In vitro cholesterol synthesis in freshly isolated mononuclear cells of human blood: effect of in vivo administration of clofibrate and/or cholestyramine

Donald J. McNamara, Nicholas O. Davidson, and Sally Fernandez

The Rockefeller University, New York, NY 10021

Abstract The rate of incorporation of [2-^{14}C]acetate into cholesterol has been measured in freshly isolated peripheral blood mononuclear leukocytes from patients on various hypolipidemic drugs that affect whole body cholesterol synthesis. These studies have demonstrated a significant two-fold increase in mononuclear cell cholesterol synthesis rates in patients receiving cholestyramine, a response measurable after 10 days of drug treatment. Mononuclear cell cholesterol synthesis rates were also measured in four groups of patients on the following drug regimens: *1*) no medication, *2*) clofibrate (2 g/day), *3*) cholestyramine (16 g/day) or *4*) both clofibrate and cholestyramine. The results demonstrated that the rate of acetate incorporation into cholesterol was significantly greater in the mononuclear cells from patients receiving either cholestyramine ($P < 0.005$) or clofibrate plus cholestyramine ($P < 0.001$), as compared to controls. Patients receiving clofibrate alone did not differ significantly from controls in their rates of mononuclear cell cholesterol synthesis. Factors other than plasma lipoprotein and lipid levels appeared to be responsible for the elevated sterol synthesis rates observed in all patients receiving cholestyramine. **McNamara, D. J., N. O. Davidson, and S. Fernandez.** In vitro cholesterol synthesis in freshly isolated mononuclear cells of human blood: effect of in vivo administration of clofibrate and/or cholestyramine. *J. Lipid Res.* 1980. **21:** 65–71.

Supplementary key words lymphocytes · monocytes · squalene · methyl sterol · sterol balance

Current methods for quantitatively measuring daily cholesterol synthesis rates in man involve either sterol balance measurements (1) which require a long-term stay in a metabolic ward, or assays of the isotope kinetics of cholesterol (2, 3) or of squalene (4). A modification of the squalene kinetic method requires the measurement of the fractional conversion of mevalonic acid to cholesterol; this has significantly reduced the amount of isotopic material administered and the time for analysis (5). While each of these methodologies has certain advantages, all require a metabolic steady state for appropriate interpretation of the results, and they are time-consuming and expensive; they cannot be readily applied to a large outpatient population nor can they be utilized for repetitive tests in the same patient.

In general, radioisotopic methods have limited applicability since they are not appropriate for determining the effect of diet and/or drug interventions on the rate of whole body cholesterol synthesis in premenopausal women or children because of potential hazards of radioactivity. Thus, a safe, relatively rapid, inexpensive, non-radioisotopic method for measuring whole body cholesterol production is clearly needed. Such a system would facilitate investigations of individual patient responsiveness to drug and/or dietary interventions thought to affect whole body cholesterol synthesis.

Recent studies have demonstrated that isolated human blood mononuclear leukocytes respond to many of the factors known to regulate cholesterol synthesis which have previously been studied in human skin fibroblasts in cell culture (6–11). Young and Rodwell (12) have recently reported that freshly isolated rat leukocytes exhibit physiological control of 3-hydroxy-3-methylglutaryl coenzyme A reductase (mevalonate: NADP oxidoreductase, EC 1.1.1.34) in response to drug and dietary manipulations. Comparison of reductase activities in rat leukocytes and rat liver preparations demonstrated that the leukocyte reductase levels responded in parallel with hepatic reductase to some of the known effectors of whole body cholesterol synthesis: fasting, cholesterol feeding and cholestyramine administration (12).

The present study was designed to test whether changes in cholesterol synthesis rates occurred in freshly isolated blood mononuclear cells of patients receiving medications thought to alter whole body cholesterol synthesis rates. The studies utilized freshly isolated peripheral blood mononuclear cells incubated

in 66% autologous plasma, under conditions in which the cells were neither induced nor transformed, conditions theoretically representative of the in vivo physiological state. The data demonstrate that assay of mononuclear leukocyte cholesterol synthesis rates in vitro qualitatively reflect in vivo conditions of cholesterol homeostasis.

