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Studies on the Influence of Dietary Fat on the fatty Acid Composition of Serum Lipids in Humans

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With 2 figures and 5 tables

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For a nation-wide prevention of cardiovascular disease there is to-day strong medical evidence that the relative content of polyunsaturated fatty acids in the diet should be increased as far as possible, at the expense of saturated fatty acids (1). The most well documented biochemical effect of such a change is a lowering of serum cholesterol. Yet, the mechanism by which saturated fatty acids increase and unsaturated fatty acids decrease serum cholesterol remains obscure. The effect of dietary fatty acids on serum cholesterol might be mediated by changes in the fatty acid composition of the different lipid classes in serum. *Schrade* et al. (2) reported that the serum lipids of patients with hyperlipidemia contained relavitely more saturated and monounsaturated fatty acids and less polyunsaturated fatty acids than the serum lipids of healthy male controls. Recently *Spritz* and *Mishkel* (3) proposed that for steric reasons less lipid can be accommodated by the lipoprotein molecules when saturated and monounsaturated acyl chains are substituted by polyunsaturated ones. In the present investigation we have studied the fatty acid composition of serum triglycerides, cholesterol esters, free fatty acids and phospholipids in healthy male Swedes during dietary induced changes of serum cholesterol.

Methods

Experimental subjects and dietary regimen

The dietary experiment was carried out on 22 healthy male persons, the age of 28–73 years. Mean body weight ± SE at the beginning and at the end of the experiment was 81.3 ± 2.4 kg and 79.4 ± 2.4 kg respectively. The participants received 3 meals per day, breakfast, lunch and dinner, which were served in a separate dining room at the Lund University Hospital.

The meals were composed of a basal diet of lowest possible fat content to which was added two different kinds of fat. The basal diet consisted of vegetables, fruit, potatoes, rice, spaghetti, peas, beans, whites of eggs, sugar, bread, fruit soups, oat-meal porridge, skimmed milk, coffee or tea. Hydrogenated coconut oil (HCO) or Lactovit® oil (90 % soy oil + 10 % cotton seed oil) was added in an amount corresponding to 40 % of the total calories. The fat was administered as a "cheese" spread and in the form of filled milk. The oils were

Table 1. Fatty acid composition (weight %) of dietary oils

Fatty acid	Hydrogenated coconut oil	Lactovit oil
Lauric	52.0	–
Myristic	21.8	–
Palmitic	13.7	12.8
Stearic	12.5	2.1
Oleic	–	22.9
Linoleic	–	55.7
Linolenic	–	6.4

also used in frying and cooking and as ingredients in e.g. mayonnaise and salad dressings. The fatty acid composition of the dietary fats is given in table 1.

The experimental subjects were divided into 2 groups, A and B, with approximately the same distribution. Group A comprised 10 persons and group B 12 persons. During the first experimental period of 4 weeks group A was given the HCO-diet and group B the Lactovit-diet. The diets were then shifted between the two groups and the experiment continued for another 3 weeks. During a third period of 10 days group A continued on the Lactovit-diet and group B on the HCO-diet but each participant received in addition 6 egg yolks per day, corresponding to approximately 1.4 g of cholesterol. The total amount of fat in the diet was kept at a level corresponding to 40 % of the total calories as during the previous periods.

Chemical analysis

Samples of blood serum were obtained in the morning before breakfast. Determination of cholesterol (4) and triglycerides (5) was done before the beginning of the experiment and then every week. The fatty acid composition of serum triglycerides, cholesterol esters, free fatty acids and phospholipids was determined in five individuals before the experiment and then in 3 individuals of each group at the end of each dietary period.

Determination of fatty acid composition

Samples of serum, were extracted with 20 vols. of chloroform/methanol 2/1 (v/v). The extracts were equilibrated with 0.4 vols. of 2 % KH_2PO_4 in water. Aliquots of the chloroform-phases thus obtained were subjected to thin-layer chromatography on 0.50 mm thick layers of Silica Gel G (E. Merck, Darmstadt). The plates were developed in diethyl-ether/petroleum ether/acetic acid 1/9/0.1 (v/v). The lipid fractions were visualized under UV-light after the plates had been sprayed with a solution of 0.2 % dichlorofluorescein (Fluka, Basel) in ethanol. The following fractions were obtained: cholesterol esters, tryglycerides, unesterfled fatty acids and, at the origin of the chromatogram, phospholipids. The lipid fractions were scraped directly into test tubes and 2 ml of absolute methanol containing 2 % sulfuric acid was added. The stoppered tubes were kept at 65° for 3 hours. By this treatment complete transesterification of the lipid fractions was achieved. The resultant fatty acid methyl esters were extracted into light petroleum ether (b.p. 40–60°) after addition of water to the reaction mixture. The methyl esters were purified by thin-layer chromatography on Silica Gel G with benzene/hexane 1/1 (v/v) as the moving phase. They were visualized with dichlorofluorescein as described above and then eluted quantitatively with a mixture of diethyl-ether/petroleum ether 5/95 (v/v).

The composition of the methyl esters was determined by gas-liquid chromatography on a Pye Argon Chromatograph equipped with an electronic integrator. Stationary phase was 15% ethyleneglycol succinate on 100–120 mesh, acid-washed Celite. The column was operated at 176°. Standard mixtures of fatty acid methyl esters from the National Heart Institute were used for calibration of the instrument. Identification of the peaks on the chromatogram was based on comparison of retention times with those of authentic standards.

