THE INFLUENCE OF LONG-TERM HIGH-FAT DIETS ON THE SERUM-LIPID PATTERNS OF WHITE AND BANTU SOUTH AFRICAN SUBJECTS IN A PRISON RESEARCH STUDY*

A. ANTONIS and I. BERSOHN, *South African Institute for Medical Research, Johannesburg*

Throughout the world ischaemic heart disease is the leading cause of death after 35 years of age. Evidence indicates that during the present century the incidence of the disease has increased markedly, and the mortality rate is still rising. In the USA 22·6% of the total mortality is due to ischaemic heart disease, while the figure for New York in 1955 was 33% (27,493 out of 81,612 deaths being due to ischaemic heart disease). In South Africa there is a notable difference between the prevalence of the disease amongst the White and Bantu populations. Amongst the White group the incidence is high. In an analysis of mortality trends from cardiovascular disease among the White population of South Africa during the period 1926 - 1954, it has been shown that the proportional mortality rate from ischaemic heart disease has increased by no less than sixteenfold. Formerly accounting for just under 1% of the total mortality, it now accounts for just over 16%.[1] Amongst the Bantu population on the other hand, ischaemic heart disease is an extreme rarity.

Studies on the epidemiology of ischaemic heart disease have shown that there is a marked difference in the prevalence of the disease in population groups subsisting on diets of varying lipid composition. In general, population groups having a high fat-calorie intake tend to have a higher prevalence of the disease than those groups subsisting on low fat-calorie diets. This is exemplified in South Africa by the notable difference between the incidence of coronary heart disease amongst the White group and the Bantu races. Amongst the White group the incidence is high and perhaps ranks amongst the highest in the world. In the Bantu coronary heart disease is an extreme rarity and very few cases of myocardial infarction are encountered in hospital practice.

From 1957 till 1960 a research project on diet and metabolism relating to cardiovascular disease was carried out on long-term White and Bantu prisoner volunteers at the Pretoria Central Prison by the South African Council for Scientific and Industrial Research in collaboration with the South African Institute for Medical Research. During the course of this investigation a study was made of the influence of short- and long-term dietary manipulation on serum-lipid concentration and composition. White and Bantu subjects were investigated while subsisting on a Bantu-type diet, as well as on a diet similar in composition to that consumed by the White population.

Subjects Investigated and Dietary Changes

None of the volunteers included in the study showed clinical manifestations of ischaemic heart or other disease. Electrocardiography and exercise tolerance tests were performed regularly under the supervision of a physician specialist. All volunteers underwent full biochemical screening, which included complete lipid studies, liver-function tests and serum-protein electrophoresis, as well as full blood counts and ESR investigations at regular intervals. Only volunteers whose screening tests confined them within the accepted range of normality were included in this investigation. However, a wider range for liver-function tests had to be accepted for the Bantu volunteers, since by White standards only very small numbers of the South African Bantu population would be considered as normal; nevertheless, no volunteers were accepted whose liver-function tests were grossly abnormal, and in all cases the 'bromsulphalein' dye-retention tests at the end of 45 minutes (5 mg. per kg. body weight) were within normal limits.

All volunteers were long-term prisoners whose ages ranged from 21 to 54 years (Whites: 29 to 54 years; mean, 38 ± 7 years. Bantu: 21 to 50 years; mean, 37 ± 9 years). White volunteers performed a normal day's work involving carpentry and typographical or other artisan labour. Bantu volunteers carried out less skilled labour, such as cleaning and sweeping. In no case was hard labour, such as stone-breaking, performed by any of the volunteers. Before the project was initiated volunteers had subsisted for periods of from 1 month to 15 years on a normal prison diet very similar in composition to the basal diet used in this study (Whites: 4 months to 15 years; mean 31 ± 33 months. Bantu: 1 month to 56 months; mean 14 ± 13 months).

The total food intake contained approximately 3,000 calories per day, and diets were maintained at this level throughout the experimental period.

The protein content of the diet was maintained throughout at approximately 15% of the total daily caloric intake.

Diets were manipulated in order to assess the influence of the following changes in the fat and carbohydrate content:

1. Change from low-fat high-carbohydrate to high-fat low-carbohydrate calories.

2. Different kinds of fat at the 40% caloric level.

3. Change from high-fat low-carbohydrate to low-fat high-carbohydrate calories.

Diet 1. The volunteers began the experimental course on this diet, which contained approximately 15% of fat calories from the fats and oils of the meat and vegetable foods, and 70% of carbohydrate calories, and is similar in composition to the usual urban Bantu diets.