MATERIALS AND METHODS

Materials

[2-^{14}C]sodium acetate (51.4 mCi/mmol) and [1,2-^{3}H]cholesterol (40 Ci/mmol) were purchased from New England Nuclear Corp., Boston, MA. Phosphate buffered saline and RPMI-1640 media were obtained from Grand Island Biological Co., Grand Island, NY. Lymphoprep was supplied by Accurate Chemical and Scientific Corp., Hicksville, NY and siliclad from Clay Adams, Parsippany, NJ.

Isolation of mononuclear leukocytes

Peripheral blood mononuclear leukocytes were isolated according to the method of Böyum (13) as follows: 23 ml of venous blood was mixed with 3 ml of 5 mM Na_2EDTA in 50 mM $KHPO_4$, pH 6.5, and diluted 1:1 with phosphate buffered saline (pH 7.5). The diluted whole blood (4 volumes) was layered over Lymphoprep (3 volumes) and centrifuged at 400 *g* for 30 min at 4°C. The mononuclear cell-rich interface was removed, washed once with phosphate-buffered saline and resuspended in 1.5 ml RPMI-1640 media. All operations were performed at 4°C; the equipment used for all manipulations was either siliconized glass or plastic.

Measurement of cholesterol synthesis rates

Each 25-ml Ehrlenmeyer incubation flask contained (in a final volume of 2.5 ml): 1.0 ml of resuspended mononuclear cells in RPMI-1640 ($1.5–2.5 \times 10^7$ cells), 1.5 ml of autologous plasma obtained at the time of the test, and 20 μCi [2-^{14}C]sodium acetate (0.39 μmol). Each flask was stoppered and incubated at 37°C for 6 hr in a metabolic shaker (100 cycles per min). Incubations were terminated by addition of 5.0 ml of 5N KOH in methanol and 3×10^4 dpm of [1,2-^{3}H]cholesterol was added as an internal recovery standard.

Samples were saponified for 2 hr at 70°C, and the non-saponifiable lipids were extracted three times with 20 ml hexane. The cholesterol, methyl sterols and squalene fractions were isolated by thin-layer chromatography on Silica gel G plates developed in hexane–diethyl ether–glacial acetic acid 70:30:2 (v/v/v); fractions were scraped into scintillation vials and counted in a Packard Tri-Carb scintillation counter. All values were corrected for procedural losses by the recovery value of the [^{3}H]cholesterol standard (average recovery 87%). The results were calculated as [2-^{14}C]acetate incorporation into cholesterol or related sterols (pmol incorporated/hr/10^7 cells).

Analytical methods

Sterol balance measurements were performed as previously described (1) using dietary β-sitosterol as an internal standard to correct for losses of neutral sterols during intestinal transit (14) and chromic oxide to correct for fecal flow variations and stool recovery (15). Sterol balance data represent the difference between dietary cholesterol intake and daily excretion of neutral and acidic steroids in feces; in the metabolic steady state these data approximate the daily rate of whole body cholesterol synthesis and are expressed as negative numbers (intake-output, in mg/day).

Plasma lipid concentrations were determined by the methods of Block, Jarrett, and Levine (16) for cholesterol and Kessler and Lederer for triglycerides (17) using the Auto Analyzer II (Technicon Instruments Corp., Tarrytown, NY).

Differential white blood cell counts were performed manually on Wright stained smears (Ames Hema-Tek I stainer) using oil immersion light microscopy.

Out-patients

Two normolipidemic volunteers and 31 patients from the Center for Prevention of Premature Arteriosclerosis (CPPA) at The Rockefeller University Hospital were recruited for the study. Informed consents were obtained from each patient after appropriate review and approval of the study protocol by The Rockefeller University Institutional Review Board. The CPPA patients were hyperlipidemic males maintained on a low-cholesterol diet (<300 mg/day), of which 35% of the calories are composed of fat with a P/S ratio of 2. All patients in this study had failed to normalize their plasma lipid levels after 6 months of dietary treatment and were subsequently randomized into one of four treatment groups. Each patient had been in the drug/diet phase of the program for over 1 year prior to the initiation of this study. The four study groups consisted of: *1*) control patients; *2*) clofibrate-treated (2 g/day); *3*) cholestyramine-treated (16 g/day); and *4*) patients treated with both cholestyramine and clofibrate. The type of patient in each group is shown in Table 2. All blood samples were taken after a 12- to 14-hr fast, and the mononuclear cells were isolated for assay within 2 hours.