Results and discussion

The concentration of total cholesterol and triglycerides in serum are given in fig. 1. As expected, the ingestion of hydrogenated coconut oil was associated with levels of serum cholesterol that were equal to or higher than the starting values. The highly unsaturated Lactovit oil reduced the concentration of cholesterol by 50–75 mg/100 ml. When the diets were shifted between the two groups the cholesterol levels changed significantly already during the first week. The influence of the dietary fat on the level of serum triglycerides was less clear-cut than the influence on the cholesterol concentration. During the first period the concentration of triglycerides in the group on the HCO-diet stayed above that of the Lactovit oil-group but the levels were not reversed after shifting the diets between the two groups. There was also a large scattering between individual values of triglyceride concentration. The addition of cholesterol in the form of egg yolks caused a marked increase in the serum cholesterol in the group on the Lactovit oil-diet while there was a much less pronounced effect on the already high cholesterol level in the HCO-group.

The fatty acid composition of serum lipids in each of the 6 subjects studied is presented in figs. 2A–2F. Mean values and the statistical significans of induced changes are given in tables 2–5.

When the subjects were on a free diet each lipid class exhibited a characteristic fatty acid composition. Cholesterol esters contained mainly linoleic and oleic acid, triglycerides oleic and palmitic acid. The free fatty acids were similar to triglycerides except for lower levels of oleic acid and an increased concentration of stearic acid. In the phospholipids palmitic and stearic acid accounted for slightly more than half of the total fatty acids. These general features are in agreement with previous reports by, among others, *Schrade* et al. (2) and *Goodman* and *Shiratori* (6). The feeding of hydrogenated coconut oil to group A during the first experimental period induced only minor changes in the composition of cholesterol esters and phospholipids. In the triglycerides myristic acid increased markedly. Linoleic and linolenic acid disappeared completely from the free fatty acid fraction. In group B, which received the Lactovit diet during the first period, linoleic acid increased conspicuously in all lipid fractions. The highest level, 80%, was attained in the cholesterol esters while the greatest relative increment from 10 to 40% occurred in the triglycerides. Shifting the diets between the two groups resulted in almost complete reversal of the fatty acid spectra after three weeks. However, the final equilibrium may not have been reached since the linoleic acid level in group B did not decline to the same low values as attained in group A during the first period, when hydrogenated coconut oil was given. Also in group A during the second period the levels of linoleic acid were somewhat lower than in group B during the first period. In both dietary groups the addition of 6 egg yolks to the diets caused only small changes in the fatty acid patterns.

It is well established that adipose tissue triglycerides are the main source of the plasma free fatty acids in the fasting state. However, the presently observed dietary influence on the composition of the plasma free fatty acids can hardly be fully accounted for by a corresponding alteration in the fatty acid composition of the adipose tissue triglycerides since the experimental diets were given for a period of 3–4 weeks. Possibly an overnight fast was insufficient to clear the adipose tissue completely of recently assimilated chylomicron fatty acids. In the rat such acids are released prior to the bulk of fatty acids in the adipose tissue during lipolysis (7).

The dietary induced alterations in the fatty acid composition of plasma cholesterol esters, triglycerides and phospholipids observed in the present study are of the same order of magnitude as those recently reported by *Spritz* and *Mishkel* (3) in a comparable experiment. These authors also found that the ratio lipid/protein was decreased in the low-density lipo-

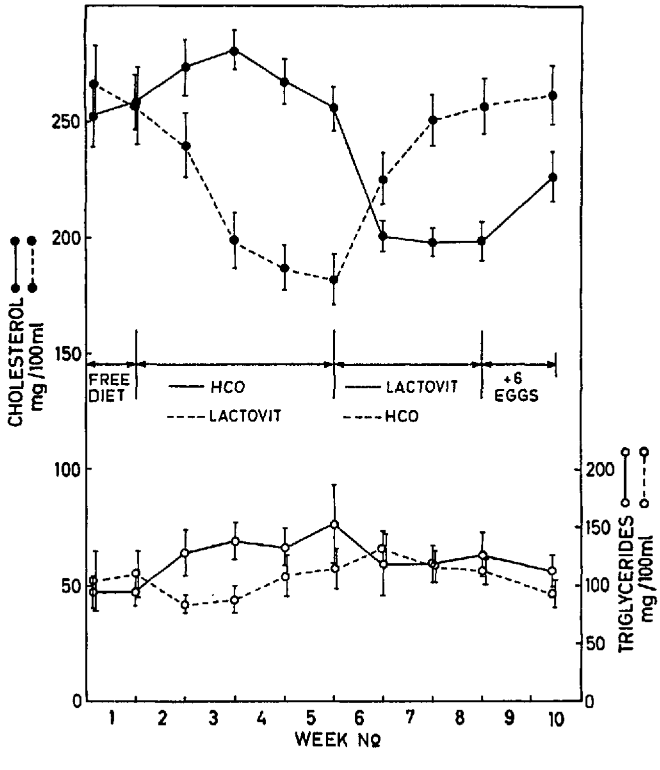

Fig. 1. Concentration of total cholesterol and triglycerides in serum. The plotted data are mean values ± SE for group A (–) and for group B (- - -).

Table 2. Fatty acid composition of serum cholesterol esters (weight%).
The figures represent mean values.
Student's t-test was applied to determine the statistical significans of the difference between two consecutive values.