Diet 2. The fat-calorie content of diet 1 was next increased to 40% by isocaloric substitution of different oils and fats for carbohydrate, which was reduced to 45% of the total calories. The volunteers were divided

*This study forms part of the programme of a prison research project on diet and metabolism relating to cardiovascular disease, conducted by the South African Council for Scientific and Industrial Research, and is published with their permission.

into 3 groups, the first having sunflower-seed oil (containing 63% linoleic acid) as the fat substitute, the second partially hydrogenated sunflower-seed oil (containing no linoleic acid), and the third butter. The last-mentioned diet closely resembles that consumed by the White population.

Diets 3 and 4. The fat composition of diet 2 was manipulated at the 40% caloric level, initially to contain a 50 : 50 mixture of sunflower-seed oil and butter (diet 3), and subsequently to contain a 75 : 25 mixture of sunflower-seed oil and butter (diet 4). These two diets were successively consumed immediately after diet 2 and just before diet 5.

Diet 5. Volunteers were now redivided into two groups. Those who had received the butter in diet 2 now received oil instead, while those who received the oil were given butter instead; and the volunteers who had initially received the partially hydrogenated oil were given either oil or butter substitutions.

Diet 6. All the volunteers were finally put on this diet, which was the same as diet 1.

Duration periods of diets. Diet 1 — 39 weeks. Diet 2 — 51 weeks. Diets 3 and 4 — 18 and 9 weeks, respectively. Diet 5 — 8 weeks. Diet 6 — 32 weeks.

RESULTS

SERUM CHOLESTEROL AND PHOSPHOLIPID CONCENTRATION

The mean concentrations of the various serum-lipid components of the different diets are shown in Table I. Prisoners were maintained on the initial base diet (diet 1, 15% fat calories) for 9 months in order to obtain statistically valid results. The mean basal lipid levels of both racial groups of prisoners were very similar and very much lower than for a comparable European population not in prison, agreeing more closely with those of the Bantu population (*cf.* Table II).

After the first dietary change (to diet 2) the mean total serum-cholesterol level for the sunflower-seed-oil groups (combined White and Bantu) fell by about 10%; the corresponding changes for the hydrogenated-oil and butter groups were increases of about 20% and 45%, respectively. Serum-phospholipid levels showed similar but smaller changes of about − 15%, + 5% and + 20%, respectively. As a result, the cholesterol/phospholipid (C/P) ratios increased on all the high fat-calorie diets. The change was the greatest for the butter group and least for the sunflower-seed-oil group, that in the hydrogenated-fat group being intermediate. Similar changes were shown in the beta-lipoprotein cholesterol and phospholipid levels, again resulting in raised C/P ratios. In general, the percentage of total cholesterol and total phospholipid in the beta-lipoprotein tended to rise slightly on the butter diet, remain steady on the hydrogenated-oil diet, and fall slightly on the sunflower-seed-oil diet. The percentage of total serum lipids in the beta-lipoprotein showed the same pattern as the other lipids. The ratios serum-cholesterol ester/total cholesterol (not shown in the table) were unaffected by any of the dietary changes.

The changes shown above took place fairly rapidly, and stable levels of serum lipids were reached within a few weeks on diet 2. Over the long-term periods of nearly 2 years, however, a number of significant findings emerged which were not apparent during the early stages of the experiment. Whereas the short-term feeding of dietary fats at the 40% caloric level had raised or lowered serum-cholesterol and phospholipid levels (depending on the nature of the dietary fat), with simultaneous alteration in the C/P ratio, the effects of this change, particularly with the oil diets, were small. After 1½ years on the high-fat diets, however, the C/P ratios were markedly raised even on sunflower-seed oil, and in all cases the initial mean ratio of 0·8 normal for prisoners of both races (corresponding fairly closely to that of the non-prisoner Bantu population) had increased to a mean ratio of almost 1·0, which corresponds to that of the older normal White population and also of patients with ischaemic heart disease.