Statistical analysis

Statistical analysis was carried out using a Hewlett-Packard 97 calculator and the Student *t* test program for two unpaired means supplied in the Hewlett-Packard Stat Pac I.

RESULTS

Fractionation of blood cells and assay

Initial studies of the in vitro incorporation of [2-^{14}C]acetate into cholesterol in freshly isolated leukocytes (12) demonstrated that these cells possess a low but measurable incorporation rate. Upon further fractionation of the total white blood cell population by Ficoll-Hypaque density centrifugation (13) into mononuclear and polymorphonuclear leukocytes, the observations of Fogelman et al. (18) that most of the sterol synthesis activity resided in the mononuclear cell fraction were confirmed. In all subsequent studies the mononuclear cells (containing both lymphocytes and monocytes) were isolated by Ficoll-Hypaque density fractionation, and assayed for in vitro cholesterol synthesis activity.

The rate of incorporation of [2-^{14}C]acetate into cholesterol in the mononuclear cells from control patients was linear with time for at least 6 hr (**Fig. 1**); with respect to cell number, there was linearity up to 2.5 × 10^7 cells/incubation (**Fig. 2**). Similar data were obtained using cells from patients receiving cholestyramine. The results depicted in Fig. 1 demonstrate the precursor–product relationship between the methyl sterols and cholesterol. The rate of incorporation into methyl sterols was initially rapid but

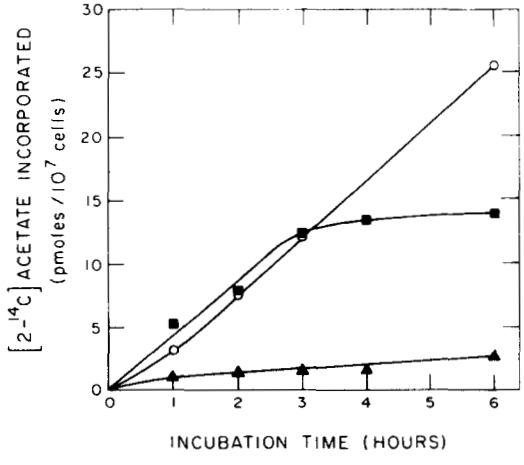

Fig. 1. Time-course of incorporation of [2-^{14}C]acetate into squalene (▲), methyl sterols (■), and cholesterol (○) in freshly isolated human mononuclear leukocytes in vitro. Cells were obtained from a patient in the control group.

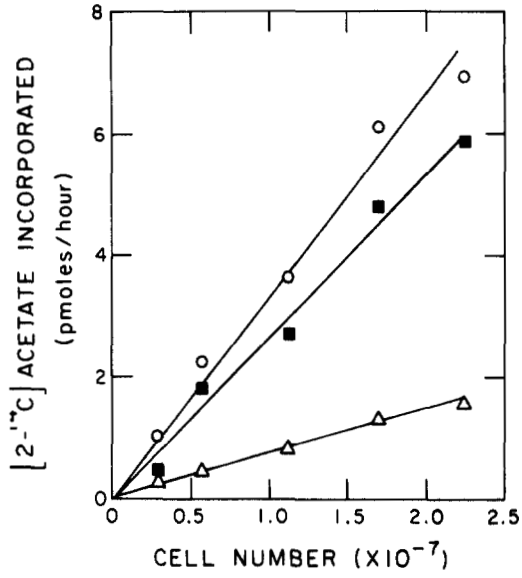

Fig. 2. Effect of cell number on incorporation rate of [2-^{14}C]-acetate into squalene (▲), methyl sterols (■), and cholesterol (○), in freshly isolated human mononuclear leukocytes in vitro. Cells were obtained from a patient in the control group.

a plateau was reached after 4 hours. For total cholesterol radioactivity there was an initial lag followed by a linear increase. On the basis of these kinetic parameters, subsequent assays of the incorporation of [^{14}C]acetate into cholesterol were fixed at 2–2.5 × 10^7 cells incubated for 6 hr at 37°C in 66% autologous plasma.

Initial studies of analytical reproducibility were performed on patients on the metabolic ward of The Rockefeller University Hospital and on hospital staff. In seven tests of intra-assay variability in five patients, the average coefficient of variation for duplicate tests was 9.9% (range 2.9–15.7%). Inter-assay variability was tested in four patients over a 3-week period; the mean coefficient of variation for three to five analyses was 16.0% (range 3.2–25.6%).

