Iranian J. Publ. Hlth.
Summer 1977, Vol. 6, No. 2

SOME EFFECTS OF ORAL CONTRACEPTIVES ON SERUM FATTY ACIDS DISTRIBUTION

H. Mohammadiha*
S.S. Nour-Azar**

ABSTRACT

Serum total fatty acids were estimated in controls and women using the oral contraceptives Eugynon (containing 0.5 mg. of neurgestron and 0.05 mg. ethinyl oestradiol) and Lyndiol (containing 2.5 mg. of linostrol and 0.05 mg. of ethinyl oestradiol) for periods ranging from 8 to 110 weeks. The serum total fatty acids level was found to be approximately 70% higher in the women using oral contraceptives, the difference being predominantly in the myristic and palmitoleic acid contents respectively. No significant difference was seen between the effect of Eugynon and Lyndiol usage upon blood fatty acids.

INTRODUCTION

Many women who use oral contraceptive agents demonstrate physiological changes which should be studied in greater detail, in order to determine whether such changes have undesirable sequelae. Increases in serum lipids have been reported in subjects taking oral contraceptives[1,2,3,4,5].

Elevated serum lipid levels are characteristic in the last trimester of pregnancy[6] and it is well established that serum lipids differ in pre-menopausal woman compared with men of the same age, as the menopause heralds a change in the serum lipids towards the male pattern[7].

* Department of Biochemistry of School of Basic Medical Sciences, University of Teheran, Teheran, Iran.
** Pharmacy Dr.

Occlusive vascular disease, secondary to atherosclerosis, is less frequent in pre-menopausal women than men, but after the menopause the incidence tends to be similar[8,9]. The possibility that the relative immunity of pre-menopausal woman to severe artherosclerosis is related to their pattern of circulating lipids has stimulated a number of studies on the effects of gonadal hormones and their synthetic analogues on the serum lipids and lipoproteins[10,11]. Such investigations have usually been on men or post-menopausal women, and no systematic investigation of the effects of natural or synthetic sex steroids upon the serum-lipids in pre-menopausal women has been reported.

The determination of individual serum fatty acid levels is becoming increasingly relevant clinically and was employed in this investigation to study the effects of oral contraceptive usage on serum fatty levels. The method is rapid and convenient as it involves gas liquid chromatographic determination of the fatty acids as their methyl esters.

MATERIAL AND METHOD

The subjects were healthy women who had been attending Ministry of Health Clinics for oral contraceptive protection for periods ranging from 8 to 110 weeks. Their full medical histories, blood pressures, body weights and nutritional habits have been noted at their first attendance and this data was used to select healthy subjects for the study. Seventy-eight women aged 20 to 49 years old in the weight range 46 to 79 kg., were finally chosen. Fifty-eight of these were taking Eugynon (0.5 mg. of neurgestron and 0.05 mg. of ethinyl oestradiol) and 20 were taking Lyndiol (2.5 mg. of linostrol and 0.05 mg. of ethinyl oestradiol). Thirty healthy women in the same age and similar weight range who had never used oral contraceptives were selected as controls.

Blood samples were collected at regular intervals from the whole group and each was treated as follows:

The blood was collected in a clean tube and centrifuged after coagulation was complete; the serum was removed and stored in a refrigerator until extraction a few days later. The lipids were extracted using a modified Folch's method[12] and the organic phase removed and evaporated to dryness under reduced pressure. The dry residue was dissolved in methanolic sodium hydroxide (5 ml.,N/2), and refluxed for 5 min.; methanolated boron trifluoride (5 ml.) was added and refluxing continued for a further two minutes[13]; n-heptane, (2 ml.) was added and heating continued for another minute [14,15]. After cooling under cold water, saturated sodium chloride solution was added and the heptane phase allowed to settle out. The aqueous phase was discarded and the heptane phase dried

under nitrogen addition of a little anhydrus sodium sulphate.