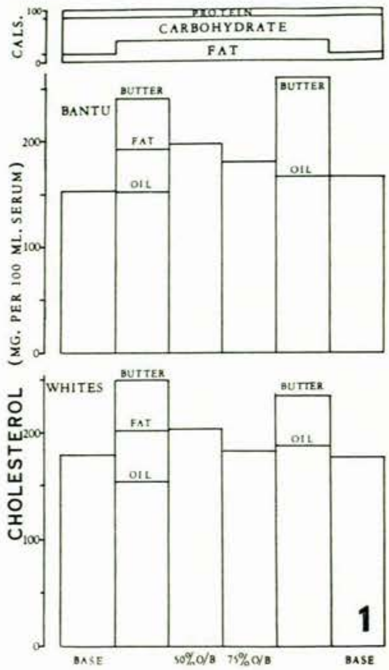
These results show (1) that saturated dietary fats at the 40%

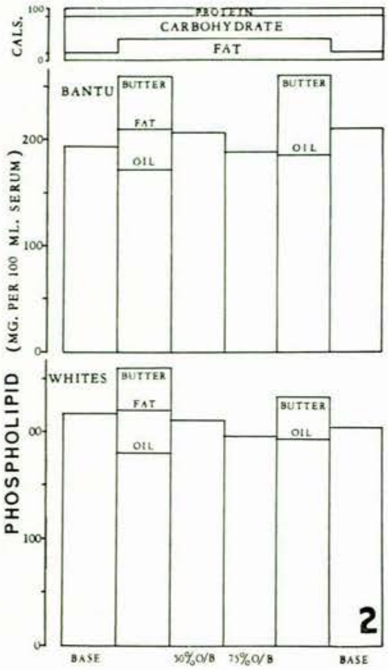
TABLE I. INFLUENCE OF DIET ON MEAN SERUM - LIPID PATTERNS IN WHITE AND BANTU VOLUNTEERS

Diet	Duration (weeks)	Extra fat calories	Cholesterol (mg./100 ml.)	Phospholipid (mg./100 ml.)	Ratio C/P	β-cholesterol (mg./100 ml.)	β-cholesterol %	β-phospholipid (mg./100 ml.)	β-phospholipid %	Ratio βC/βP	β-lipoprotein lipid %
White volunteers											
15% fat calories	39	—	179	216	0·83	134	75	136	63	0·99	65
40% fat calories	51	Butter	249	259	0·96	197	79	171	66	1·15	75
		Fat	202	219	0·92	160	79	145	66	1·11	80
		Oil	154	179	0·86	108	70	104	58	1·04	67
	18	50% O/B	205	210	0·97	152	75	137	65	1·11	70
	9	75% O/B	182	195	0·93	133	73	117	60	1·14	65
	8	Oil	187	192	0·97	137	73	116	61	1·18	70
		Butter	234	232	1·01	187	80	153	66	1·22	73
15% fat calories	32	—	176	203	0·87	132	75	120	59	1·10	71
Bantu volunteers											
15% fat calories	39	—	156	194	0·80	114	73	120	62	0·95	62
40% fat calories	51	Butter	240	260	0·92	185	77	164	63	1·13	74
		Fat	192	210	0·91	138	72	128	61	1·08	74
		Oil	154	172	0·89	104	68	100	58	1·04	67
	18	50% O/B	197	207	0·95	141	72	126	61	1·12	68
	9	75% O/B	180	188	0·96	128	71	107	57	1·20	61
	8	Oil	166	185	0·90	110	66	100	54	1·10	62
		Butter	259	261	0·99	194	75	162	62	1·20	73
15% fat calories	32	—	166	210	0·79	126	76	118	56	1·07	68

TABLE II. LIPID PATTERNS OF BANTU AND WHITE SUBJECTS (31-40) YEARS) (MG. PER 100 ML. SERUM)

	Natural Bantu diet ± 15% fat calories	*Natural White diet ± 40% fat calories*	*'Bantu type' prison diet: 15% fat calories*		*'White type' prison diet: 40% fat calories (butter)*	
	Bantu subjects	*White subjects*	*Bantu prisoners*	*White prisoners*	*Bantu prisoners*	*White prisoners*
Cholesterol	150-160	220-230	156	179	240	249
Phospholipid	220-230	250-265	194	216	260	259
C/P ratio	0·70	0·90	0·80	0·83	0·92	0·96
% cholesterol in β-lipoprotein	69%	75%	73%	75%	77%	79%
% total lipid in β-lipoprotein	60%	70%	62%	65%	74%	75%





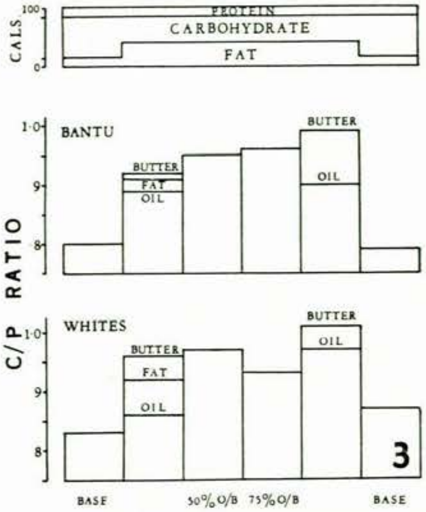


Fig. 1. Influence of dietary fat on serum-cholesterol levels in European and Bantu volunteers.
Fig. 2. Influence of dietary fat on phospholipid levels in Bantu and European volunteers.
Fig. 3. Influence of dietary fat on the cholesterol/phospholipid ratio in Bantu and European volunteers.