Fatty acid	Dietary regimen (group A)			
	Free diet (n = 2)	HCO-diet (n = 3)	Lactovit-diet (n = 3)	Lactovit-diet + 6 eggs (n = 3)
Lauric	0.0	0.0	0.0	0.0
Myristic + Myristoleic	1.6	3.5	0.4**)	1.1*)
Palmitic	13.0	11.9	8.4	9.8
Palmitoleic	5.6	10.8	2.6*)	2.7
Stearic	0.9	1.0	0.4	1.2**)
Oleic	24.6	20.8	9.5*)	12.5
Linoleic	49.9	48.3	76.0**)	67.7
Arachidonic	4.6	3.4	2.7	4.9

Fatty acid	Dietary regimen (group B)			
	Free diet (n = 3)	Lactovit diet (n = 3)	HCO diet (n = 3)	HCO diet + 6 eggs (n = 3)
Lauric	0.0	0.0	0.6**)	0.0**)
Myristic + Myristoleic	1.7	0.0*)	4.0**)	3.1
Palmitic	12.9	6.9**)	11.2**)	12.0
Palmitoleic	4.1	1.2*)	6.0**)	4.8
Stearic	0.5	0.1*)	1.1*)	1.3
Oleic	20.8	7.8**)	15.2**)	17.3*)
Linoleic	56.8	81.4**)	57.8**)	55.4
Arachidonic	3.1	2.5	4.1	5.8

*) $0.01 < P < 0.05$ for the difference between this value and the preceding value for this fatty acid

**) $P < 0.01$ for the difference between this value and the preceding value for this fatty acid

protein fraction during feeding of polyunsaturated fats. It was tentatively proposed that the binding of cholesterol to the lipoproteins is sterically hindered by the presence of polyunsaturated fatty acids. In the present experiment a marked increase in plasma cholesterol occurred when cholesterol was added to the highly unsaturated Lactovit diet. This increase in plasma cholesterol was in fact accompanied by slightly decreased levels of polyunsaturated fatty acids in the plasma lipids, notably in the

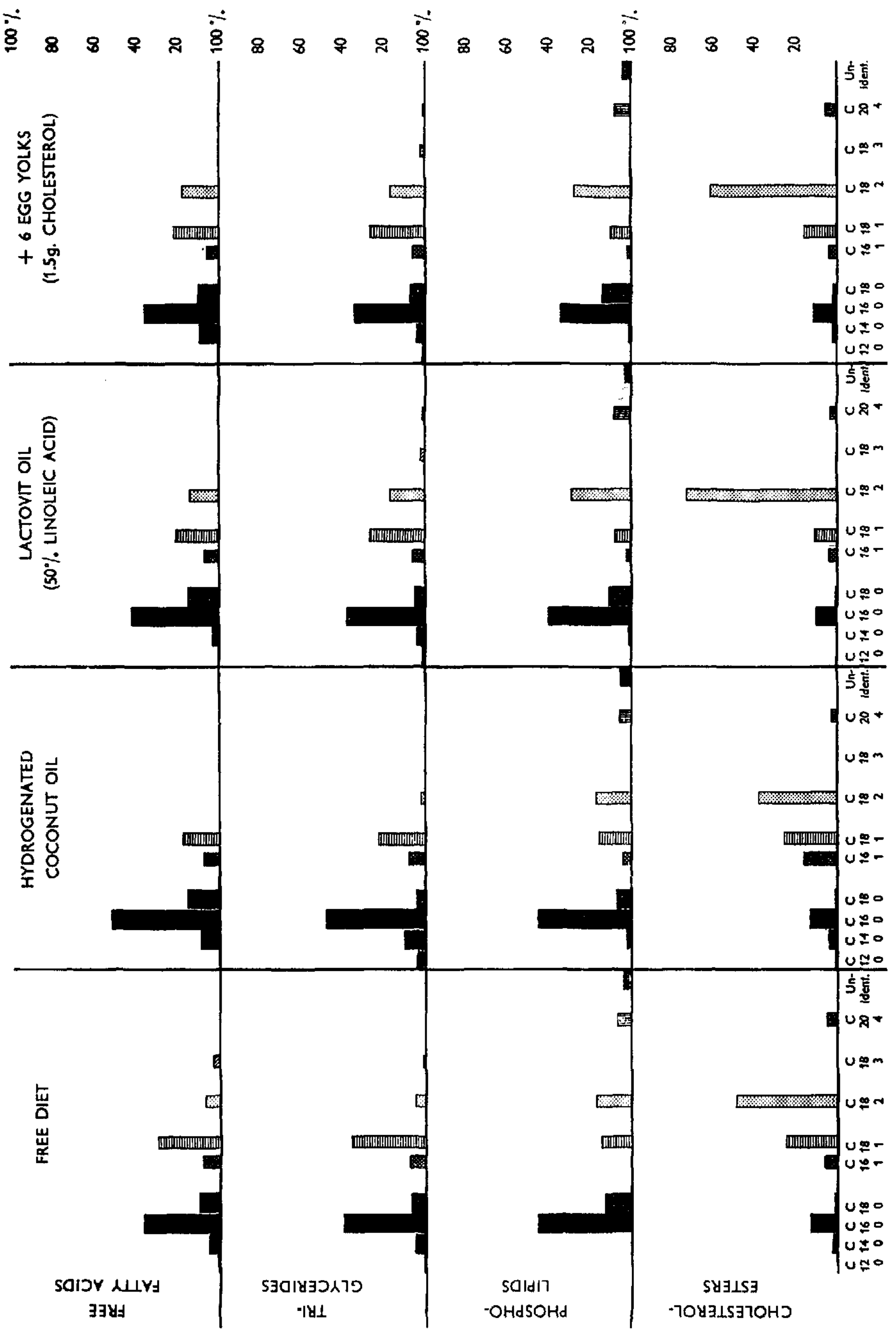

Fig. 2a. Fatty acid composition of subject H. B. (group A).
The fatty acids are designated by number of carbon atoms and number of double bonds.