1 μl. of this solution which contained the methyl esters of the fatty acids was analysed by gas liquid chromatography.

This was carried out using a Varian 17040/10 series gas chromatograph fitted with a steel column, 5 ft. long and ¼ in. in diameter packed with 20% D.E.G.S. on 60—80 mesh chromosorb W—AW, and a flame ionisation detector. Operating temperatures and gas flows were as follows: injector 250°C, column 170°C and detector 290°C; nitrogen carrier gas 17 ml/min., hydrogen 40 ml/min. and air 400 ml/min. Satisfactory sensitivity was achieved with an attenuation of 128. The fatty acid contents were calculated by comparison of the peak heights obtained with those from known amounts of oleic or stearic acids. Details of this method and the calculation are being published separately.

RESULTS

The total fatty acid contents in serum from women who had been taking oral contraceptives for three months was found to be significantly higher than those of the controls and was found to reach a maximum after six to 12 months. The average increase in total fatty acid content compared with the controls over this period was 87% and there was a slight decrease after 18 months and a slight increase after two years of continuous oral contraceptive treatment. A maximum was presumably reached because of metabolism or excretion of excess hormone or metabolism or excretion of the excess fatty acids in the serum.

Fig. 1 shows a typical gas chromatogram obtained from the fatty acid mixture extracted from the serum of a woman control and Fig. 2 shows the corresponding chromatogram from the serum of a woman who had been taking oral contraceptive for two years. In Fig. 3 for convenience in comparison Fig. 1 has been superimposed on Fig. 2, the higher content of fatty acids is clearly seen particularly in the C16:0 and C16:1 and C18:1 peaks (i.e. palmitic, palmitoleic and oleic acids respectively).

The percentage of increase is clearly shown in Fig. 4 where the increase in average total fatty acid (compared with the controls) is plotted against the time spent on oral contraceptive treatment, the rapid rise in serum fatty acid during the first six months of treatment is particularly noticeable. The graph also shows the maximum reached after 12 months and the slight fall from this over the next year of treatment. This slight fall, from 92 to 68% may be due to some readjustment to the hormone metabolism.

Figure 5 shows all the individual fatty acid increases on the same graph for ease of comparison, and it can be seen that there is a rapid

increase in the first six months followed by a levelling off, and this was found to be the case for each of the six fatty acids.

Table 1 shows the actual numerical values of the percentage increases in fatty acid contents obtained from the 78 subjects. Each figure represents the average value for each acid from the whole group of patients over the two year study period.

Table 2 shows the statistical analysis of the serum fatty acid contents of the control group; the low standard error shows the results to be statistically significant.

Table 3 shows the statistical analysis of the results obtained to be statistically significant although the standard errors were slightly larger than those in the control group. The P value was found to be less than 0.01.

Table 4 shows the age distribution for the whole group of women concerned and reasonable similarity between the control group and the subjects. The percentage of controls in the 40–49 age ranges is higher than the corresponding percentage in the subjects, so the increase in serum fatty acids in the subjects is not age related.

Similarly Table 5 shows the weight distribution for the whole group but here more marked differences are seen between the controls and the subjects as the controls were all below 69 kg. body weight, but 7½% of the subjects were in the 70–79 kg. weight range. It is thought that there is some connection between weight increase and oral contraceptive treatment and the level of serum fatty acids is on average higher in obese subjects than normal people but this level is not more than 10%(16). The level of triglycerides in the blood is often moderately raised in obesity(17).

No significant correlation was found between increase in serum fatty acid and age or weight of the subjects concerned, nor was there any significant difference due to type of oral contraceptive being taken. The results clearly show a significant overall increase in serum fatty acids resulting from treatment of healthy women with Eugynon or Lyndiol and that this increase reaches a maximum within 12 months of treatment which decreases only slightly after another year of treatment. The largest increases were found in the myristic and palmitoleic acids.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Professor A. Baghdiantz for his encouragement and helpful advice during this work.