Effect of cholestyramine treatment on mononuclear cell cholesterol synthesis

Having characterized the kinetic and statistical properties of the assay system, studies were undertaken to test the hypothesis that human blood mononuclear cells exhibit altered rates of cholesterol synthesis in patients on hypolipidemic drugs known to affect whole body cholesterol synthesis. Cholestyramine-treatment results in a significant increase in daily cholesterol synthesis as measured by either sterol balance methods (19) or by squalene kinetics (4, 5) and, as shown in **Table 1,** also results in a significant increase in the rate of [2-^{14}C]acetate incorporation into cholesterol in freshly isolated mononuclear cells. In this study, daily negative sterol balance was increased

TABLE 1. Effect of cholestyramine treatment on the rate of mononuclear cell cholesterol synthesis: comparison to sterol balance measurements[a]

Treatment	Plasma Lipids (mg/dl)[b]		Sterol Balance[c]	[2-^{14}C]Acetate → Cholesterol
	Cholesterol	Triglycerides		
			mg/day	*pmol/hr/10^7 cells*
Control	317 ± 5 (5)	163 ± 30 (5)	1180 ± 124 (8)	3.94 ± 0.95 (5)
Cholestyramine	271 ± 7 (3)	120 ± 17 (3)	2018 ± 413 (6) $P < 0.001$	7.79 ± 0.94 (3) $P < 0.005$

[a] Studies were performed during a control period and 6 months after initiation of cholestyramine treatment (16 g/day) in a 53-year old male hypercholesterolemic. In both study periods, the patient was fed eucaloric amounts of an orally administered liquid formula containing 15% of total calories as protein, 40% fat (corn oil), and 45% carbohydrate, with the mineral and vitamin supplements previously described (20).
[b] Values represent mean ± standard deviation; number in parentheses indicates number of analyses.
[c] Continuous stool collections were made for 4 and 6 weeks, respectively, in the two periods; numbers in parentheses refer to the number of consecutive 4-day collections analyzed.

1.7-fold by 6 months of treatment with cholestyramine ($P < 0.001$), and the rate of cholesterol synthesis in mononuclear cells increased 2.0-fold ($P < 0.005$).

Fig. 3 presents the results of a second study designed to determine the time course of the cholestyramine-mediated increase in mononuclear cell cholesterol synthesis rates. The data demonstrate that during the initial 10-day time period following initiation of cholestyramine treatment there was no significant increase in mononuclear cell cholesterol synthesis; however, during the subsequent two 10-day periods cholesterol synthesis increased 1.8-fold ($P < 0.005$). Following termination of cholestyramine treatment the rate of cholesterol synthesis in the mononuclear cells rapidly returned to control levels.

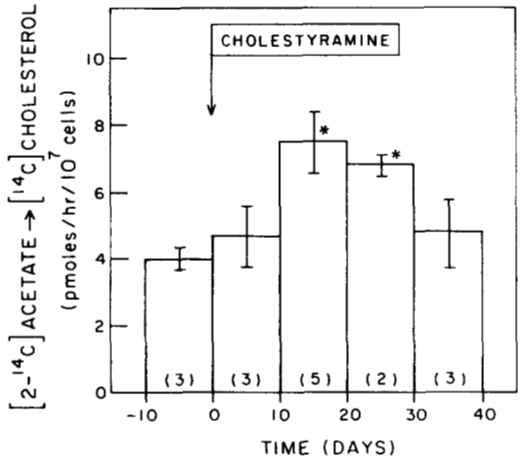

Fig. 3. Time-course of cholestyramine-induced increase in the rate of incorporation of [2-^{14}C]acetate into cholesterol in freshly isolated mononuclear cells from a patient prior to, during, and following cholestyramine treatment (16 g/day). (*: $P < 0.005$; the brackets at the top of each bar represent mean ± S.D. for the number of assays shown in parentheses at the base of each bar). During this study the patient was fed eucalorically amounts of an orally administered liquid formula containing 15% of total calories as protein, 40% fat (cottonseed oil), and 45% carbohydrate, with the mineral and vitamin supplements previously described (20).