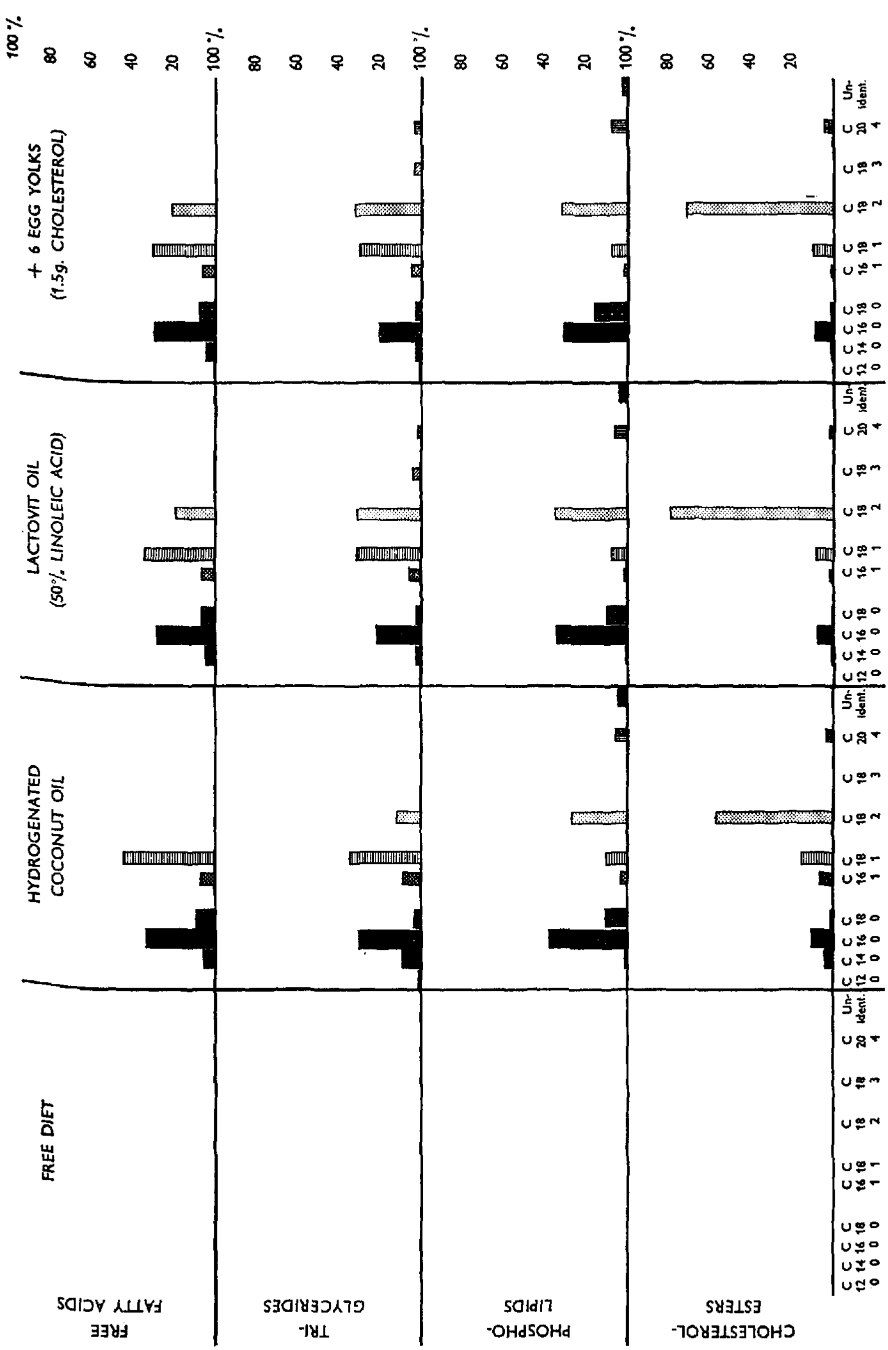

Fig. 2b. Fatty acid composition of subject H. G. (group A).
The fatty acids are designated by number of carbon atoms and number of double bonds.

Table 3. Fatty acid composition of serum triglycerides (weight%).
The figures represent mean values.
Student's t-test was applied to determine the statistical significans of the difference between two consecutive values.

Fatty acid	Dietary regimen (group A)			
	Free diet (n = 2)	HCO-diet (n = 3)	Lactovit-diet (n = 3)	Lactovit-diet + 6 eggs (n = 3)
Lauric	0.5	3.0	0.5*)	0.3
Myristic + Myristoleic	2.9	10.7*)	3.1**)	2.7
Palmitic	35.3	39.5	27.8	25.8
Palmitoleic	6.3	8.4	5.1*)	4.9
Stearic	5.2	3.9	3.3	4.5
Oleic	42.5	28.1	28.8	28.6
Linoleic	7.1	6.1	27.6*)	28.5
Linolenic	0.4	0.0	2.8**)	3.0
Arachidonic	0.0	0.0	0.9	1.8

Fatty acid	Dietary regimen (group B)			
	Free diet (n = 3)	Lactovit-diet (n = 3)	HCO-diet (n = 3)	HCO-diet + 6 eggs (n = 3)
Lauric	0.5	0.0	3.0	4.5
Myristic + Myristoleic	4.9	1.5*)	9.9	9.5
Palmitic	31.2	20.3*)	31.6**)	27.4
Palmitoleic	6.6	4.7	7.7*)	6.0
Stearic	4.0	2.4**)	3.3	5.0
Oleic	41.1	29.8**)	33.7	32.4
Linoleic	10.7	40.5**)	9.6**)	12.4
Linolenic	1.0	0.9	1.2	1.3
Arachidonic	0.0	0.0	0.0	1.6

*) $0.01 < P < 0.05$ for the difference between this value and the preceding value for this fatty acid

**) $P < 0.01$ for the difference between this value and the preceding value for this fatty acid

cholesterol ester fraction (figs. 2A, B, C). However, it cannot be decided at present whether the observed changes in fatty acid composition would be sufficient to account for an increased uptake of cholesterol by the lipoproteins according to the hypothesis of *Spritz* and *Mishkel*. It should also be noted that in spite of markedly depressed levels of polyunsaturated

Table 4. Fatty acid composition of serum free fatty acids (weight%).
The figures represent mean values.
Student's t-test was applied to determine the statistical significans of the difference between two consecutive values.