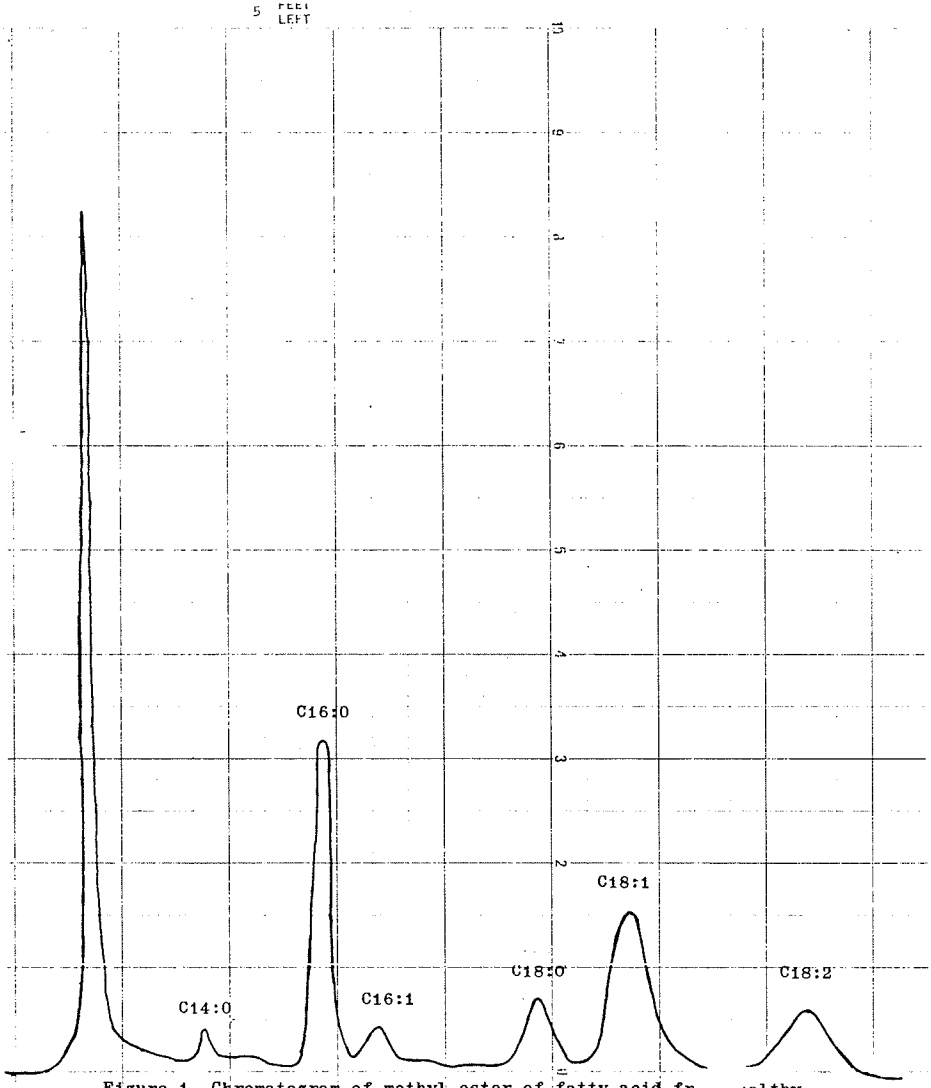

Figure 1- Chromatogram of methyl ester of fatty acid fr· ·althy human blood serum.

