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Fluctuations in Human Serum Lipoproteins During the Normal Menstrual Cycle

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1. Lipoproteins were measured in sera from 11 young women having a similar environment and diet. Sera were obtained at weekly intervals over a 12-week period. The values of the lipoproteins during periods in the normal ovulatory cycle were compared to assess their relation to reported hormone concentrations in blood.
2. Only the 4–0 s_f (HDL_2) (*d* 1·125 sodium chloride) fraction changed significantly; it increased at ovulation in ten of the 11 subjects and fell as menstruation approached.
3. There was greater variability in most of the low-density rather than the high-density lipoproteins within individuals. The lipoprotein class most characteristic for an individual was the 4–0 s_f or HDL_2 fraction.

The influence of sex hormones on serum lipoproteins was first demonstrated by Gofman *et al.* (1950). They observed lower quantities of the 20–10 s_f fraction with density less than 1·0635 g./ml. in sera from young women than from young men.

Russ, Eder & Barr (1951), using Cohn's method 10 (Cohn *et al.* 1950), noted a difference in amount of cholesterol between young men and young women in plasma α- and β-lipoprotein fractions. On the basis of these observations, the authors concluded that women had greater quantities of α-lipoproteins whereas men had more β-lipoproteins in plasma. In post-menopausal women, the quantities of α-lipoproteins were decreased, thus also indicating an hormonal influence. Barr, Russ & Eder (1952) observed an increase in cholesterol associated with the α-lipoproteins and a decrease in β-lipoproteins after the administration of oestrogens to men who had suffered myocardial infarction. After cessation of treatment this distribution was reversed.

Eilert (1953) showed that some blood lipids could be controlled by sex hormones. She reported increases in serum phosphorus with decreases in serum cholesterol after the oral administration of oestradiol for relief of menopausal symptoms. Conversely, Glass, Engelbert, Marcus, Jones & Gofman (1953), administering small doses of oestradiol orally, reported no change in the amounts of the 20–12 s_f or 100–20 s_f lipoprotein classes (with densities less than 1·0635 g./ml.) in either men or women after 3 months' treatment. Robinson, Higano & Cohn (1957) reported an increase in the ratio of cholesterol from β- to α-lipoprotein with age in normal women on whom oophorectomies had been performed before the natural menopause. More recently, Robinson, Cohn & Higano (1962) reported a decrease in β-lipoprotein cholesterol and an increase in phospholipids and α-lipoprotein cholesterol in post-menopausal women after the daily administration of 5 mg. of ethynyloestradiol 3-methyl ether (Enovid). Berezin & Studnitz (1957*a*,*b*), using electrophoresis techniques, observed an increase in α-lipoproteins after the administration of oestrogen and a decrease in α-lipoproteins after the administration of androgen to women.

Two reports have been concerned with blood lipid measurements relative to the menstrual cycle. Oliver & Boyd (1953) reported that the lowest concentrations of both cholesterol and phospholipid coincided with the phase in the menstrual cycle when oestrogen production was maximal, and Adlercreutz & Tallqvist (1959) indicated recurrent changes of total cholesterol concentrations in serum during the cycle, the highest values being at the ovulatory period.

Serum contains many lipoproteins whose quantitative compositions, structures and roles in metabolism are different; their origins are probably not the same (Vandenheuvel, 1962). Therefore it is no

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longer entirely pertinent to measure constituents of lipoprotein molecules and relate them to intact lipoproteins as they circulate in blood. Most .data previously reported dealing with effects of sex hormones on lipoproteins have been derived from measurements of lipid constituents of lipoproteins. On the basis of these results conclusions have been made pertaining to the lipoproteins as molecules or classes of molecules. This may have led to erroneous and conflicting conclusions.

The purpose of the present work has been to measure lipoproteins as molecular entities, or as classes, in relation to the reported fluctuation of hormones during the normal ovulatory cycle.

