triglyceride (TG), 44.5% cholesteryl ester (CE), 8.6% free cholesterol (FC), and 17.8% phospholipid (PL) by weight. LDL-2 contained 22.1% protein, 3.8% TG, 44.2% CE, 11.2% FC, and 18.7% PL by weight.

LDL frozen without cryopreservative has a characteristic abnormal spectrophotometric pattern (325–900 nm) (7), and can be seen in electron micrographs to contain a large number of fused and aggregated particles (7). In the present study, and as we previously reported (7), LDL frozen to −70°C with sucrose both short-term and long-term showed spectrophotometric and gel elution profiles indistinguishable from that of control nonfrozen LDL (data not shown). Electron microscopy of LDL samples frozen for 6 months at −70°C in the presence of sucrose demonstrated that they were of normal size and similar to nonfrozen control LDL-2 (**Fig. 2**) mean (± SD) particle diameter (n = 100) was 20.6 ± 2.9 nm versus 21.1 ± 1.8 nm, respectively. Thus, similar to our previous study (7), physical and chemical analysis of fresh versus cryopreserved LDL demonstrated no changes in frozen LDL, nor any losses of LDL after thawing.

Turnover studies

Cryopreserved and fresh human LDL were labeled with ^{125}I or ^{131}I and injected simultaneously into cynomolgus monkeys. A representative decay curve for a single monkey is given in **Fig. 3.** In all cases, frozen and

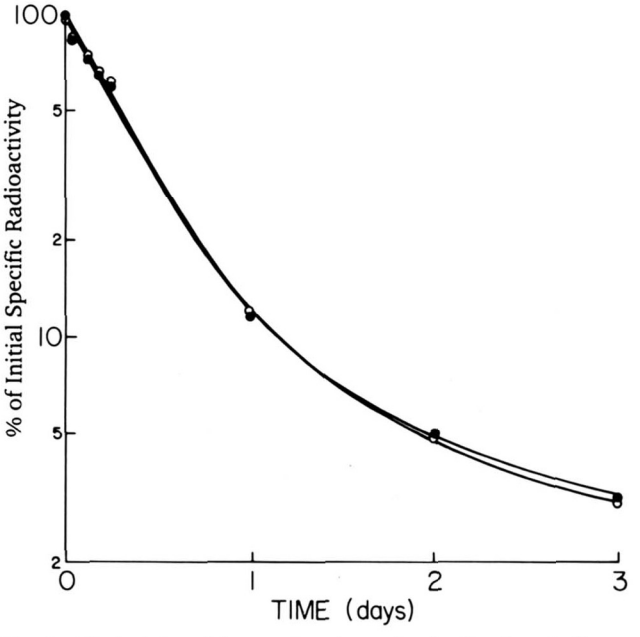

Fig. 3. Typical plot of data used to determine fractional catabolic rate (FCR) of fresh (open circles) and frozen (closed circles) human LDL in cynomolgus monkey. FCR in pools/day were determined by fitting the data with a two-pool model. Solid lines represent model-generated curves.

TABLE 2. Fractional catabolic rates of fresh and frozen human LDL in cynomolgus monkeys

Animal	Turnover 1 Frozen (20 h) FCR[a] (Fresh)	FCR (Frozen)	Difference[b]	Turnover 2 Frozen (6 months) FCR (Fresh)	FCR (Frozen)	Difference
1	0.89	0.86	0.03	1.11	1.09	0.02
2	1.28	1.44	−0.16	3.18	2.99	0.19
3	1.68	1.67	0.01	1.54	1.53	0.01
4	1.82	1.97	−0.15	1.73	1.97	−0.24
5	1.90	1.93	−0.03	2.17	2.31	−0.14
6	2.90	3.01	−0.11	2.02	2.32	−0.30
7	3.02	3.16	−0.14	8.26	8.03	0.23
8	3.22	3.27	−0.05	4.43	4.15	0.28
Mean ± SD	2.09 ± 0.88	2.16 ± 0.86	−0.08 ± 0.07	3.06 ± 2.35	3.05 ± 2.22	0.01 ± 0.22

Fresh or frozen human LDL, labeled with either ^{131}I or ^{125}I, were injected into eight cynomolgus monkeys to test the effect of sucrose cryopreservation on LDL FCR. Turnover 1: comparison of fresh LDL-1 with LDL-1 frozen 20 h. Turnover 2: comparison of fresh LDL-2 with LDL-1 frozen for 6 months.

