Ultracentrifugal Analysis of Lipoproteins During Adaptation to High Carbohydrate Diet in Type II Hyperlipoproteinemia and Normals (36205)

DAVID H. BLANKENHORN, FRANK T. LINDGREN, JULIUS JENSEN, AND LIN C. JENSEN

Cardiology Section, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California 90033, and Donner Laboratory and Donner Pavilion, University of California, Berkeley, California 94720

Acute changes in plasma lipids occur during high carbohydrate feeding in man. The rate of change of lipid level and the magnitude of this change vary according to dietary test conditions (1, 2). The effect of genotype and degree of obesity (3) as well as the nature of the preceding diet also influence these changes. In a previous report (4), we have described a 5-day regimen with test conditions producing an immediate fall in total cholesterol level, increase in triglyceride level, and a change in lipoprotein electrophoretic pattern. In normals and in patients with Type II hyperlipoproteinemia, the decrease in total cholesterol averages 5% of original level each day and the increase in triglyceride level is accompanied by an increase in pre-beta (alpha$_2$) lipoprotein concentration. We wish to report here ultracentrifugal lipoprotein patterns compared on the second and fifth day of the test regimen.

Subjects and Methods. Age, sex, and clinical diagnosis of study subjects is given in Table I. All subjects, except one, were drawn from the large hypercholesterolemic family first described by Alvord (5). The adult females all menstruated regularly, did not take oral contraceptives, and were in the midportion of a menstrual cycle during the period of this study. The 15-year old female menstruated; the two 11-year old females did not.

All studies were performed in the University of Southern California Clinical Research Center. No subjects took any medication known to alter serum lipids. The study protocol has

TABLE I. Normal Subjects and Type II Patients Studied by Ultracentrifugal Analysis.

Patients	Age	Sex	Position in Pedigree[a]	Remarks	Total Cholesterol mg/100 ml[b]	Triglyceride mg/100 ml[b]
Normal Subjects:						
J.G.	11	Fe	D-IV-40	Pair 1, daughter	131	79
D.G.	35	Fe	D-III-12	Pair 1, mother	149	85
H.W.	57	Fe	G-II-3		196	238
Type II Patients:						
M.W.	11	Fe	B-IV-22	Pair 2, daughter	380	220
E.W.	32	Fe	B-III-12	Pair 2,mother	390	158
C.E.	15	Fe	H-III-17	Pair 3, daughter	297	150
S.E.	40	Fe	H-II-7	Pair 3, mother	339	250
M.L.	49	M	C-III-8		381	208
S.R.	43	M	E-III-1		462	318
O.J.	43	M	—		407	165

[a] Position in the pedigree of Alvord's family: For example, D-IV-40 is the 40th member of the 4th generation in Family Branch D.

[b] Second Day Value (Tuesday).

TABLE II. Tuesday and Friday Lipoprotein Concentrations.

	HDL				LDL and VLDL							
	$F_{1.20}$ (3.5–9)		$F_{1.20}$ (0–3.5)		S''_f 0–12		S''_f 12–20		S''_f 20–100		S''_f 100–400	
	Tues.	Fri.	Tues.	Fri.	Tues.	Fri.	Tues.	Fri.	Tues.	Fri.	Tues.	Fri.
Normal Subjects:												
J.G.	162	62	173	183	256	142	14	1	12	48	0	28
D.G.	168	120	194	231	227	188	10	30	4	74	3	12
H.W.	18	17	126	189	369	189	59	40	154	335	115	293
Type II Patients:												
M.W.	4	3	186	131	848	720	74	55	125	79	35	78
E.W.	72	0	176	122	878	638	113	75	129	79	17	18
C.E.	51	8	171	151	570	435	9	26	86	114	49	143
S.E.	14	8	151	161	768	573	87	98	162	240	110	205
M.L.	2	0	146	103	837	709	58	64	90	97	34	82
S.R.	36	33	274	266	1169	767	99	143	167	249	50	107
O.J.	0	1	200	106	927	706	90	67	105	201	22	171

been described in detail previously (4). In brief, each subject's entire food intake consisted of a daily 100 g oral glucose load and an additional ration of liquid formula diet, adjusted to produce a caloric intake of 42 cal/kg/day. The liquid formula contained 338 g of carbohydrate, 55 g of protein, and 5 g of residual milk fat per liter and provided a total of 1620 cal/liter.