Base diet contains approximately 15% fat calories; high-fat diets contain approximately 40% fat calories; 50 and 75% O/B refer to mixtures of oil and butter. Fat refers to hydrogenated sunflower-seed oil; B to butter; and oil (O) to sunflower-seed oil.

fat-calorie level, which elevate the serum-cholesterol concentration, do not elevate the serum-phospholipid concentration to the same extent, and (2) that unsaturated dietary oils (at a high calorie level) which lower the serum-cholesterol concentration, lower the serum-phospholipid concentration to a greater extent. In each case the result is a greater proportion of cholesterol to phospholipid. Results are shown graphically in Figs. 1, 2 and 3. This change is reflected exactly in the beta-lipoproteins. When the diets were again changed back to the low fat-calorie level (15%), the base levels of cholesterol and phospholipid were readily resumed, the C/P ratios returning to their previously low levels. It would therefore appear that the ratio of cholesterol to phospholipid in the serum is directly related to the amount of fat in the diet (since even on the sunflower-seed-oil diet the C/P ratios were high). The reasons for these changes are not clear, but their importance may lie in the role the phospholipids play in stabilizing the beta-lipoprotein complex, since phospholipids appear to be necessary for maintaining insoluble lipids in stable aqueous emulsions.

INFLUENCE OF DIETARY FAT ON SERUM-TRIGLYCERIDE CONCENTRATION

(*a*) *Changes produced by an Increase in the Proportion and Alteration in the Nature of the Dietary Fat Calories*

The results (see Fig. 4) show that White and Bantu subjects when subsisting for long-term periods on a particular diet (either high or low in fat caloric content) have fasting serum-triglyceride levels not significantly different from each other, and, furthermore, the composition of their serum-triglyceride fatty-acid components is almost identical. Changes, whether in the nature of the calories (by substitution of fat for carbohydrate) or in the type of fat consumed at the 40% caloric level, produced an identical response in each racial group. We have also shown that faecal lipid excretion is also similar in the two racial groups,[2] and it is apparent that no significant racial differences exist in the mechanisms of fat transport, utilization and excretion, when these groups are habituated to the same diet.

On the base diet containing 15% of fat calories the mean fasting serum-triglyceride level of both groups showed a progressive response to increase in the fat caloric content from 15 to 40%. The replacement volunteers included towards the end of the study consumed the high-fat diet for 8 weeks only, and their fasting serum-triglyceride levels at the end of this period were still low. Although basal values were not obtained for this group before the initiation of the high-fat diet, no significant changes occurred when they were subsequently habituated to the low-fat diet. This might indicate that the immediate response to an increase in the proportion of fat calories was negligible, a finding also confirmed by other workers.[3] In the early part of this study values were estimated at relatively long periods after the change in the proportion of the dietary fat calories. Twenty-two weeks after the change to hydrogenated oil or butter, the fasting serum-triglyceride levels were just significantly higher than the corresponding levels on the 15% fat-calorie diet; by 51 weeks, however, the increase was highly significant. The period of habituation to the saturated fat diet was only for 1 year, but even in this relatively short period significant changes had occurred, and the mean serum-triglyceride levels of both racial groups (124·1 and 127·1 mg. per 100 ml., respectively) were higher than the upper limit of normal (114 mg. per 100 ml. serum) suggested in our previous study.[4] In contrast to their response to the saturated-fat diets, both groups of prisoners, when consuming the sunflower-seed-oil diet, maintained their low basal serum-triglyceride levels (Whites 81·9 and Bantu 83·0 mg. per 100 ml., respectively).