[a] Fractional catabolic rates (FCR) are given in pools/day.

[b] Difference between FCR of fresh and frozen LDL for each animal.

fresh LDL had very similar or identical decay curves. In each study, the radiolabels were crossed over in half of the animals and initial volumes of distribution were calculated to determine whether tracer type or cryopreservation resulted in any initial rapid clearance of LDL in vivo. In both studies, ^{131}I-labeled LDL showed evidence of rapid LDL removal (5–10% in study 1, 25–35% in study 2), but these losses were independent of whether the LDL was fresh or frozen (data not shown).

LDL FCR were determined for each study by fitting a two-pool model. In each animal for each tracer, a two-pool model provided excellent fit to the radioactivity data, with residual errors averaging 8.4%. LDL FCR (pools/day) are presented in **Table 2.** Mean LDL FCR of fresh and frozen LDL were similar within either turnover study period (2.09 ± 0.88 vs. 2.16 ± 0.86 (short-term), and 3.06 ± 2.35 vs. 3.08 ± 2.22 (long-term; mean ± SD)). Differences in each animal between FCR of fresh and frozen LDL ranged from −0.16 to 0.03 pools/day with a standard deviation of 0.07 in the first study, and from −0.30 to 0.28 pools/day with a standard deviation of 0.22 in the second study. The difference was significant in study 1, suggesting a slightly faster clearance of frozen LDL, but the difference was quite small, about 3.6% of the FCR of fresh LDL. In study 2, the average difference was practically zero and not significant. On a percentage basis, the average in study 2 was still small, about 2.2% of the FCR of fresh LDL. Maximum differences were less than 15% in both studies.

Correlation between the FCR of fresh and frozen LDL is shown in **Fig. 4.** The upper panel shows the FCR of fresh LDL-1 versus short-term frozen LDL-1, and the lower panel shows FCR of fresh LDL-2 versus long-term frozen LDL-1. Correlations of FCR of fresh and frozen