MATERIALS AND METHODS

Subjects. Eleven normal women ranging in age from 25 to 44 years were selected. The subjects were members of a religious order engaged in educational and medical endeavours and were thus entirely co-operative and understanding of the research project in which they were involved. They had undergone general physical examinations at regular intervals before this study and were ostensibly free of systemic disease. Special attention was directed toward pertinent data on the individual's menstrual cycles and of possible endocrine disorders. The progression of the menses during the study was rigorously recorded for each subject, all of whom had regular ovulatory cycles.

The subjects had a common diet each day, and no obese or underweight subjects were included in the study.

Analysis of serum. After a 12hr. period without food, sufficient blood was drawn to yield at least 7ml. of serum. Samples were taken on the same day each week and kept at 4°. Ultracentrifugal fractionation procedures were started within a few days after the serum samples were obtained. The lipoproteins were removed sequentially by stepwise increases in solution densities, which permitted separation of the lower-density from the higher-density lipoprotein groups (Barclay, Barclay, Terebus-Kekish, Shah & Skipski, 1963*b*). This technique resulted in three low-density fractions: chylomicra, and lipoproteins with densities less than 1·006 and less than 1·0635g./ml.; and three high-density fractions: lipoproteins with densities less than 1·125, less than 1·21 and greater than 1·21g./ml. The last fraction was not studied in relation to the menstrual cycle. These fractions were separated further by means of the analytical ultracentrifuge into those floating in solution of density 1·006g./ml.: 400–100, 100–20 and 20–0s_f; those in solution of density 1·0635g./ml.: 20–10, 10–0 and 3–0s_f or HDL_1; those in solution of density 1·125g./ml.: 20–12, 12–4 and 4–0s_f or HDL_2; those in solution of density 1·21g./ml.: HDL_3 (Lindgren & Nichols, 1960; Barclay *et al.* 1963*b*). (s_f=Svedberg flotation units at the densities specified at 26°.) No chylomicra were observed in the serum from any subject.

Adjustment of results to periods in the menstrual cycle. The analysis was repeated on the 11 subjects for 12 weeks spanning about three complete menstrual cycles. Average values were then determined for each subject at the following periods during the cycle: days 1–6, menstruation; days 7–12, follicular or preovulatory phase; days 13–18, ovulation; days 19–24, progestational phase; days 25–30, beginning of menstruation in the next cycle. The observations of Brown (1959) and Roy (1962) show that blood oestrogen concentrations are highest at day 14 and lowest during menses. The intra-subject variability and the lipoprotein values during the phases of the cycles were analysed statistically.

RESULTS AND DISCUSSION

Fig. 1 illustrates the fluctuations of the mean values for three classes of low-density lipoproteins (*d* 1·006g./ml. in unaltered serum) as the menstrual cycle progressed. There was no 400–100s_f fraction measurable in any serum sample throughout the experiment. Most of the subjects only occasionally had values for the 100–20s_f fraction that were above zero, as the low mean values in Table 1 show. The 20–0s_f class comprised almost the total lipoproteins of density less than 1·006. Although three of the subjects did not have increased amounts at day 14, the remaining subjects had increased amounts, which resulted in a peak (Fig. 1). The mean value at day 14 was 13mg./100ml. of serum, which is nearly twice the value at day 0 (menstruation).

Fig. 2 shows the concentrations of low-density lipoproteins with densities between 1·006 and 1·0635g./ml. These were present in serum in the greatest amounts, the majority in the 10–0s_f (*d* 1·0635 sodium chloride) class, which remained relatively stable throughout the cycle in all but two subjects. There was no fluctuation in this component in two subjects, but in five subjects the concentrations fell (substantially in subject 6) at day 14, resulting in a depression of the mean. Of the other four subjects, subject no. 3 had a marked increase in the 10–0s_f fraction at day 14; the other three had very slight increases at this period.