Fatty acid	Dietary regimen (group A)			
	Free diet (n = 2)	HCO-diet (n = 3)	Lactovit-diet (n = 3)	Lactovit-diet + 6 eggs (n = 3)
Lauric	1.2	0.4	0.0	0.0
Myristic + Myristoleic	4.3	8.2	4.1*)	4.9
Palmitic	37.4	43.1	33.6	31.9
Palmitoleic	6.7	7.1	6.2	5.1
Stearic	8.6	12.3	9.6	8.8
Oleic	32.6	28.9	27.9	26.6
Linoleic	7.0	0.0**)	18.6**)	21.7
Linolenic	2.0	0.0**)	0.0	1.0

Fatty acid	Dietary regimen (group B)			
	Free diet (n = 3)	Lactovit-diet (n = 3)	HCO-diet (n = 3)	HCO-diet + 6 eggs (n = 3)
Lauric	0.6	0.0**)	2.7*)	0.0*)
Myristic + Myristoleic	4.5	4.0	7.7	10.8
Palmitic	30.1	30.5	35.2	30.6
Palmitoleic	6.7	6.0	6.1	7.6*)
Stearic	9.7	8.6	9.9	9.3
Oleic	36.1	27.7	27.8	27.8
Linoleic	9.6	23.2**)	10.3**)	13.9
Linolenic	2.5	0.0**)	0.0	0.0

*) $0.01 < P < 0.05$ for the difference between this value and the preceding value for this fatty acid

**) $P < 0.01$ for the difference between this value and the preceding value for this fatty acid

fatty acids in the serum during the feeding of hydrogenated coconut oil, the addition of cholesterol to this diet did not appreciably raise the already high level of serum cholesterol. Possibly the previous feeding of hydrogenated coconut oil had already induced a maximal value in the lipid/protein ratio of the lipoproteins.

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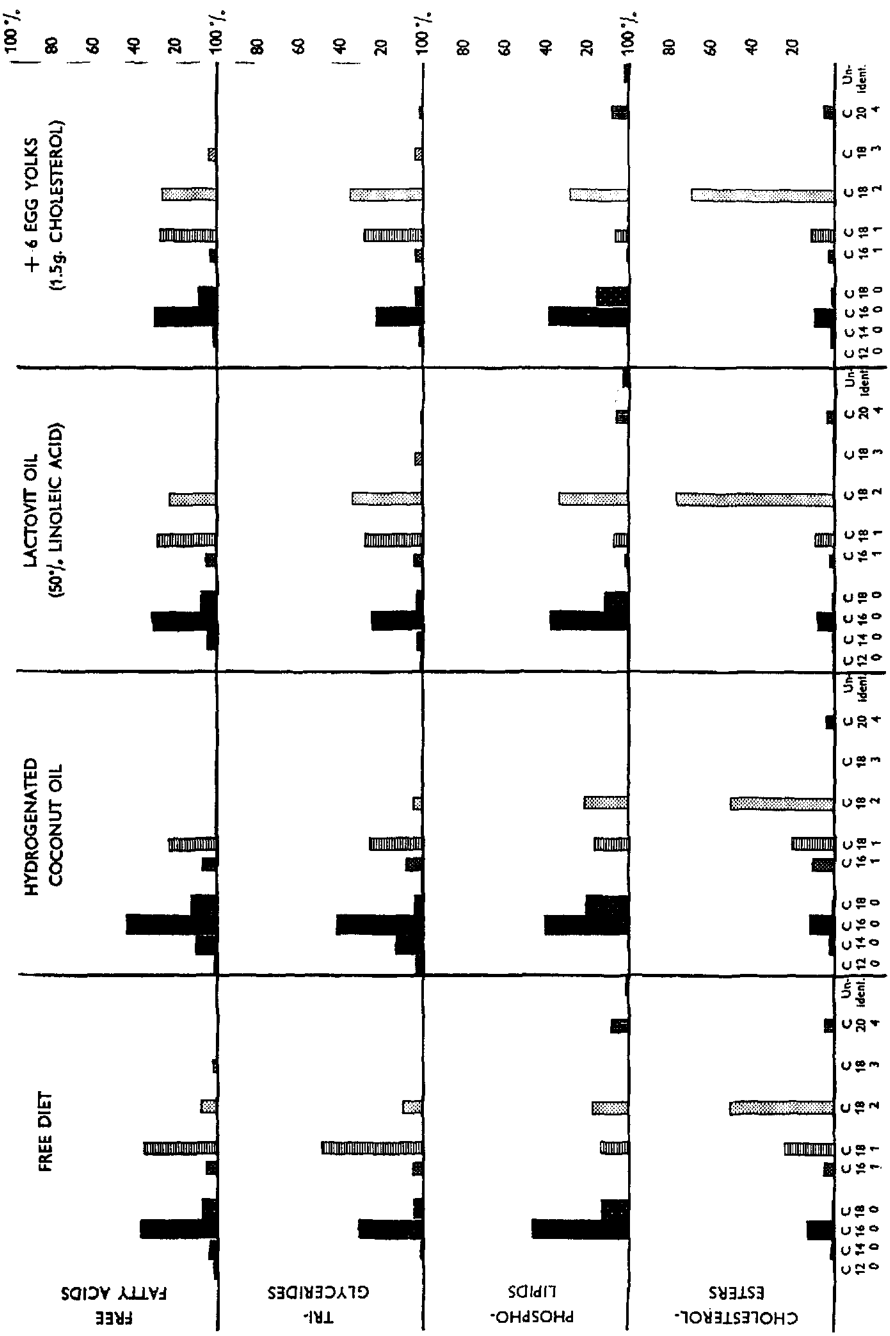

Fig. 2c. Fatty acid composition of subject A. H. (group A).
The fatty acids are designated by number of carbon atoms and number of double bonds.