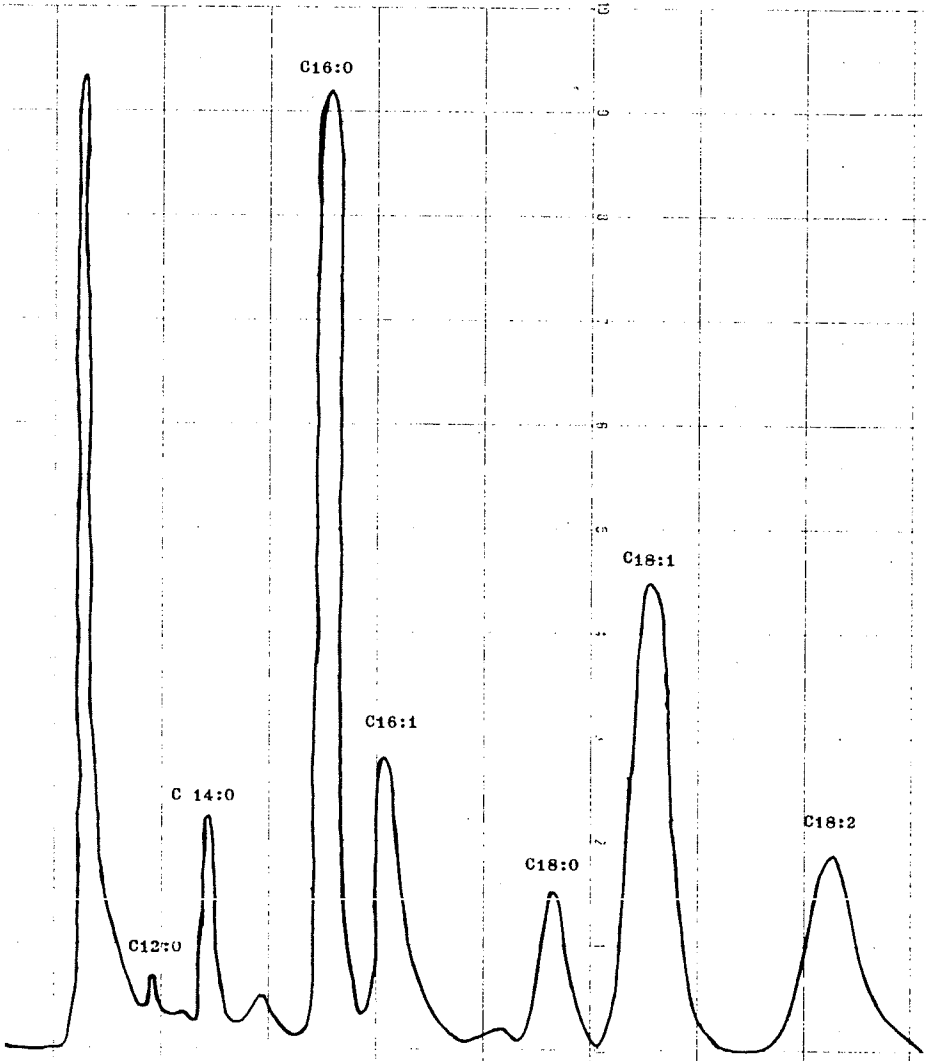

Figure 2- Gas chromatogram of the methyl esters of fatty acids extracted from subjects taking oral contraceptives.