Outpatient studies

In order to test further the hypothesis that hypolipidemic drugs affect the rate of cholesterol synthesis in mononuclear cells, an out-patient population was tested two to five times over a 4-month period.

Table 2 presents the results of these studies. The in vitro rate of acetate incorporation into cholesterol in isolated mononuclear cells was significantly elevated in patients receiving either cholestyramine or cholestyramine plus clofibrate. Patients receiving cholestyramine alone exhibited a 1.6-fold increase in cholesterol synthesis activity as compared to controls ($P < 0.005$), and patients receiving both medications had a 1.5-fold increase in synthesis ($P < 0.001$). However, the results in patients receiving clofibrate alone did not differ significantly from those in controls. **Fig. 4** presents the mean incorporation rates of

TABLE 2. Rates of cholesterol synthesis in freshly isolated mononuclear cells from 35 outpatients on three lipid-lowering regimens (compared unblindly to a control group)

Medication Group[a]	*n*	[2-^{14}C]Acetate → Cholesterol	*P*
		pmol/hr/10^7 cells	
I Control	9	4.51 ± 0.63[b]	
II Clofibrate	10	4.57 ± 1.47	NS
III Cholestyramine	10	7.24 ± 2.10	<0.005
IV Clofibrate + Cholestyramine	6	6.77 ± 1.54	<0.001

[a] The medication groups were comprised as follows:

	Hypercholesterolemic	Hypertriglyceridemic	Mixed hyperlipidemic	Normolipidemic
I	5	1	1	2
II	4	1	5	0
III	5	0	5	0
IV	1	3	2	0

[b] Values represent means ± one standard deviation of the means of two–five tests per patient (average = 3.5 tests/patient).

individual patients (average of 3.5 tests per patient): it illustrates the individual variability of responses to the medications and the overlaps among the four groups. Deletion of the two highest values from the cholestyramine-treated group calculations resulted in a mean incorporation rate of 6.33 ± 1.01 pmol/min/10^6 cells that was significantly different from the mean rate in controls ($P < 0.001$).

When the four medication groups were further subdivided into the three major hyperlipidemic phenotypes (hypercholesterolemic, hypertriglyceridemic, and mixed hyperlipidemic) and incorporation rates analyzed in these three subgroups as a function of treatment regimen, no significant differences were observed nor was any demonstrable relationship to plasma lipid levels (**Fig. 5**). While the control population's plasma cholesterol concentrations ranged between 125–320 mg/dl, no significant differences in in vitro cholesterol synthesis rates of the mononuclear cells were observed.

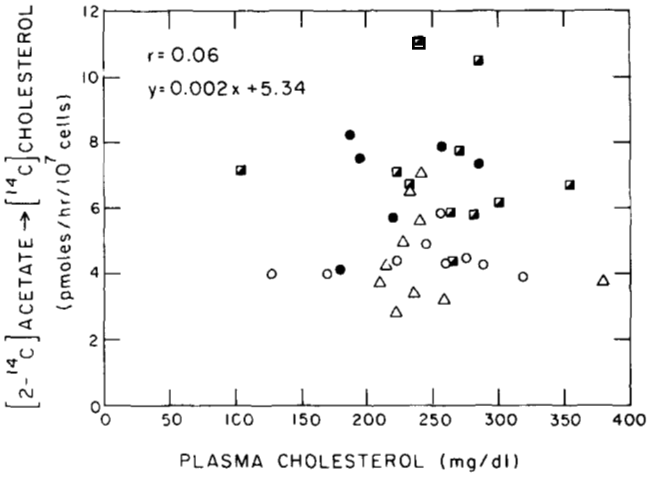

Fig. 5. Relationship between plasma cholesterol concentration and the in vitro rate of incorporation of [2-^{14}C]acetate into cholesterol in freshly isolated mononuclear cells. Each point represents the mean of two to five analyses per patient (average = 3.5) for each individual patient in the four drug-treatment groups; (○) control, (△) clofibrate, (◪) cholestyramine, and (●) clofibrate plus cholestyramine.

DISCUSSION

Previous studies have documented the clinical applicability of utilizing isolated white blood cells in screening for familial hypercholesterolemia in man (6–11). Studies in the rat (12) and rabbit (21) demonstrated that drug- or dietary-induced changes in hepatic sterol synthesis rates are reflected by parallel changes in freshly isolated white cell cholesterol synthesis measured in vitro.