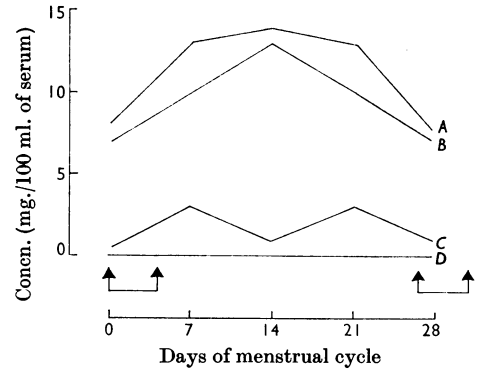

Fig. 1. Serum lipoproteins of density less than 1·006g./ml. during the course of a normal menstrual cycle. The results are the means of 11 subjects, over a 12-week period. *A*, Total lipoproteins $d < 1·006$; *B*, 20–0s fraction; *C*, 100–20s fraction; *D*, 400–100s ,fraction.

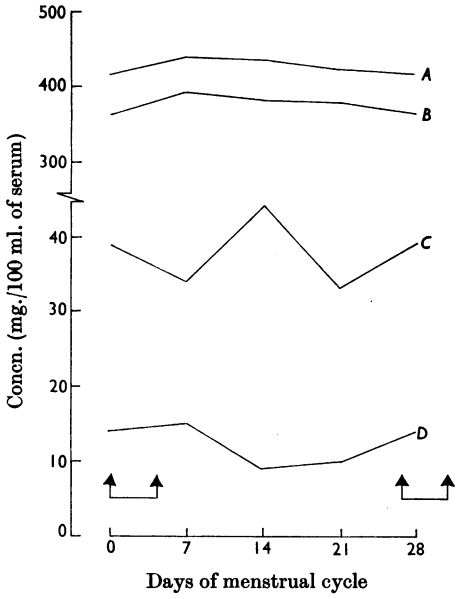

Fig. 2. Serum lipoproteins of density less than 1·0635g./ml. during the course of a normal menstrual cycle. The results are the means of 11 subjects, over a 12-week period. *A*, Total lipoproteins $d < 1{\cdot}0635$; *B*, 10–0 s_f fraction; *C*, 3–0 s_f (HDL_1) fraction; *D*, 20–10 s_f fraction.

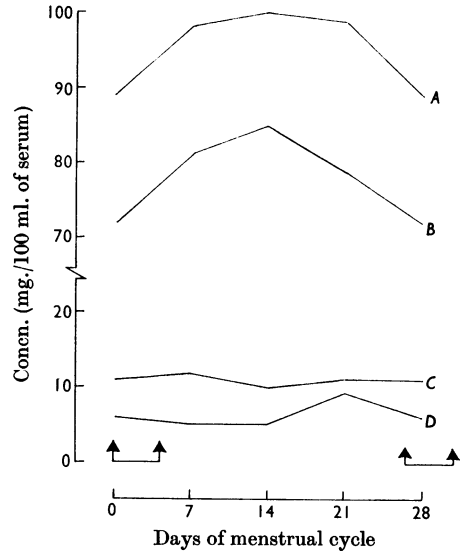

Fig. 3. Serum lipoproteins of density less than 1·125g./ml. during the course of a normal menstrual cycle. The results are the means of 11 subjects, over a 12-week period. *A*, Total lipoproteins $d < 1{\cdot}125$; *B*, 4–0 s_f (HDL_2) fraction; *C*, 12–4 s_f fraction; *D*, 20–12 s_f fraction.

The 20–10 s_f (d 1·0635 sodium chloride) class showed a depression at mid-cycle in ten subjects, whereas the values for the 3–0 s_f (d 1·0635 sodium chloride) fraction, considered to be HDL_1, rose and fell throughout the cycle in all subjects. The highest and lowest mean values were not significantly different.

The greatest concentration in the high-density lipoprotein fraction floating in a solution of d 1·125 sodium chloride (1·0635–1·125g./ml.) was in the 4–0 s_f or HDL_2 fraction (Fig. 3). There was an increase in HDL_2 on day 14 in ten of the 11 subjects. The total values at day 14 (107mg./100ml.) and day 0 (72mg./100ml.) are significantly different ($P = 0{\cdot}05$).