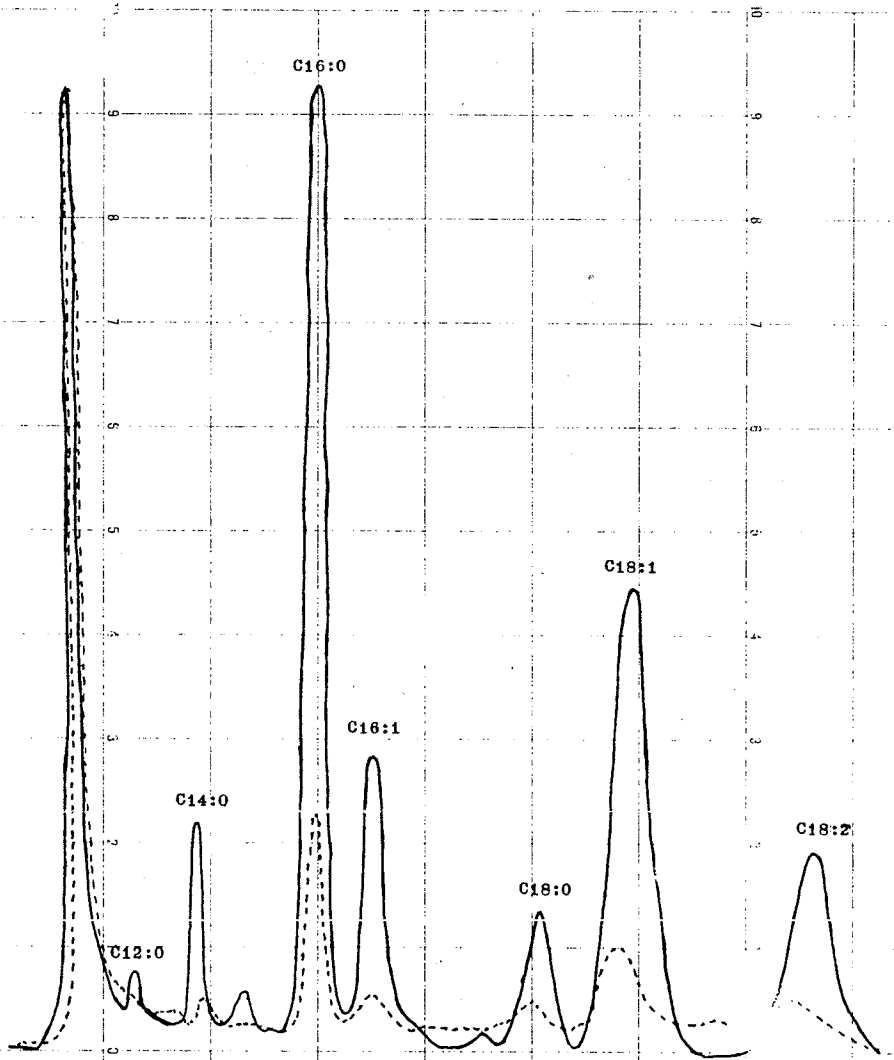

Figure 3- Gas chromatogram of the methyl esters of fatty acids extracted from controls (---------) and the subjects (————)。

Fig. 4 — Percentage increase in total serum fatty acids during two years of oral contraceptive treatment

