notes on methodology

Minimal within-day variation of high density lipoprotein cholesterol and apolipoprotein A-I levels in normal subjects

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Summary Blood was drawn from twenty normal hospital employees after a 12–14 hr fast (9:30 AM), and at 4 hr (1:30 PM) and 7 hr (4:30 PM) thereafter while test subjects consumed their habitual diet. Absorptive chylomicronemia was reflected in average triglyceride increases of 30% and 53% at 4 hr and 7 hr, respectively. No significant changes in total (<1%) and high density lipoprotein (<0.1%) cholesterol concentrations were observed. Moreover, levels of the major high density lipoprotein apolipoprotein, apoA-I, varied by less than 3%. Blood samples, randomly obtained during standard working hours, appear adequate for quantitation of high density lipoprotein cholesterol and apolipoprotein A-I levels.—**Henderson, L. O., A. L. Saritelli, E. LaGarde, P. N. Herbert, and R. S. Shulman.** Minimal within-day variation of high density lipoprotein cholesterol and apolipoprotein A-I levels in normal subjects. *J. Lipid Res.* 1980. **21:** 953–955.

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Chylomicronemia after a fatty meal raises the serum triglyceride concentration (1) and increases the total serum and high density lipoprotein (HDL) phospholipid levels (2). Serum cholesterol levels, in contrast, vary little during the course of the day (3–6) and while inter-individual differences in variability are known to exist (7–10) these have not been explained by known physiological variables, meals, or time of day.

Less is known of the effects of active fat absorption on serum levels of HDL cholesterol and the major HDL protein, apolipoprotein A-I (apoA-I). Havel, Kane, and Kashyap (2) noted small reciprocal changes in the cholesterol transported in serum HDL_2 (δ 1.063–1.125 g/ml) and HDL_3 (δ 1.125–1.21 g/ml) after a single meal containing 100 g fat. ApoA-I has been identified in association with human (11) and rat (12, 13) lipoproteins of intestinal origin. However, the contribution of this apoA-I to the total serum pool and the degree of post-absorptive variation have not been defined. The studies described here were undertaken to define the variability of both HDL cholesterol and apoA-I levels in normal subjects tested during standard working hours. This information was required to determine if non-fasting serum samples were suitable for population studies of HDL cholesterol and apoA-I concentrations.

METHODS

Lipid, lipoprotein, and apolipoprotein measurements. Cholesterol, HDL cholesterol, and triglyceride were quantitated by enzymatic methods on a Gilford 3500 Computer Directed Analyzer (Gilford Instrument Laboratories, Oberlin, OH) as previously described (14). A modification of the Lipid-Research Clinic protocol (14) employing 0.5 ml of serum was used in the heparin-$MnCl_2$ precipitation procedure for the HDL cholesterol determination. Plasma samples, generously supplied by the Center for Disease Control Lipid Standardization Laboratory, were employed as primary cholesterol standards and used to standardize a large secondary standard plasma pool. Cholesterol and HDL cholesterol analyses over a 3-month period had coefficients of variation of 2.0% ($\bar{X}$ = 160 mg/dl; N = 107 runs) and 4.2% ($\bar{X}$ = 52 mg/dl; N = 16 runs), respectively. Triglyceride analysis of a standardized plasma pool had a coefficient of variation of 3.7% ($\bar{X}$ = 142 mg/dl; 7 runs). ApoA-I levels were measured by radioimmunoassay (14).

Serum samples. Serum samples from 16 male and 5 female hospital employees, aged 25–63, were obtained at three time periods: *1*) 9:30 AM, after a 14-hr fast, *2*) 1:30 PM, and *3*) 4:30 PM. Volunteers were instructed to eat an habitual breakfast and lunch after initial sampling. Informed consent was obtained from all study subjects and all denied taking medication known to affect lipid levels. This study conforms with a protocol approved by The Miriam Hospital Human Experimentation Committee.

Statistical methods. The statistical significance of differences in cholesterol, triglyceride, HDL-cholesterol, and apolipoprotein A-I levels was analyzed by the paired *t*-test (15).

Abbreviations: HDL, high density lipoproteins of $1.063 < \delta < 1.21$ g/ml; apoA-I, apolipoprotein A-I.

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RESULTS

Serum cholesterol. Comparison of mean serum cholesterol levels found in the twenty volunteers at 1:30 PM and 4:30 PM with those found while fasting demonstrated no significant differences (**Table 1**). Fasting values were lowest in eight individuals while seven others had lowest concentrations at 4:30 PM. The mean within-day variation was 4% while the maximum variation was 8% (subject 1).

Serum triglycerides. The mean triglyceride level, as expected, was lowest at 9:30 AM and significantly higher 4 hr and 7 hr later. Two subjects (numbers 19 and 20) demonstrated their highest triglyceride levels while fasting. The relative change in triglyceride levels was quite variable but no dietary restrictions were imposed on study subjects and estimates of inter-individual variability cannot be made.