On the second day (Tuesday) and the fifth day (Friday) of formula feeding, 40 ml of fasting blood was drawn and allowed to clot at room temperature. Serum was separated, preserved with Merthiolate 1/5,000, packed in crushed ice, and shipped by air to Berkeley, California. Ultracentrifugal analysis was performed by the method of Ewing *et al.* (6) with schlieren presentation by cathode ray tube plots (7). This latter technique allows for continuous logarithmic low-density lipoprotein spectra plots as well as difference plots for both individuals and populations. Also, to de-

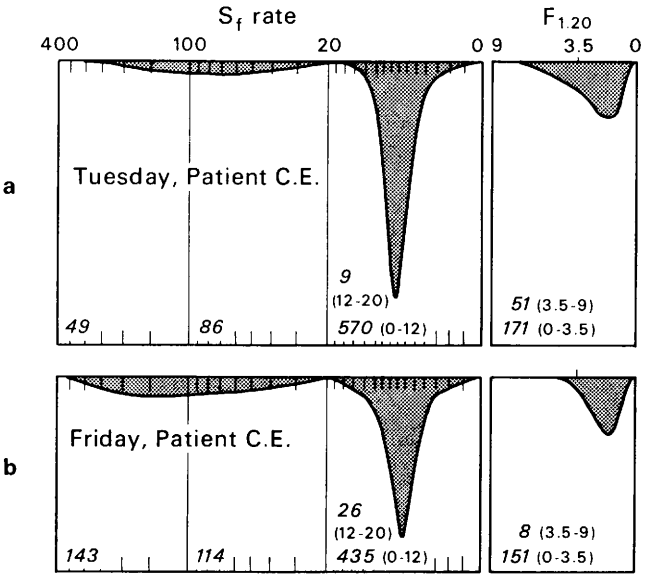

FIG. 1. Lipoprotein patterns on Tuesday and Friday of Type II patient C.E., a 15-year-old girl. Unless otherwise indicated, low-density lipoproteins are plotted on a logarithmic scale and high-density lipoproteins are plotted on a linear scale. All concentrations given are mg/100 ml.

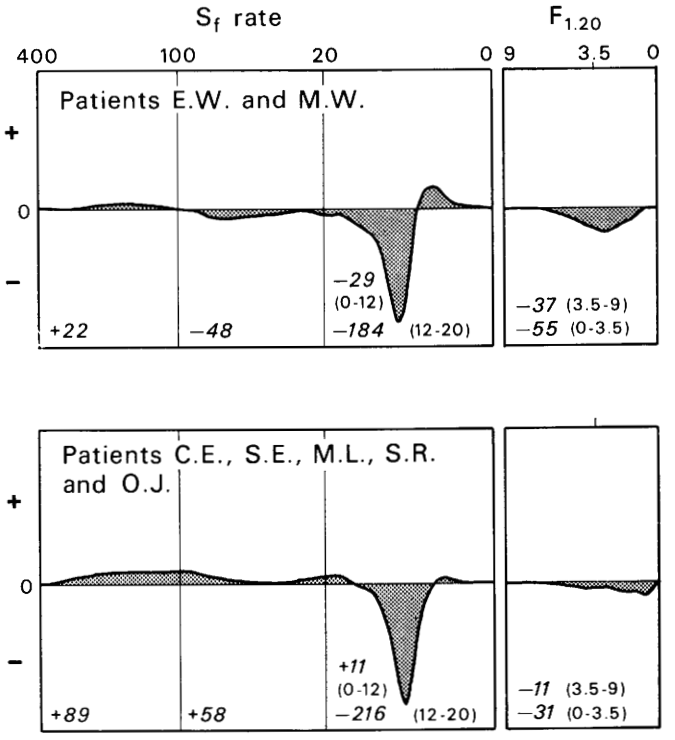

FIG. 2. (Upper) A Tuesday–Friday difference plot of low- and high-density lipoprotein classes for subjects M.W. and E.W. To produce this figure, Tuesday and Friday concentrations of the two subjects were averaged and the average for Friday was subtracted from the average for Tuesday. Area above the baseline indicates increased lipoprotein concentration on Friday.

