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INVESTIGATIONS INTO
THE EFFECTS OF CYCLICAL RHYTHM AND HORMONAL CONTRACEPTION
ON SERUM FAT-MOBILIZING ACTIVITY, GLYCEROL, CHOLESTEROL AND BLOOD GLUCOSE

By

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ABSTRACT

The effects were investigated of cyclical rhythm and hormonal contra-
ception on serum fat-mobilizing activity, glycerol, cholesterol and whole
blood glucose during 2 menstrual cycles in a group of normally menstruat-
ing young women and a second group of young women using hormonal
contraception. A control group of normal young men was also investigated.
There was no evidence of any change in mean level of any of the para-
meters measured, among the follicular, ovulatory and luteal phases. No
cyclical pattern was discernable in the male subjects. The mean value for
serum cholesterol concentration in women using hormonal contraception
was higher than the value for the untreated human female group. The
overall mean value for serum glycerol concentration in the women was
significantly $(0.01 > P > 0.001)$ higher than the mean value obtaining
in the men.

Serum and plasma possess the capacity to mobilize triglyceride from adipose
tissue incubated *in vitro* (*Recant et al.* 1963; *Burns et al.* 1967; *Curtis-Prior*
1973). The potency of the fat-mobilizing factor has been shown to be adequate
for the fulfilling of normal energy demands in terms of free fatty acid (FFA)

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Table 1 a.

Basic data of Sub-group A - normal women.

Subject	Age (years)	Height (m)	Weight (kg)	Ponderal index	Skin fold thickness (cm)	Normal cycle length (days)
1	20	1.65	53.1	13.3	1.0	2S
2	24	1.69	52.2	13.9	1.9	30
3	24	1.68	61.7	12.7	1.9	28
4	28	1.73	56.3	13.9	0.9	28
5	22	1.60	62.1	12.1	2.3	30
6	24	1.57	54.0	12.6	2.3	30
7	30	1.52	54.0	12.2	2.0	2G
Mean values ± SEM	24.6 ± 1.3	1.63 ± 0.03	56.2 ± 1.6	13.0 ± 0.3	1.8 ± 0.2	28.6 ± 0.6

availability from adipose reserves in the rat (*Curtis-Prior & Hanley* 1973). Since plasma FFA concentrations are increased during the luteal phase of the menstrual cycle (*Reinke et al.* 1972) and under conditions of hormonal contraception (*Seng et al.* 1969; *Wynn et al.* 1966) it was decided to follow

Table 1 c.

Basic data of Sub-group C - normal men.

Subject	Age (years)	Height (m)	Weight (kg)	Ponderal index	Skin fold thickness (cm)
12	26	1.73	56.7	13.9	0.5
13	28	1.75	64.9	13.3	0.6
14	24	1.83	75.8	13.1	0.7
15	31	1.73	77.1	12.4	1.2
Mean values ± SEM	27 ± 1.5	1.76 ± 0.02	68.6 ± 4.8	13.2 ± 0.3	0.75 ± 0.16

Table 1 b.
Basic data of Sub-group B — normal women using hormonal contraception.

Subject	Age (years)	Height (m)	Weight (kg)	Ponderal index	Skin fold thickness (cm)	Normal cycle length (days)	Contraceptive therapy (Proprietary name)
8	30	1.70	63.5	12.9	2.2	28	Eugynon
9	26	1.57	53.9	12.6	1.2	28	Minovlar
10	22	1.62	56.7	13.1	1.2	30	Orthonovum
11	26	1.57	54.4	12.6	1.1	28	Gynovlar
Mean values ± sem	26 ± 1.6	1.62 ± 0.03	57.1 ± 2.2	12.8 ± 0.1	1.4 ± 0.3	28.5 ± 0.5	

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the activity of the fat-mobilizing factor, in serum, during the menstrual cycle in normal women, women using hormonal contraception and a control group of normal men. It was also planned to correlate observed changes in serum fat-mobilizing activity with possible fluctuations in circulating levels of glycerol, glucose and cholesterol.

MATERIALS AND METHODS

Human subjects

Volunteers were selected from the scientific and technical staff of the hospital campus to form an approximately homogenous group as regards height, weight and age. The general group was sub-divided as indicated to form: Sub-group A – normal women (Table 1 a), Sub-group B – normal women using hormonal contraception (Table 1 b), and Sub-group C – normal men (Table 1 c).

The basic data obtained from the volunteers included skinfold thickness (SFT) and their ponderal index (P. I.), which is calculated as height in inches divided by the cube root of body weight in pounds (Seltzer 1966). These data provided further evidence of the homogeneity of the volunteer group as a whole.

Blood sampling

Sampling was carried out during the follicular, ovulatory and luteal phases of menstruation over a period of 2 cycles. The male subjects were sampled weekly for 8 weeks. After an overnight fast, 20 ml of blood was on each occasion withdrawn from either left or right cubital vein. Two ml of whole blood was used for glucose determination by a glucose oxidase method (Huggett & Nixon 1957), and the remaining blood allowed to clot for 60 min at room temperature and 60 min at +4°C. Portions of the serum obtained following centrifugation were used for estimation of free glycerol (Weiland 1963) and cholesterol (Levine & Zak 1964). The residual bulk of serum was stored frozen at –20°C prior to bioassay for lipolytic activity.