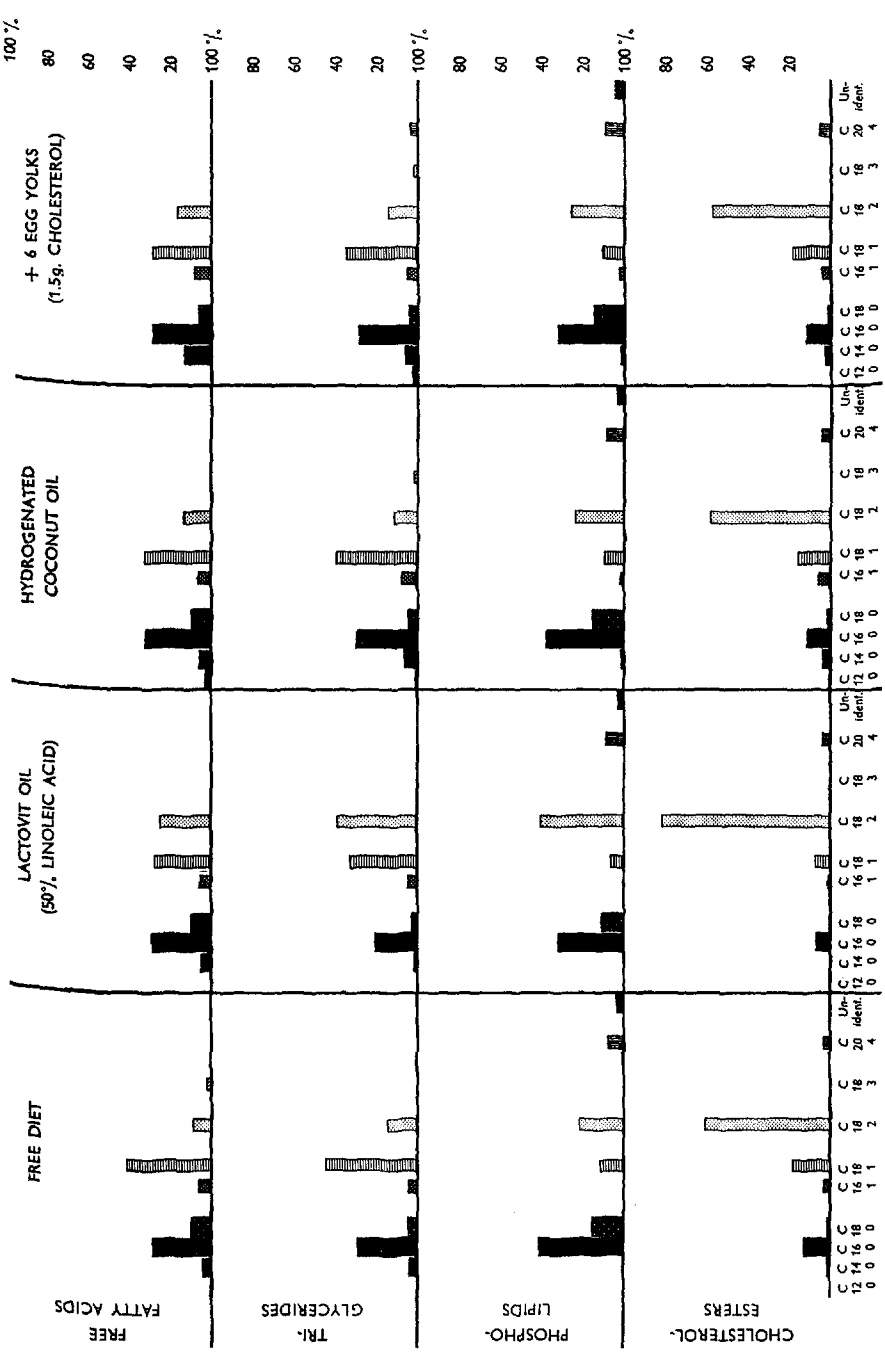

Fig. 2d. Fatty acid composition of subject N. N. (group B).
The fatty acids are designated by number of carbon atoms and number of double bonds.