The two minor components in this fraction, 20–12 and 12–4 s_f (d 1·125 sodium chloride), change little throughout the cycle. Neither had patterns that resembled those of the other lipoproteins measured. Data concerning these two discretely floating components have been published by Barclay, Terebus-Kekish, Skipski & Barclay (1965).

A further indication that oestrogens may play a more specific role in the metabolism of HDL_2 is that normal young women have greater quantities of HDL_2 than are observed in men. On the other hand, men have somewhat greater quantities of

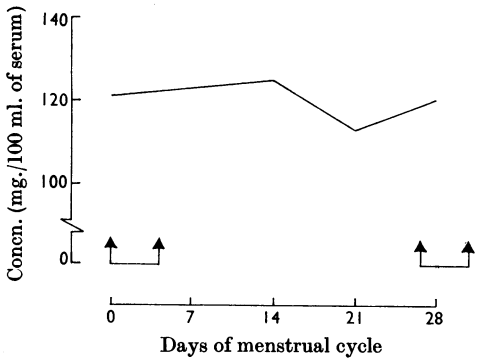

Fig. 4. Total serum lipoproteins of density less than 1·21g./ml. (HDL_3 fraction) during the course of a normal menstrual cycle. The results are the means of 11 subjects, over a 12-week period.

HDL_3 than women (Barclay, Barclay & Skipski, 1963*a*).

The values for the high-density lipoprotein HDL_3, which is separated in a solution of density 1·21g./ml., are in Fig. 4. On day 14 there was a slight increase followed by a gradual reduction in quantity from day 14 to day 21. There was, however, no marked correlation between the high and low values with the periods in the cycle.

In Table 1 are presented the means and ranges of

Table 1. *Serum lipoproteins in weekly samples from normal young women*

Experimental details are given in the text. Results are given as means ± S.D., with the ranges in parentheses. All values are given as mg./100 ml. of serum.

Subject no.	Age (years)	*d* 1·006 g./ml. 100–20 s_f	*d* 1·006 g./ml. 20–0 s_f	*d* 1·0635 g./ml. 20–10 s_f	*d* 1·0635 g./ml. 10–0 s_f	*d* 1·0635 g./ml. HDL_1
1	35	1·0± 3·0 (0–9)	10± 9 (0–28)	2± 3 (0–10)	358± 52 (300–443)	43±20 (10–76)
2	31	0·4± 1·0 (0–4)	3± 8 (0–27)	8± 9 (0–24)	405± 76 (228–460)	47±31 (5–105)
3	44	0·5± 1·0 (0–2)	11± 8 (0–18)	6± 9 (0–19)	432±105 (297–547)	48±44 (9–106)
4	38	0	3± 4 (0–12)	6± 7 (0–19)	309± 92 (140–484)	33±28 (5–96)
5	29	6·0±11·0 (0–35)	12±13 (0–36)	11±14 (0–46)	330± 76 (230–452)	48±16 (19–62)
6	34	2·0± 4·0 (0–13)	10±10 (0–28)	13±25 (0–84)	421± 73 (346–506)	28±20 (5–63)
7	25	0·4± 0·8 (0–2)	5± 7 (0–14)	2± 3 (0–10)	292± 38 (233–363)	37±20 (10–67)
8	25	0·5± 1·0 (0–4)	14±22 (0–69)	3± 2 (0–5)	278± 46 (174–340)	31±15 (5–53)
9	41	0·4± 1·0 (0–2)	5± 3 (0–9)	27±21 (5–56)	399± 63 (333–502)	20±18 (5–48)
10	43	6·0± 7·0 (0–22)	25±12 (9–55)	41±26 (0–75)	555± 31 (504–605)	39±47 (0–143)
11	37	8·0± 6·0 (0–37)	10± 9 (0–23)	12±12 (0–38)	387± 36 (339–436)	34±28 (9–86)
Total means	35	2	10	12	394	37