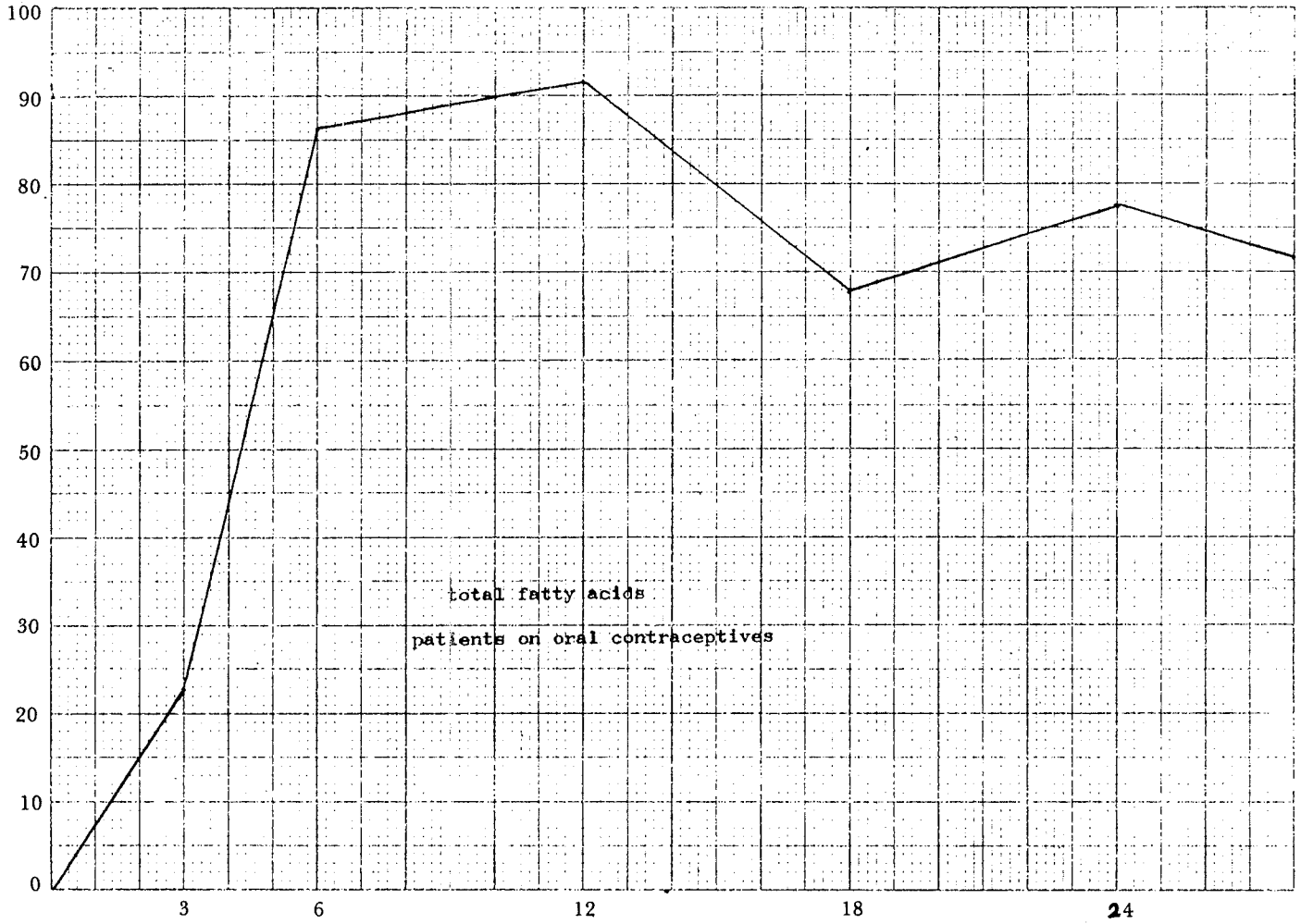

Fig. 5 — Percentage increase in individual serum fatty acids during two years of oral contraceptive treatment