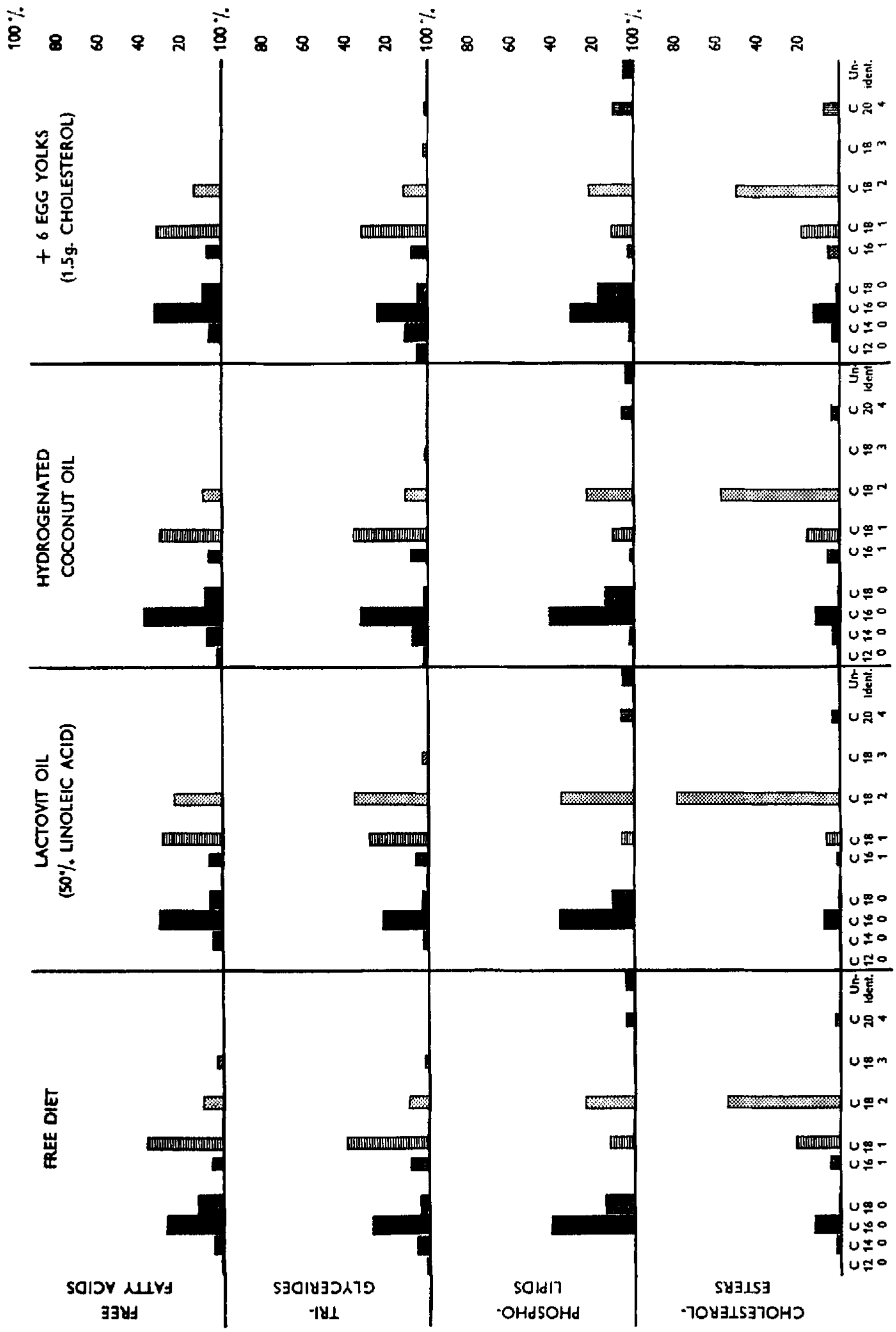

Fig. 2e. Fatty acid composition of subject G. P. (group B).
The fatty acids are designated by number of carbon atoms and number of double bonds.

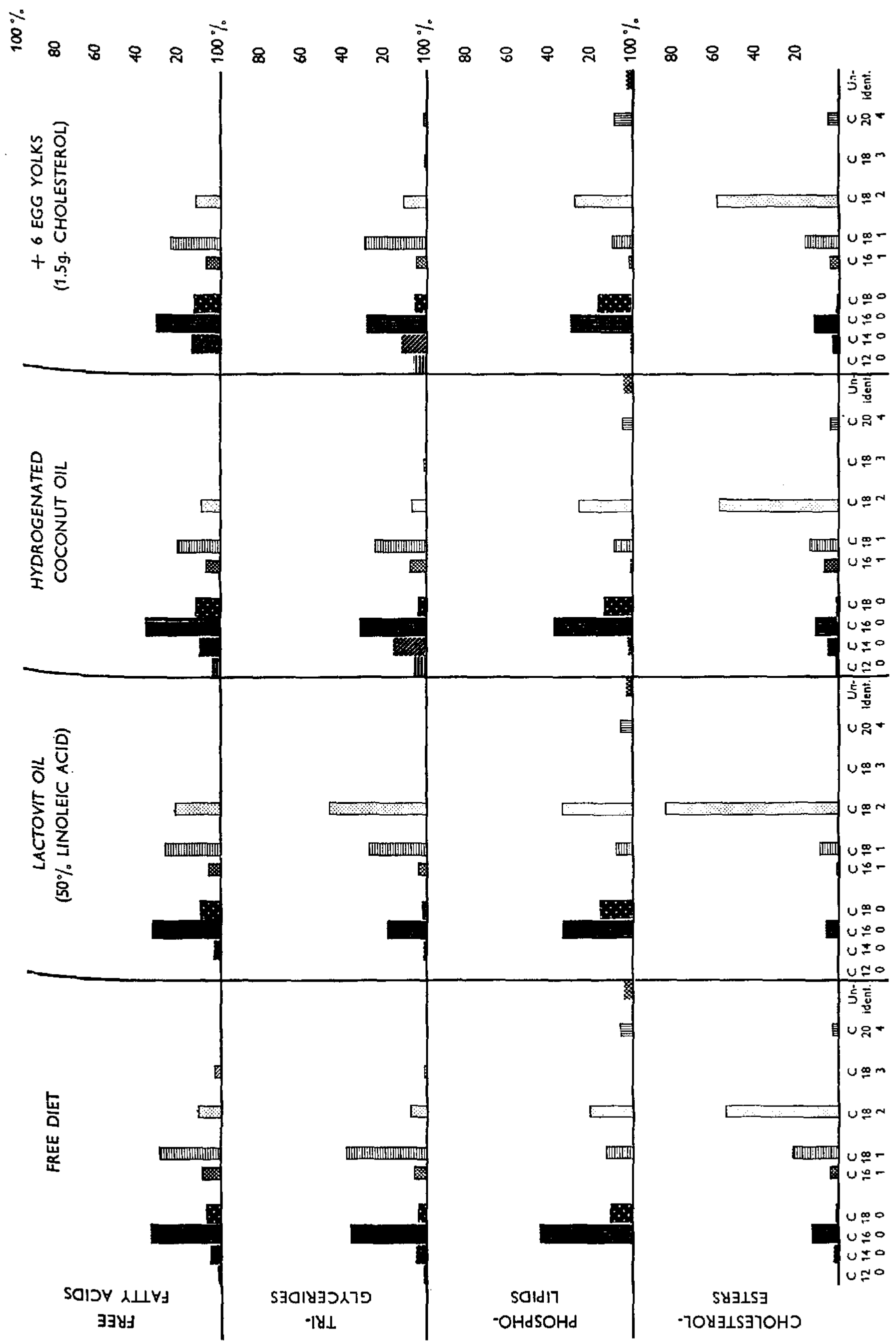

Fig. 2f. Fatty acid composition of subject G. S. (group B).
The fatty acids are designated by number of carbon atoms and number of double bonds.