(Lower) Similar data giving a Tuesday–Friday difference plot of low- and high-density lipoprotein classes for all Type II patients except M.W. and E.W. Note the different response of the S^0_f 20–100 lipoproteins.

fine changes in the fast-moving S^0_f 100–400 class lipoproteins more accurately because they are of special interest here, all individual low-density lipoprotein data have been corrected for the acceleration phase of each analytic run (8).

Results. Table II presents lipoprotein data for $F_{1.20}$ (0–3.5), $F_{1.20}$ (3.5–9), S^0_f 0–12, S^0_f 12–20, S^0_f 20–100, and S^0_f 100–400. Table II indicates that all subjects, both normal and Type II patients, showed a consistent change in S^0_f class 0–12 and S^0_f class 100–400. The decrease in S^0_f 0–12 was significant, $p < .01$; the increase in S^0_f 100–400 was also significant, $p < .01$. Less consistent patterns of change were seen in $F_{1.20}$ (0–3.5) which increased in normal subjects, but generally decreased in Type II patients and $F_{1.20}$ (3.5–9) class lipoprotein which decreased in all subjects with initial levels above 50 mg/100 ml. S^0_f 12–20 class lipoproteins showed no consistent change; S^0_f 20–100 class lipoproteins showed a consistent increase in all subjects except one mother–daughter pair described more fully below. Figure 1 illustrates second day (Tuesday) and fifth day (Friday) patterns of patient C.E. whose changes were typical of all Type II patients excluding the exceptional mother–daughter pair.

Mother–daughter pair #2, who were otherwise indistinguishable from other Type II patients, exhibited a unique behavior of S^0_f 20–100 class lipoproteins. In all other subjects, both normal and Type II, S^0_f 20–100 class lipoproteins increased. In this mother–daughter pair, S^0_f 20–100 decreased. Figure 2 presents Tuesday–Friday difference plots comparing mother–daughter pair #2 (E.W. and M.W.) with all other Type II patients. If patients E.W. and M.W. are excluded, the in-

TABLE III. Characteristics of the Major S^0_f 0–12 Components.

	S_f rate(svedbergs)		Hydrated density (g/ml, 26°)		Molecular weight (10^6 daltons)	
	Tues.	Fri.	Tues.	Fri.	Tues.	Fri.
Normal Subjects:						
J.G.	7.99	7.01	1.0256	1.0276	2.53	2.27
D.G.	8.22	7.72	1.0246	1.0269	2.54	2.54
H.W.	4.81	2.12	1.0349	1.0476	1.83	1.35
Type II Patients:						
M.W.	6.12	5.95	1.0311	1.0318	2.17	2.15
E.W.	5.99	5.48	1.0320	1.0330	2.20	2.01
C.E.	6.89	6.10	1.0292	1.0313	2.37	2.18
S.E.	5.66	4.33	1.0304	1.0372	1.86	1.79
M.L.	5.94	5.84	1.0316	1.0318	2.09	2.13
S.R.	6.00	6.22	1.0305	1.0293	2.05	2.04
O.J.	6.55	6.03	1.0291	1.0303	2.19	2.04

crease in lipoprotein class S^0_f 20–100 is significant, $p < .01$.

Table III presents characteristics of the major S^0_f 0–12 component; *e.g.* S^0_f rate, hydrated density, and molecular weight. Several subjects showed striking changes in these variables. All significant changes involved lower S^0_f rates, higher hydrated densities, and generally lower molecular weights in the Friday samples. Further, with the exception of patient E.W., such changes were found in subjects in whom marked elevations of S^0_f 20–100 and/or S^0_f 100–400 class lipoprotein occurred. These data are consistent with the observed inverse relation between S^0_f 20–400 concentration and S^0_f rate of the major beta lipoprotein component (6).

Discussion. Effects of high carbohydrate diet on plasma lipids and lipoproteins can begin a few hours after high carbohydrate feeding (1) and will continue for months if the diet is continued (9). Although long term effects of high carbohydrate diet have been extensively studied (3, 10–12) short term changes have received less attention. Under the conditions of this experiment, consistent and significant short term changes in ultracentrifugal lipoprotein patterns have been found. These are a fall in concentration of S^0_f 0–12 class lipoproteins and an increase in S^0_f 100–400 class lipoproteins. The change in concentration of S^0_f 0–12 class lipoproteins is accompanied by a shift toward higher hydrated density and lower molecular weight in this class.