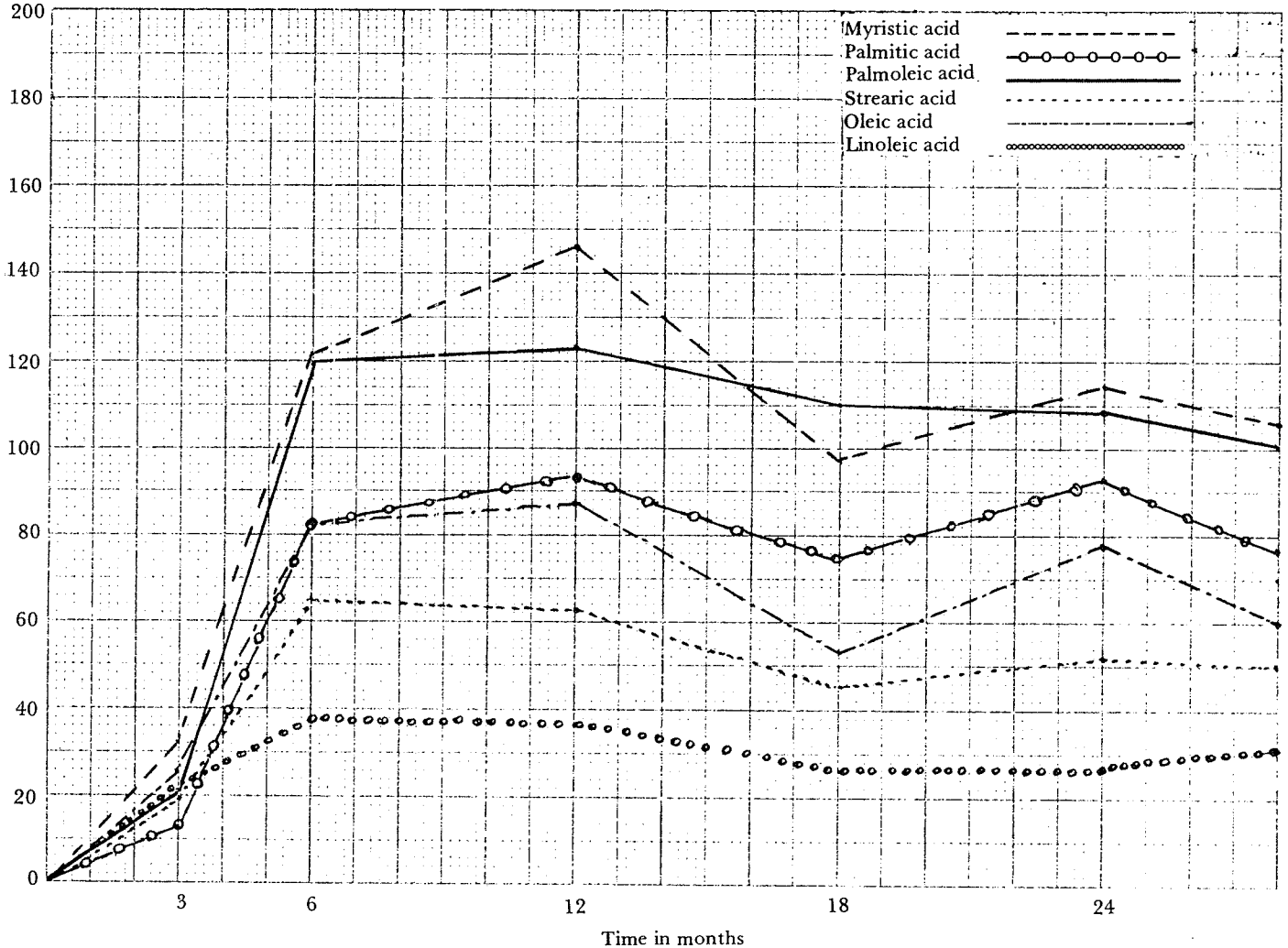

Table 1
Percentage increase in serum fatty acids during two years treatment with oral contraceptives

Fatty acid	Average between 8 to 10 weeks	Percentage after					
		3 months	6 months	12 months	18 months	24 months	26 months
Myristic C14:0	103.2	32.8	122.2	146.6	96.7	114.7	106.2
Palmitic C16:0	71.3	12.8	82.9	93.6	74.3	87.0	77.3
Palmitoleic C16:1	97.8	20.6	120.1	123.7	110.6	109.9	101.0
Stearic C18:0	49.3	20.0	65.5	62.0	44.8	52.3	50.9
Oleic C18:1	64.7	25.2	83.1	87.6	53.3	78.6	60.2
Linoleic C18:2	29.9	21.4	37.5	36.4	26.0	26.3	31.5
Total Fatty Acids	69.3	22.2	86.2	91.7	67.8	78.1	71.2

Table 2
Statistical analysis of serum fatty acid determination of control group

Fatty acid	Number of tests	$\bar{X}$	S.D.	S.E.
Myristic	30	1.02	0.35	0.06
Palmitic	30	7.66	1.64	0.3
Palmitoleic	30	0.95	0.24	0.04
Stearic	30	1.45	0.41	0.07
Oleic	30	3.80	1.04	0.19
Linoleic	30	2.92	1.03	0.19

Table 3
Statistical analysis of serum fatty acid determination of treated group

Fatty acid	Number of tests	$\bar{X}$	S.D.	S.E.
Myristic	78	2.13	1.07	0.12
Palmitic	78	13.63	6.33	0.72
Palmitoleic	78	1.92	1.06	0.12
Stearic	78	2.20	0.85	0.10
Oleic	78	6.34	2.42	0.27
Linoleic	78	3.83	1.50	0.17