Subject no.	Age (years)	*d* 1·125 g./ml. 20–12 s_f	*d* 1·125 g./ml. 12–4 s_f	*d* 1·125 g./ml. HDL_2	*d* 1·21 g./ml. HDL_3
1	35	5·0± 8 (0–19)	7±6 (0–19)	26± 6 (15–35)	99±21 (75–131)
2	31	7·0±10 (0–27)	7±5 (0–16)	27±12 (15–45)	118±22 (98–170)
3	44	7·0± 9 (0–18)	3±3 (0–7)	43±14 (23–49)	107±14 (88–119)
4	38	7·0± 9 (0–27)	10±6 (0–17)	47±19 (23–88)	110±23 (54–134)
5	29	1·0± 2 (0–5)	26±9 (17–38)	73±16 (49–98)	152±28 (97–178)
6	34	7·0± 8 (0–18)	4±6 (0–14)	89±21 (51–118)	127±21 (81–156)
7	25	0·4± 1 (0–4)	36±9 (24–52)	89±22 (54–122)	121±20 (93–149)
8	25	0	1±3 (0–7)	125±37 (57–176)	122±17 (95–141)
9	41	13·0±13 (0–31)	7±6 (0–13)	96±22 (64–113)	110±33 (62–151)
10	43	6·0± 9 (0–23)	13±7 (0–19)	100±19 (88–128)	133±21 (87–144)
11	37	10·0±10 (0–29)	2±3 (0–9)	168±23 (137–215)	144±22 (110–174)
Total means	35	5	14	82	122

Table 2. *Distribution of total cholesterol and phospholipids in different lipoprotein fractions*

All values are given as percentages of the values for total serum.

Compounds	Total serum	Lipoprotein fractions *d* < 1·006	*d* < 1·0635	*d* < 1·125	*d* < 1·21	*d* > 1·21
Total cholesterol	(100)	2·7	56·4	31·1	8·0	2·1
Phospholipids	(100)	0·5	39·5	42·8	13·8	3·4

serum lipoproteins in all the subjects for the 12-week period. The values are listed in order of increasing ranges of HDL_2 (*d* 1·125 sodium chloride), the only lipoprotein species that was significantly affected by the menstrual cycle.

Each lipoprotein is a definite species in that it has its own specific structure (Wolfe, 1962) and chemical composition (Havel, Eder & Bragdon, 1955; Freeman, Lindgren & Nichols, 1963; Polonovski & Paysant, 1963; V. P. Skipski & M. Barclay, unpublished work). All data indicate that each lipoprotein fraction is composed of many lipids and all fractions contain cholesterol (free and esterified) as well as different phospholipids. Thus the differences in chemical composition between all the fractions of normal serum are quantitative rather than qualitative. Apparently the chemical composition of each lipoprotein species in the normal individual is more or less characteristic; at least the fractions with density between 1·006 and 1·0635 g./ml. and the high-density lipoproteins have relatively stable compositions.

The different fractions of lipoproteins contribute some amounts of all lipids to the total lipid content of serum. The relative amount that is contributed by each fraction is determined by the composition of the fraction and its concentration in serum. Table 2 shows the relative amounts of cholesterol and phospholipid in serum. It is obvious that measurement of total cholesterol and especially of phospholipid in serum would not in itself show the concentrations of the different lipoprotein species.

Simultaneous measurements of total cholesterol and phospholipid may indirectly indicate which fraction is affected, e.g. increase of lipoproteins with density between 1·006 and 1·0635g./ml. will cause an increase in the total cholesterol/phospholipid ratio, whereas an increase of the high-density lipoproteins will cause a decrease in this ratio. However, to get a significant change in the cholesterol/phospholipid ratio, drastic changes in the amounts of lipoproteins are necessary. The relatively mild changes that occur in most lipoprotein concentrations during the menstrual cycle could not be determined with any accuracy by the chemical analysis of cholesterol and phospholipid on total serum. Thus, although Oliver & Boyd (1953) reported decreases in both cholesterol and phospholipid in serum at day 14 of the menstrual cycle, the HDL_2 fraction, which contains the largest proportion of phospholipid, actually increased, and the low-density lipoprotein fraction (1·006–1·0635g./ml.), which contains the largest proportion of cholesterol, did not decrease in most of our subjects at that period.