Table 5. Fatty acid composition of serum phospholipids (weight%). The figures represent mean values. *Student's* t-test was applied to determine the statistical significans of the difference between two consecutive values.

Fatty acid	Dietary regimen (group A)			
	Free diet (n = 2)	HCO-diet (n = 3)	Lactovit-diet (n = 3)	Lactovit-diet + 6 eggs (n = 3)
Myristic + Myristoleic	0.0	1.0	0.6	0.6
Palmitic	46.1	41.1	37.4	34.8
Palmitoleic	0.0	2.4	1.4	1.5
Stearic	12.9	12.8	10.6	15.0
Oleic	14.1	14.3	7.6*)	7.9
Linoleic	17.3	21.7	32.9*)	29.4
Arachidonic	7.3	3.8	6.4	8.0
Unidentified	2.4	2.9	3.1	2.8

Fatty acid	Dietary regimen (group B)			
	Free diet (n = 3)	Lactovit diet (n = 3)	HCO-diet (n = 3)	HCO-diet + 6 eggs (n = 3)
Myristic + Myristoleic	0.0	0.0	1.4**)	1.6
Palmitic	42.1	33.7**)	39.0*)	30.8**)
Palmitoleic	0.0	0.0	1.4*)	2.3*)
Stearic	13.6	12.3	14.5	16.4
Oleic	12.1	6.7**)	9.6**)	10.6
Linoleic	22.2	36.6**)	24.1**)	25.1
Arachidonic	6.2	7.1	6.3	9.3
Unidentified	3.7	3.5	3.7	3.9

*) $0.01 < P < 0.05$ for the difference between this value and the preceding value for this fatty acid

**) $P < 0.01$ for the difference between this value and the preceding value for this fatty acid

Summary

Two groups of healthy male Swedes were fed an experimental diet providing 40% of the total calories as fat. One group received hydrogenated coconut oil (HCO) and the other group a mixed vegetable oil (Lactovit®) rich in linoleic acid. The diets were fed during 4 weeks and were then shifted between the groups. After another 3 weeks 6 egg yolks per day were added to the diets and the experiment continued for 10 days. The concentrations of total cholesterol and triglycerides in serum were determined every week. The fatty acid composition of serum cholesterol esters, triglycerides, free fatty acids and phospholipids was determined before the experiment and at the end of each dietary period. Changing the dietary fat from HCO to Lactovit caused a rapid decrease in serum cholesterol and increased significantly the proportion of linoleic acid in all serum lipid fractions. The opposite effects were recorded when changing from Lactovit to HCO. The highest proportion of linoleic acid, 80%, occurred in the cholesterol esters during the feeding of Lactovit. Linoleic acid disappeared completely from the free fatty acids on the HCO-diet. The addition of egg yolks to the diet increased serum cholesterol when Lactovit was the dietary fat but did not affect significantly the already high level of cholesterol on the HCO-diet. A clear-cut influence of the dietary fat on the concentration of serum triglycerides was not obtained.

Zusammenfassung

Zwei Gruppen gesunder schwedischer Männer erhielten eine experimentelle Diät mit einem Fettgehalt von 40% der Totalkalorien. Die eine Gruppe bekam als Fett hydriertes Kokosöl (HCO) und die andere Gruppe ein gemischtes Pflanzenöl (Lactovit®) mit einem hohen Gehalt von Linolsäure. Nach 4 Wochen fand ein Diätwechsel zwischen den beiden Gruppen statt und das Experiment wurde noch 3 Wochen fortgesetzt. Danach wurden der Diät noch 6 Eidotter täglich zugefügt und das Experiment nach 10 Tagen beendet. Die Konzentrationen von Gesamtcholesterin und Triglyceriden in Serum wurden wöchentlich einmal bestimmt. Vor dem Experiment und nach jeder Diätperiode wurde die Fettsäureverteilung von Cholesterinestern, Triglyceriden, unveresterten Fettsäuren und Phospholipiden in Serum bestimmt. Der Übergang von der HCO-Diät zur Lactovitdiät ging mit einer schnellen Senkung der Cholesterinkonzentration einher und die Proportion von Linolsäure erhöhte sich signifikant in sämtlichen Serumlipiden. Umgekehrte Veränderungen ergaben sich beim Austausch von Lactovit mit HCO. Die höchste Proportion von Linolsäure in den Serumlipiden, 80%, wurde in den Cholesterinestern nach den Lactovitperioden beobachtet. Während der HCO-Diätperiode enthielten die unveresterten Fettsäuren keine Linolsäure. Die Konzentration von Cholesterin in Serum erhöhte sich nach Zusatz von Eidottern zur Lactovitdiät. Bei HCO-Diät hatten Eidotter keinen Einfluß auf die schon hohe Cholesterinkonzentration. Ein deutlicher Einfluß der Diätfette auf die Konzentration der Serumtriglyceride konnte in diesem Experiment nicht festgestellt werden.

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