Table 4
Age distribution of subjects (i.e. women taking oral contraceptives) and control

Age (year)	Subjects		Control	
	Number	Percent	Number	Percent
20–24	14	17.5	4	13.4
25–29	16	20.5	6	20.0
30–34	22	28.2	8	26.6
35–39	12	15.3	3	10
40–44	8	10.7	6	20
45–49	6	7.8	3	10
Total	78	100	30	100

Table 5
Weight distribution of subjects (i.e. women taking oral contraceptives) and control

Weight (kg.)	Subjects		Control	
	Number	Percent	Number	Percent
45–49	8	10.2	2	6.7
50–54	14	17.9	4	13.4
55–59	11	14.3	13	43.3
60–64	24	30.8	6	20
65–69	15	19.2	5	16.6
70–74	4	5.1	–	–
75–79	2	2.5	–	–
Total	78	100	30	100

REFERENCES

1. Smith, J.L. et al (1975) Effects of oral contraceptives steroids on vitamin and lipid levels in serum. Amer. J. Clin. Nutri. 28: 371–376.
2. Aurell, M., Cramer, K. and Rybo, G. (1966) Serum lipids and lipoproteins during long-term administration of an oral contraceptive. Lancet, 1:291.
3. Doar, J.W.H. and Wynn, V. (1970) Effect of obesity, glucocorticoid, and oral contraceptive therapy on plasma glucose and blood pyruvate levels. British Medical J. 1:149.
4. Hazzard, W.R., Spiger, M.J. et al (1969) Studies on the mechanism of increased plasma triglyceride levels induced by oral contraceptives. New England J. of Medicine, 280:471.
5. Wynn, V., Doar, J.W.H. and Mills, G.L. (1966) Some effects of oral contraceptives on serum lipid and lipoprotein levels. Lancet, 2:720.
6. de Lalla, O.F., Gofmann, J.W. and Page, I.H. (1961) Blood and other body fluid, edited by Allman, P.L. and Dittmer, D.S., Washington. D.C. 64.
7. Kuku, S.B. and Akinyanyu, P.A. (1973) Fasting serum lipids and serum lipoprotein distribution during contraceptive therapy in Nigerians. J. Obstet. & Gynae. British Comm. 80:750–53.
8. Carter, A.B. (1963) Strokes (Abridged) Proc. Royal Soc. Med. 56:9(483).
9. Kannel, W.B. et al (1971) Serum cholestrol, lipoproteins, and the risk of coronary heart disease. The Framingham Study, Ann. Inter. Med. 74:1.
10. Russ, E.M. et al (1951) Protein-lipid relationships in human plasma in normal individuals, Am. J. Med. 11:468.
11. Russ, E.M., Eder, H.A. and Barr, D.P. (1955) Influence of gonadel hormones on protein-lipid relationships in human plasma. Am. J. Med. 19:4.
12. Folch, J., Lees, M. and Stanley, S. (1957) A simple method for isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497.
13. Martinelli, et al (1960) First observation on fatty acids of the lipids and lipid fraction of normal human blood by means of gas chromatography, Ball. Soc. Ital. Biol. 36:1698–1700.

14. Halgreen, B. and Svanborg, A. (1962) The separation of free fatty acids from human plasma lipids. Gas chromatographic analysis of the free fatty acid composition in healthy human individuals. Scand. J. Clin. Lab. Invest. 14:179.
15. Wijingarden, D. Van (1967) Modified rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chem. 39:848–849.
16. Opie, L.H. and Walfish, P.G. (1963) Plasma free fatty acid concentration in obesity. New Engl. J. Med. 268:757.
17. Albrik, M.J. and Meigs, J.W.A. (1965) The relationship between serum triglycerides and skinfold thickness in obese subjects. Ann. N.Y. Acad. Sc. 131:673.