The present study was designed to test the hypothesis that the peripheral blood mononuclear cells' rate of cholesterol synthesis in vitro might qualitatively reflect the in vivo rate of whole body cholesterol synthesis. In order to mimic the in vivo condition in this in vitro assay, the mononuclear cells were isolated rapidly at 4°C and incubated in 66% autologous plasma for a brief time-period. No effectors of cell growth or modulators of cholesterol synthesis rates were added, and cholesterol synthesis in the cells was not induced by prior incubation in lipid-free serum (6, 11). While it is known that biochemical changes can occur during the isolation and incubation periods, the present system appears to approximate the in vivo state reasonably well.

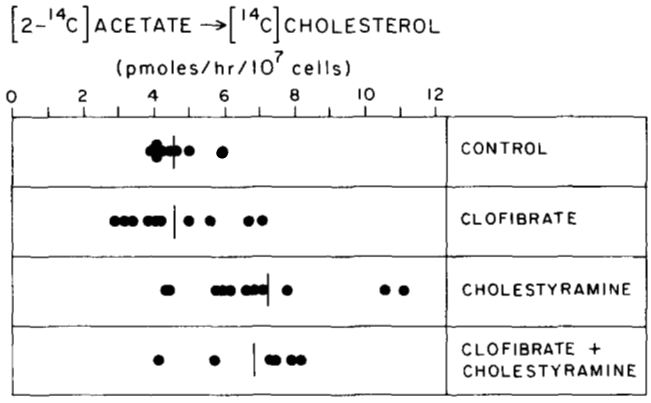

Fig. 4. Rates of incorporation of [2-^{14}C]acetate into cholesterol in freshly isolated mononuclear leukocytes in vitro in individual patients in four drug-treatment groups. Each solid circle represents the mean of two to five analyses per patient (average = 3.5 duplicate analyses per patient), and the average value for each of the four groups is shown as a horizontal bar.

The results of the present study demonstrate that in man the mononuclear cells do indeed qualitatively reflect changes in rates of whole body cholesterol synthesis known to be caused by specific hypolipidemic drugs. Cholestyramine dosage leads to significant increases in daily cholesterol synthesis as measured either by sterol balance (19) or by squalene kinetics (4, 5), and we show here that the freshly isolated mononuclear leukocytes of cholestyramine-treated patients express elevated rates of acetate incorporation into cholesterol. Administration of clofibrate alone resulted in a rate of synthesis indistinguishable from that of controls; however, combined treatment with cholestyramine and clofibrate resulted in an elevated rate of cholesterol synthesis in mononuclear cells. Clofibrate exerted little effect on mononuclear cell cholesterol synthesis rates, a finding that stands in contrast to a previous report from this laboratory (22) which suggested an inhibition of whole body sterol synthesis by clofibrate.

Control factor

The question of what physiological factors account for the differences in cholesterol synthesis observed in the mononuclear cells from patients receiving cholestyramine has not been addressed. At least one possibility can be excluded: plasma low-density lipoprotein levels are apparently not involved in the observed changes in the cholesterol synthesis activity of freshly isolated mononuclear leukocytes (11, 23–25). Lymphocytes from patients with homozygous familial hypercholesterolemia exhibit the same basal rate of acetate incorporation into cholesterol as cells from normolipidemic patients, and the expected phenotypic expression of non-repressible rates of cholesterol synthesis was observed only after the cells were induced in vitro in lipoprotein-free media (11). Similarly, lymphocytes from patients with abetalipoproteinemia express cholesterol synthesis rates that are the same (23) or two-fold greater (11) than cells from controls; however, these cells can be induced an additional six-fold upon incubation in lipoprotein-free-media (11).

The only documented situations in which elevated rates of sterol synthesis in freshly isolated mononuclear cells have been reported are in certain types of acute leukemia (24, 25) and in patients with infectious mononucleosis (25); the causes of these elevated rates are unknown. Neither in the present study nor in studies of familial hypercholesterolemia, abetalipoproteinemia, infectious mononucleosis or acute leukemia is there any observed relationship between plasma cholesterol concentrations and the rate of cholesterol synthesis in the freshly isolated mononuclear cells.