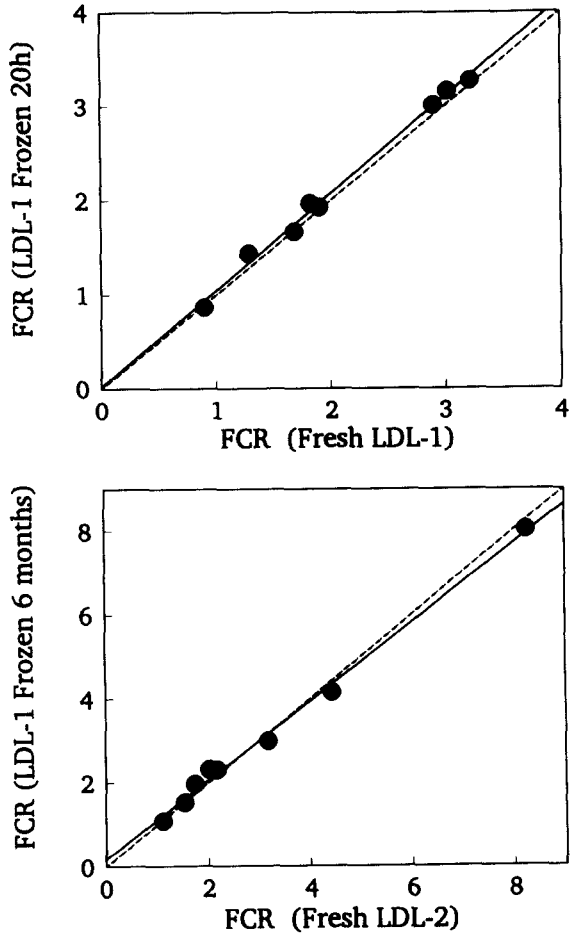

Fig. 4. Correlation plots of FCR of cryopreserved LDL versus fresh LDL. Upper panel: Turnover study 1 (fresh LDL-1 vs. LDL-1 frozen 20 h; R^2 = 0.994). Lower panel: Turnover study 2 (fresh LDL-2 vs. LDL-1 frozen 6 months; R^2 = 0.991). The dashed line represents the line of identity.

LDL were extremely high; R^2 values were 0.994 and 0.991 for short-term and long-term frozen LDL, respectively.

We also compared the results of the two separate turnover studies, which were separated in time by 6 months. The correlation plot of FCR of fresh LDL-1 versus 6-month frozen LDL-1 is shown in **Fig. 5.** As explained below, when the data for animals 2 and 7 (open symbols, Fig. 5) are excluded from analysis, correlation between study periods 1 and 2 was very close (R = 0.87).

The mean FCR for the eight monkeys in study 2 was almost 1 pool/day higher than that of study 1 performed 6 months earlier (3.05 vs. 2.12 pools/day, respectively). This difference is predominantly the result of large increases in FCR of animals 2 and 7 (Table 2). Another three animals (1, 5, and 8) also showed increases in FCR in the second study period, but these increases were considerably smaller. It is possible that an antibody response to the first LDL injection was responsible for the differences in FCR (see Discussion). When animals 2 and 7 were excluded from analysis, mean FCR for the first and second study became very close (2.12 vs. 2.19 pools/day). Importantly, despite the increase in FCR in the second period, the FCR of fresh and frozen LDL in these two animals were still virtually the same (Table 2).

DISCUSSION

Previous studies have clearly shown that freeze-thawing of LDL results in irreversible alterations of its physical and biological properties (6, 7, 17, 18). We have recently demonstrated in our laboratory that sucrose can be used as a cryopreservative to maintain the physical and biological properties of LDL over long periods of storage (−70°C for > 1 year) (7). This study provides an additional critical evaluation of the efficacy of sucrose cryopreservation, i.e., the ability to maintain LDL kinetic characteristics in vivo.

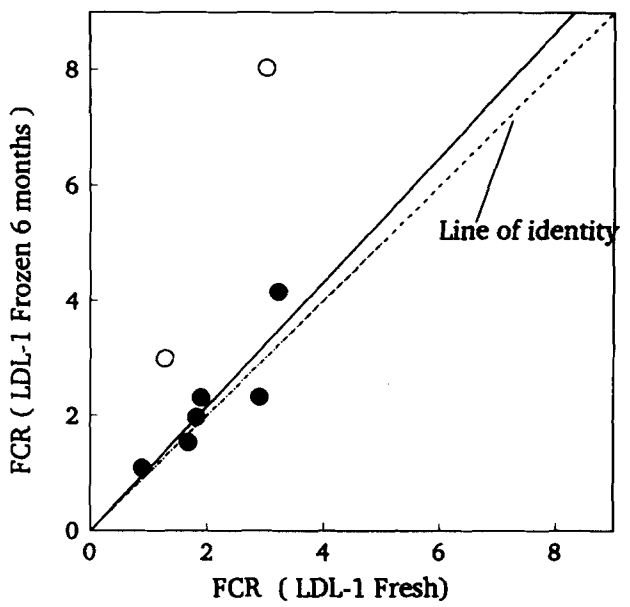

Fig. 5. Comparison of FCR obtained in turnover study 1 and turnover study 2. Correlation plot of FCR of fresh LDL-1 versus LDL-1 frozen 6 months (R = 0.87). The FCR of two animals, numbers 2 and 7, (open symbols) were excluded in this plot from the determination of the regression line and correlation coefficient (see Discussion).