High density lipoprotein cholesterol. No clear diurnal trend in HDL cholesterol levels was observed. Eight subjects had highest levels while fasting whereas ten others had higher levels at later time points and two showed no change at all (Table 1). The mean maximum variation was 3 mg/dl. The greatest variation, in general, occurred in those subjects exhibiting larger fluctuations in triglyceride levels (subjects 7, 14, and 17). However, greater than average changes were also observed in subjects with small variation in serum triglycerides (e.g., subjects 2, 12, 16, and 19). Similarly, no simple correlation with changes in serum cholesterol levels was observed.

Apolipoprotein A-I. Apolipoprotein A-I levels were quantified in half of the study subjects as an independent measure of HDL mass. The mean levels at fasting and non-fasting time points did not differ significantly (Table 1), although slightly higher levels were found at 1:30 PM and 4:30 PM. ApoA-I and HDL cholesterol levels were highly correlated ($r = 0.63$; $P < 0.01$), but no statistically significant correlations between changes in apoA-I and within-day changes in HDL cholesterol ($r = 0.10$) and serum triglycerides ($r = 0.14$) were found.

DISCUSSION

Chylomicronemia might affect HDL levels in a number of ways. Apolipoprotein A-I is the major protein of both serum HDL and chylomicrons (11) and Glickman et al (16) reported that a single fatty

TABLE 1. Within-day variation of cholesterol, triglyceride, HDL-cholesterol, and apoA-I concentrations

Subject (Age, Sex)	Cholesterol			Triglyceride			HDL-Cholesterol			Apolipoprotein A-I		
	9:30 AM	1:30 PM	4:30 PM	9:30 AM	1:30 PM	4:30 PM	9:30 AM	1:30 PM	4:30 PM	9:30 AM	1:30 PM	4:30 PM
	(mg/dl serum)											
1 (25, F)	172	170	159	39	46	59	72	74	71	138	142	143
2 (37, M)	196	198	194	106	77	113	43	47	47	106	105	108
3 (28, F)	165	163	157	79	145	142	47	46	43	119	117	126
4 (36, M)	201	201	190	83	89	162	38	40	38	113	113	114
5 (28, M)	216	214	213	83	75	131	32	33	32	97	101	100
6 (25, F)	161	165	162	38	73	38	42	42	43	126	126	123
7 (63, M)	246	236	249	102	212	186	40	35	37	99	94	99
8 (33, M)	171	171	180	67	104	37	81	77	80	127	138	120
9 (30, M)	192	194	192	112	139	149	34	33	33	106	122	115
10 (37, M)	165	165	166	104	137	142	48	48	48	141	144	138
11 (28, M)	147	153	152	42	58	99	46	47	48			
12 (26, M)	171	175	180	66	59	99	41	44	45			
13 (31, M)	157	161	152	58	137	106	37	36	35			
14 (27, M)	140	135	143	87	171	210	47	42	42			
15 (28, M)	170	170	168	55	69	113	47	51	48			
16 (25, F)	165	176	171	56	76	76	46	50	51			
17 (34, M)	225	211	215	91	248	234	54	47	47			
18 (30, F)	241	241	238	134	201	180	55	55	55			
19 (33, M)	193	178	187	85	57	70	48	44	44			
20 (25, M)	170	173	175	69	54	44	47	47	48			
Mean	183	182	182	78	111	119	47	47	47	117	120	119
S.D.	29.8	27.3	28.6	25.9	59.5	56.8	11.9	11.5	11.8	15.5	17.3	14.7
Δ[a]		−0.7	−1.1		33.4	41.5		−0.2	−0.3		2.8	1.5
S.E.M.		1.4	1.4		10.7	10.0		0.7	0.7		2.0	1.5
Paired *t*		0.5	0.8		3.1	4.2		0.2	0.4		1.4	1.0
P (Paired *t*)		0.3	0.2		<0.001	<0.001		0.4	0.3		0.1	0.2

[a] Δ, Corresponds to changes relative to fasting values at 9:30 AM.

meal increased apoA-I levels by 17 mg/dl in normal subjects. Surface lipid of chylomicrons, moreover, may serve as HDL precursors by mechanisms such as those reviewed by Tall and Small (17). Conversely, net transfer of cholesteryl esters from HDL to VLDL (18) and probably to chylomicrons occurs in vivo, possibly by exchange for HDL triglyceride. Thus chylomicronemia might alter HDL composition without changing HDL mass.

Study subjects were requested to consume habitual meals and snacks after fasting blood samples were obtained. The quality or quantity of food consumed was presumably dictated only by individual appetites. Information regarding diet was not elicited and no instructions concerning food intake patterns were provided to minimize the probability of conscious alteration of habits.

Our finding that HDL cholesterol, like serum cholesterol, does not vary significantly during standard working hours indicates that random blood samples are adequate for HDL quantitation at least in normal subjects. The absence of consistent within-day variation in apoA-I levels, moreover, suggests that this index of HDL concentration may also be reliably measured in non-fasting sera.

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