Taken together, the available evidence suggests that factors other than the presence of receptors for LDL or LDL itself are singularly responsible for the regulation of cholesterol synthesis in isolated mononuclear cells. It is possible that the rate of cholesterol flux in and out of circulating lymphocytes may regulate the synthetic capacity of these cells, as already demonstrated in the in vitro control of cholesterol synthesis rates of leukocytes by Fogelman et al. (26). We are currently testing this possibility.

Lymphocytes versus monocytes

Studies by Fogelman et al. (18, 27) have demonstrated that monocytes incorporate approximately five times more [2-^{14}C]acetate into sterols than lymphocytes under basal conditions; and that upon incubation in lipid-depleted medium, monocytes exhibit a 20-fold greater incorporation rate of acetate into sterols than lymphocytes. Our own studies on fractionated mononuclear cells have resulted in similar findings;[1] they suggest that, because monocytes appear to be more responsive to inductive influences than lymphocytes, these cells may account for the observed increase in sterol synthesis in cells from patients on cholestyramine. However, routine hematological analyses of the white blood cell distribution in our four groups of patients did not reveal any significant change in monocyte–lymphocyte concentrations or ratios, suggesting that the increased rates of sterol synthesis we observed were not the result of an increased concentration of any particular cell type. A definitive answer to the question of whether a single cell type accounts for the observed increase in sterol synthesis awaits further investigation.

Potential applications

The present study suggests that, for the effectors tested, the rate of cholesterol synthesis in mononuclear leukocytes reflects the expected alterations in whole body cholesterol synthesis. If this finding is substantiated in subsequent tests of other manipulations, it is reasonable to expect that such a system may potentially facilitate the testing of individual patients for qualitative changes in whole body cholesterol synthesis in response to various dietary, pharmacologic and physiological challenges, each patient serving as his own control. This approach has the advantages of economy, patient-safety, and could be applied in large numbers of patients, or for repeated tests of the any one patient who is being subjected to a series of challenges, where in the non-steady state other methods of measuring daily cholesterol synthesis rates are known to be invalid.

We gratefully acknowledge the excellent technical assistance of Serafin Prado and Florence Chu. This work was supported in part by a Grant-in-Aid from The New York Heart Association to D.J.M.; by USPHS Grants HL 06222 from the National Heart, Lung, and Blood Institute; by FR-00102 from the General Clinical Research Centers Branch, Division of Research Resources; by a Grant-in-Aid from the National Dairy Council; grants from the Herman Goldman Foundation and Weight Watchers Foundation, Inc.; and by a Career Scientist Award to D.J.M. from the Irma T. Hirschl Charitable Trust. The authors express their gratitude to Dr. E. H. Ahrens, Jr., for his enthusiastic support and encouragement of these studies.

Manuscript received 30 May 1979; accepted 31 August 1979.

[1] McNamara, D. J. Unpublished observations.