In this study we chose to determine the turnover of human LDL in cynomolgus monkeys because of the extensive prior work performed in our laboratory on the cryopreservation of human LDL, and because the effectiveness of sucrose as a cryopreservative agent for monkey LDL has not yet been established. As well, turnover of human LDL in this species has been previously characterized in studies on the metabolism of normal and cyclohexanedione-modified human LDL (19).

In the present study, human LDL frozen with sucrose as a cryopreservative either short-term (20 h) or long-term (6 months) demonstrated no detectable aggregation or fusion. When this LDL was subsequently labeled and its fractional clearance rate was determined in cynomolgus monkeys, only very small differences were seen as compared to fresh control LDL. There was, however, a noticeable period effect attributable mainly to higher FCR in animals 2 and 7. The reasons for the increase in FCR seen in these animals are not entirely clear, but are possibly related to potentiation of immune responses against the foreign human LDL from the first to second period. Although these animals both displayed marked changes in either body weight or plasma triglyceride levels, no correlations can be drawn between these changes and the higher FCR in the second period.

The turnover rates obtained in the present study with human LDL are higher than what is typically found using homologous monkey LDL. In other studies in our laboratory using four cynomolgus monkeys on a diet similar to that of the present study, mean FCR was 0.81 ± 0.07 pools/day (unpublished data). However, the FCR of human LDL reported here (range = 0.86 to 3.27 pools/day in the first period) are comparable to those obtained in a previous study in cynomolgus monkeys (range = 1.68 to 3.84 pools/day) in which the turnover of native and cyclohexanedione-modified human LDL were studied (19). The slightly lower rates in the present study may be attributable to diet; in the present study animals were fed "average American diets" containing 36% dietary fat (P/S = 0.45, 0.1% cholesterol by weight), while in the previous study (19) the animals were maintained on monkey chow diets, lower in fat and cholesterol.

In assessing the usefulness of sucrose cryopreservation as a means of preserving LDL for in vivo turnover, two major factors must be considered. First, does the cryopreserved LDL reproduce the true FCR for individual animals? And, second, does the cryopreserved LDL provide accurate results with reliable relative differ-

ences between animals or treatment groups? A measure of the validity of the FCR obtained from cryopreserved LDL is provided by the difference between fresh and frozen FCR for each animal (Table 2). In the first study period, the range of this difference was from +3.5% to −12.5% and from +6% to −14.8% in study 2. Nonetheless, in either case these small differences are well within acceptable experimental limits.

Our conclusion that the FCR obtained with cryopreserved LDL accurately reflects relative differences in LDL metabolism between animals is supported by the very close correlation between FCR of fresh and frozen LDL. In these studies, over a wide range of individual FCR, excellent correlations were obtained both comparing LDL-1 (fresh) to LDL-1 (frozen 20 h) (R^2 = 0.994), and LDL-2 (fresh) to LDL-1 (frozen 6 months) (R^2 = 0.991). As well, in each study, the ranking of animals by FCR was virtually the same with fresh or frozen LDL. When the two aberrant animals, 2 and 7, were removed from the analysis, the FCR of fresh LDL-1 in the first and frozen LDL-1 in the second study correlated well (R = 0.87), and although the correlation coefficient was not as high as the within-study correlations, the animal ranking was nonetheless still maintained.

The mechanism by which sucrose prevents LDL aggregation upon freezing, as discussed previously (7), remains unclear. It seems likely that, as is the case with phospholipid liposomes (20, 21), sucrose may form hydrogen bonds with phospholipid head groups and prevent fusion or aggregation by creating a barrier between LDL particles, effectively preventing phospholipid contact.

The results of these in vivo turnover studies add to our previous in vitro findings (7) which demonstrated that sucrose cryopreserved LDL shows no observable alterations in physical structure or cell binding behavior. On the basis of both in vitro and now in vivo results, we suggest that sucrose cryopreservation of human LDL at −70°C can be used effectively to decrease experimental variability and facilitate the design of long-term studies of LDL metabolism, both in vitro and in vivo.

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