Assay for lipolytic activity

Rat isolated fat cells were prepared in Krebs-Ringer bicarbonate buffer (pH 7.4 containing glucose 45 mg/100 ml and human albumin 3.5 g/100 ml) as described ppreviously (Curtis-Prior 1972). The fat-mobilizing activity of 1.10 ml of serum (in triplicate) previously shown to be the maximally effective concentration (Curtis-Prior & Hanley 1973) was determined by using glycerol production during a 90 min incubation as the index of lipolysis. The number of nanomoles of glycerol released was expressed in terms of the weight of total intracellular lipid determined by a modified Folch et al. (1957) technique (Curtis-Prior et al. 1969).

Table 2.

Serum fat-mobilizing activity (FMA), glycerol, cholesterol and blood glucose levels during different phases of the menstrual cycle.

Phase	FMA (nmoles mg^{-1}/90 min)	Glycerol (nmol/ml)	Glucose (mg/100 ml whole blood)	Cholesterol (mg/100 ml)
Sub-group A (normal women, mean of 7 subjects ± SEM)				
Follicular	10.5 ± 0.8	105.8 ± 14.2	73.3 ± 2.0	191.8 ± 9.2
Ovulatory	10.2 ± 0.8	111.1 ± 11.6	75.7 ± 2.4	195.1 ± 7.0
Luteal	11.0 ± 0.9	100.8 ± 14.4	75.4 ± 2.2	187.2 ± 10.4
Sub-group B (normal women using hormonal contraception, mean of 4 subjects ± SEM)				
Follicular	10.8 ± 0.5	79.5 ± 9.6	73.8 ± 2.3	229.5 ± 9.7
Ovulatory	11.9 ± 0.8	88.0 ± 16.0	72.6 ± 3.1	221.0 ± 11.7
Luteal	10.1 ± 0.9	82.5 ± 14.5	75.9 ± 1.3	220.5 ± 18.2

RESULTS

The data in Table 2 show serum fat-mobilizing activity (FMA), glycerol, glucose and cholesterol levels during different phases of the menstrual cycle for normal women (Sub-group A) and normal women using hormonal contraception (Sub-group B). Analysis of variance using a two-factor model (subjects and phase of the menstrual cycle) showed no evidence of any change in mean

Table 3.

Overall mean values ± SEM for each of the parameters measured within each Sub-group.

Sub-group	Parameter			
	FMA (nmol mg^{-1}/90 min)	Glycerol (nmol/ml)	Glucose (mg/100 ml)	Cholesterol (mg/100 ml)
A normal women	10.6 ± 0.7	105.9 ± 12.3	74.8 ± 1.8	191.4 ± 8.4
B normal women using hormonal contraception	10.9 ± 0.4	83.6 ± 12.5	74.1 ± 2.1	223.7 ± 13.1
C normal men	10.2 ± 1.1	48.0 ± 7.9	74.1 ± 5.3	214.8 ± 13.8

levels of any of the parameters measured, among the follicular, ovulatory and luteal phases. The factor "phases" was therefore ignored, and the 3 values for each subject were regarded as merely within-subject variation in a re-calculation of the data to produce a single value for each parameter shown in Table 3. Lack of evidence of any cyclic pattern in Sub-group C subjects (normal men) also allowed compilation of these data to a single value for each parameter as shown in Table 3.

There was a difference between the overall mean values of serum cholesterol for Sub-grouup A (normal women) and Sub-group B (normal women using hormonal contraception). However, this difference was only at the 7 % level of statistical significance. When Sub-groups A and B were combined and compared with Sub-group C, the only significant difference was for serum glycerol levels. Sub-group C gave a much lower value than Sub-groups A and B combined $(0.01 > P > 0.001)$.

DISCUSSION

The lack of differences in free glycerol and glucose levels at different stages of the menstrual cycle, is in agreement with the work of others (*Kaffarnik et al.* 1969; *Reinke et al.* 1972). *Reinke et al.* (1972) observed a significant rise in FFA, associated with elevated progesterone levels, during the luteal phase of the cycle. If this is the result of increased adipose tissue lipolysis, then elevation in circulating glycerol level would also be expected to occur, and increased activity of the serum lipolytic factor might be expected; neither of these phenomena was observed.

Raised serum cholesterol values in women using hormonal contraception have been reported previously (*Wynn et al.* 1966). *Stokes & Wynn* (1971) showed that the most 'progestational' formulations resulted in the highest cholesterol values, whereas the most oestrogenic led to the highest triglyceride values.

The highly significant difference $(0.01 > P > 0.001)$, between the serum glycerol concentrations of women (Sub-groups A and B taken together) and men (Sub-group C) is a most surprising finding. The normal human range of values for serum glycerol has been reported (*Eggstein* 1966) to be 0.5–1.7 mg/100 ml of serum, approximately equivalent to 54–185 nmol/ml of serum. Therefore, the women tend to life towards the upper end of the range (97.8 ± 9.3 nmol/ml) and the men at the lower end (48.0 ± 7.9 nmol/ml). We can find no other report of such a sex difference. It is difficult to reconcile this picture with the higher incidence of atherosclerosis in men compared with women, which is presumably due, at least in part, to *higher* levels of circulating lipid, rather than lower.

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ACKNOWLEDGMENTS

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