The S^0_f 100–400 class lipoprotein change produced in this experiment in Type II patients is relevant to the occasional hypertriglyceridemia encountered in Type II family studies (13). Figure 3 illustrates the similarity of lipoprotein patterns we have produced with high carbohydrate diet and a pattern previously reported by Fredrickson in a hypertriglyceridemic Type II patient. When hypertriglyceridemia is encountered in an apparently exceptional individual of a Type II family, the carbohydrate content of this individual's diet should be given consideration.

The S^0_f 20–100 class lipoprotein changes observed in this experiment have bearing on the general problem of the inheritance of hyperlipoproteinemia and lipoprotein response to change in diet. This experiment has demonstrated that significant differences in transport of acutely induced endogenous triglyceride can occur in two branches of a single Type II family. During a period of acutely rising triglyceride level mother–daughter pair #2 exhibited a change in S^0_f 20–100 class lipoproteins different than that of matched mother–daughter pairs from the same family. It is the present consensus that Type II hyperlipoproteinemia is transmitted by a single autosomal dominant gene, although this view has recently been questioned (14). If Type II is considered the result of a single genetic defect, this aberrant response suggests an additional superimposed genetic difference and the possible need for additional subclassifica-

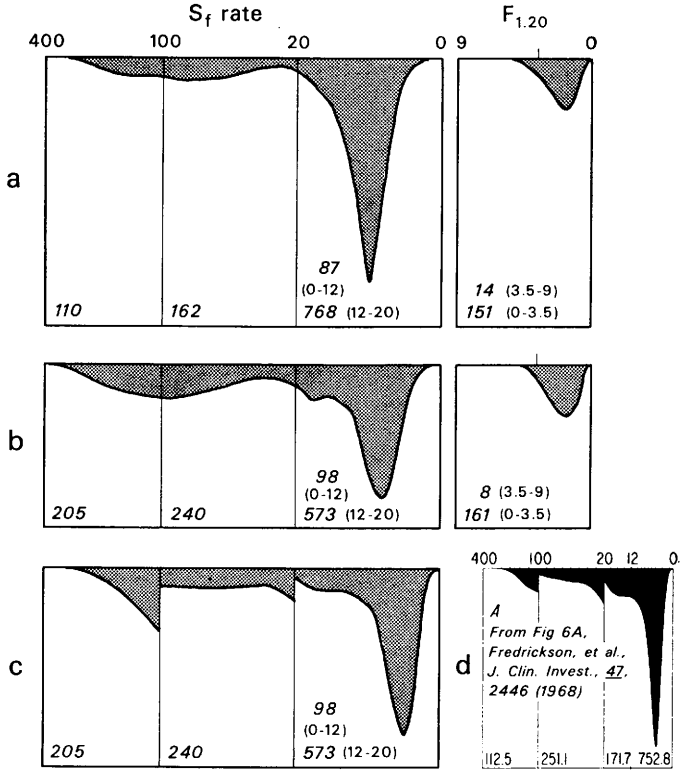

FIG. 3. Lipoprotein pattern of patient S.E. on Tuesday (a) and on Friday (b). Friday's pattern is also plotted in the more traditional linear fashion (c) to illustrate its similarity to a hypertriglyceridemic Type II patient previously reported by Fredrickson *et al.* (13).

tion of Type II is introduced. If Type II is considered the result of polygenic inheritance, minor variations in common phenotypic patterns and responses are to be expected and this finding does not suggest the need for additional subclassification.

Summary. Changes in ultracentrifugal lipoprotein patterns on the second and fifth day of high carbohydrate feeding in normals and patients with Type II hyperlipoproteinemia are reported. A significant increase in S^0_f 100–400 class lipoproteins and decrease in S^0_f 0–12 class lipoproteins occurred. S^0_f 20–100 class lipoproteins showed a significant increase in all subjects except one mother–daughter pair with Type II hyperlipoproteinemia. Less consistent patterns of change were seen in $F_{1.20}$ (0–3.5) and $F_{1.20}$ (3.5–9) class lipoproteins. S^0_f 12–20 class lipoproteins showed no consistent change.

Supported in part by U.S. Public Health Service Grant HE 07197, U.S. Public Health Service Grant HE 1882-C15, and U.S. Public Health Service General Clinical Research Center Grant RR 43.

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Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.