The current concept, based on studies with 'high-density α-lipoproteins' as a whole, is that they are influenced greatly by oestrogens and androgens. However, when these lipoproteins are separated, the preovulatory increase in ovarian hormone output appears to affect the $4–0s_f$ or HDL_2 fraction more markedly than it does the HDL_3 fraction. In fact, the increased values for the HDL_2 fraction at day 14 agree with increased α-lipoproteins, estimated by cholesterol and phospholipid determinations on fraction IV of the cold-ethanol procedure of Cohn *et al.* (1950), employed by Eder (1958) to study the effect of the administration of oestrogens to post-menopausal women.

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REFERENCES

Adlercreutz, H. & Tallqvist, G. (1959). *Scand. J. clin. Lab. Invest.* **11**, 1.

Barclay, M., Barclay, R. K. & Skipski, V. P. (1963*a*). *Nature, Lond.*, **200**, 362.

Barclay, M., Barclay, R. K., Terebus-Kekish, O., Shah, E. B. & Skipski, V. P. (1963*b*). *Clin. chim. Acta*, **8**, 721.

Barclay, M., Terebus-Kekish, O., Skipski, V. P. & Barclay, R. K. (1965). *Clin. chim. Acta*, **11**, 389.

Barr, D. P., Russ, E. M. & Eder, H. A. (1952). *Trans. Ass. Amer. Phycns*, **65**, 102.

Berezin, D. & Studnitz, W. von (1957*a*). *Acta endocr., Copenhagen*, **25**, 427.

Berezin, D. & Studnitz, W. von (1957*b*). *Acta endocr., Copenhagen*, **25**, 435.

Brown, J. B. (1959). *J. Obstet. Gynaec., Brit. Commonw.*, **66**, 795.

Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. Z., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, D., Mouton, R. F., Schmid, K. & Uroma, E. (1950). *J. Amer. chem. Soc.* **72**, 465.

Eder, H. A. (1958). *Recent Progr. Hormone Res.* **14**, 405.

Eilert, M. L. (1953). *Metabolism*, **2**, 137.

Freeman, N. K., Lindgren, F. T. & Nichols, A. V. (1963). In *Progress in the Chemistry of Fats and other Lipids*, vol. 6, p. 216. Ed. by Holman, R. T., Lundberg, W. O. & Malkin, T. New York: Pergamon Press.

Glass, S. J., Engelbert, H., Marcus, R., Jones, H. & Gofman, J. (1953). *Metabolism*, **2**, 133.

Gofman, J. M., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A. & Strisower, B. (1950). *Circulation*, **2**, 161.

Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955). *J. clin. Invest.* **34**, 1345.

Lindgren, F. T. & Nichols, A. V. (1960). In *The Plasma Proteins*, vol. 2, p. 1. Ed. by Putnam, F. W. New York: Academic Press Inc.

Oliver, M. F. & Boyd, G. S. (1953). *Clin. Sci.* **12**, 217.

Polonovski, J. & Paysant, M. (1963). *Bull. Soc. Chim. biol., Paris*, **45**, 339.

Robinson, R. W., Cohn, W. D. & Higano, N. (1962). *Amer. J. med. Sci.* **244**, 108/736.

Robinson, R. W., Higano, N. & Cohn, W. D. (1957). *A.M.A. Arch. intern. Med.* **100**, 739.

Roy, E. J. (1962). *J. Endocrin.* **25**, 361.

Russ, E. M., Eder, H. A. & Barr, D. P. (1951). *Amer. J. Med.* **11**, 468.

Vandenheuvel, F. A. (1962). *Canad. J. Biochem. Physiol.* **40**, 1299.

Wolfe, L. S. (1962). *Canad. J. Biochem. Physiol.* **40**, 1261.