REFERENCES

1. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurements of cholesterol turnover, synthesis and absorption in man, carried out by isotope kinetics and sterol balance methods. *J. Lipid Res.* **10:** 91–107.
2. Goodman, D. S., R. P. Noble, and R. B. Dell. 1973. Three-pool model of the long-term turnover of plasma cholesterol in man. *J. Lipid Res.* **14:** 178–188.
3. Samuel, P., and S. Lieberman. 1973. Improved estimation of body masses and turnover of cholesterol by computerized input–output analysis. *J. Lipid Res.* **14:** 189–196.
4. Liu, G. C., E. H. Ahrens, Jr., P. H. Schreibman, P. Samuel, D. J. McNamara, and J. R. Crouse. 1975. Measurement of cholesterol synthesis in man by isotope kinetics of squalene. *Proc. Nat. Acad. Sci. USA,* **72:** 4612–4616.
5. McNamara, D. J., E. H. Ahrens, Jr., P. Samuel, and J. R. Crouse. 1977. Measurement of daily cholesterol synthesis rates in man by assay of the fractional conversion of mevalonic acid to cholesterol. *Proc. Nat. Acad. Sci. USA.* **74:** 3043–3046.
6. Fogelman, A. M., J. Edmond, J. Seager, and G. Popjak. 1975. Abnormal induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase in leukocytes from subjects with heterozygous familial hypercholesterolemia. *J. Biol. Chem.* **250:** 2045–2055.
7. Kayden, H. J., L. Hatam, and N. G. Beratis. 1976. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and the esterification of cholesterol in human long term lymphoid cell lines. *Biochemistry.* **15:** 521–528.
8. Higgins, M. J. P., and D. J. Galton. 1977. The regulation of sterol biosynthesis in leukocytes of subjects with familial hypercholesterolemia. *Eur. J. Clin. Invest.* **7:** 309–313.
9. Higgins, M. J. P., D. S. Lecamwasam, and D. J. Galton. 1975. A new type of familial hypercholesterolemia. *Lancet.* **II:** 737–740.
10. Ho, Y. K., M. S. Brown, H. J. Kayden, and J. L. Goldstein. 1976. Binding, internalization, and hydrolysis of low density lipoprotein in long-term lymphoid cell lines from a normal subject and a patient with homozygous familial hypercholesterolemia. *J. Exp. Med.* **144:** 444–455.
11. Ho, Y. K., J. R. Faust, D. W. Bilheimer, M. J. Brown, and J. L. Goldstein. 1977. Regulation of cholesterol synthesis by low density lipoprotein in isolated human lymphocytes. *J. Exp. Med.* **145:** 1531–1549.
12. Young, N. L., and V. W. Rodwell. 1977. Regulation of hydroxymethylglutaryl-CoA reductase in rat leukocytes. *J. Lipid Res.* **18:** 572–581.
13. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **97** (Suppl. 21): 77–89.
14. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1968. Dietary β-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **9:** 374–387.
15. Davignon, J., W. J. Simmonds, and E. H. Ahrens, Jr. 1968. Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function. *J. Clin. Invest.* **47:** 127–138.
16. Block, W. D., K. J. Jarrett, and J. B. Levine. 1965. Use of a single color reagent to improve the automated determination of serum total cholesterol. *In* Automation in Analytical Chemistry. J. H. Allen, editor. Mediad Inc., New York. 345–347.
17. Kessler, G., and H. Lederer. 1965. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. J. H. Allen, editor. Mediad Inc., New York. 341–344.
18. Fogelman, A. M., J. Seager, P. A. Edwards, M. Hokum, and G. Popjak, 1977. Cholesterol biosynthesis in human lymphocytes, monocytes, and granulocytes. *Biochem. Biophys. Res. Commun.* **76:** 167–173.
19. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78:** 94–121.
20. Ahrens, E. H., Jr. 1970. The use of liquid formula diets in metabolic studies: 15 years' experience. *In* Advances in Metabolic Disorders, Vol. 4. R. Levine and R. Luft, editors. Academic Press, N.Y., 297–332.
21. Kar, S., and A. J. Day. 1978. Composition and metabolism of lipid in macrophages from normal and cholesterol-fed rabbits. *Exp. Molec. Path.* **28:** 65–75.
22. Grundy, S. M., E. H. Ahrens, Jr., G. Salen, P. H. Schreibman, and P. J. Nestel. 1972. Mechanisms of action of clofibrate on cholesterol metabolism in patients with hyperlipidemia. *J. Lipid Res.* **13:** 531–551.
23. Reichl, D., N. B. Myant, and J. K. Lloyd. 1978. Surface binding and catabolism of low-density lipoprotein by circulating lymphocytes from patients with abetalipoproteinemia, with observations on sterol synthesis in lymphocytes from one patient. *Biochim. Biophys. Acta.* **530:** 124–131.
24. Heiniger, H. J., H. W. Chen, O. L. Applegate, Jr., L. P. Schacter, B. Z. Schacter, and P. N. Anderson. 1976. Elevated synthesis of cholesterol in human leukemic cells. *J. Mol. Med.* **1:** 109–116.
25. Ho, Y. K., R. G. Smith, M. S. Brown, and J. L. Goldstein. 1978. Low-density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. *Blood.* **52:** 1099–1114.
26. Fogelman, A. M., J. Seager, P. A. Edwards, and G. Popjak. 1977. Mechanism of induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase in human leukocytes. *J. Biol. Chem.* **252:** 644–651.
27. Fogelman, A. M., J. Seager, M. Hokom, and P. A. Edwards. 1979. Separation of and cholesterol synthesis by human lymphocytes and monocytes. *J. Lipid Res.* **20:** 379–388.