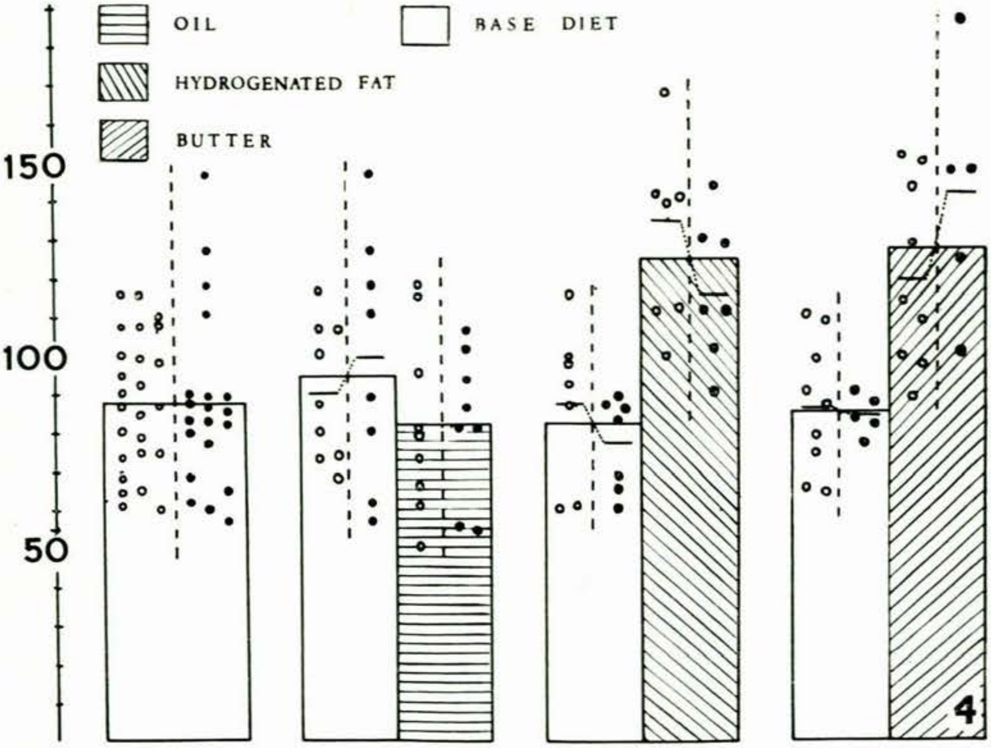


Fig. 4. Influence on fasting serum-triglyceride levels of increasing the proportion and altering the nature of the dietary fat calories.

Results are shown for the base diet (15% fat, 70% carbohydrate calories) as unshaded bars, and after a year on the high-fat diets (40% fat, 45% carbohydrate calories) as shaded bars. The first unshaded bar represents the mean for all volunteers on the base diet. The subsequent unshaded and shaded bars represent the means for each group of volunteers before and after the dietary change respectively. Open circles represent White volunteers; closed circles, Bantu volunteers. The tops of the bars represent the mean values for both groups of volunteers combined. The short bars above and below the means for the combined groups represent the means for each racial group; where no short bars are shown the means for the racial and combined group are identical.

The results in our previous study[4] of these racial groups subsisting on their natural diets indicated significant differences, particularly when older subjects were compared, both in the absolute fasting serum-triglyceride levels and in the composition of the fatty-acid components. These differences were slight when young subjects of both races were compared, but became more significant with increasing age, since the Bantu subjects evinced no age trend while the levels of the White subjects increased with increasing age. The present study indicates that the consumption of diets low in fat calories or high in their content of vegetable oils (i.e. rich in the proportion of highly unsaturated fatty acids) maintains serum-triglyceride concentrations at a low level, whereas high fat-calorie diets which are rich in their content of saturated fatty acids raise the serum-triglyceride levels. The nature of the fat calories in high fat-calorie diets is therefore of considerable importance in controlling the absolute serum-triglyceride level, and the age trend among the White subjects consequently appears to be a result of habituation to the high-saturated-fat diet; their triglyceride metabolic processes altering in some manner and leading to increased serum-triglyceride levels with increasing age — the process apparently being more rapid in some individuals than in others. Habituation to low-fat diets on the other hand has no such effect, and the low serum-triglyceride levels of the Bantu subjects (75 mg. per 100 ml. serum) appear to be primarily related to their low fat-calorie diet, an observation confirmed in the present study by the low serum-triglyceride levels of the White prisoners when habituated to the Bantu-type low fat-calorie diet (87·9 mg. per 100 ml. serum).

(b) Changes produced by Decreasing the Proportion of Fat Calories

When the proportion of dietary fat calories was reduced from 40% to 15%, certain racial and interracial differences soon became apparent (Fig. 5). While the fasting serum-triglyceride levels of 88% of the Bantu volunteers had returned to their low base levels by 12 - 14 weeks, the corresponding values for the White volunteers were still elevated, and base levels were reached by 68% of the latter only after 17 - 20 weeks. At this stage, however, the remaining 32% of the White volunteers and 12% of the Bantu volunteers still had considerably elevated levels; these finally returned to the low base levels after 32 weeks. The more rapid return to their base levels by the majority of the Bantu volunteers is probably due to the close similarity between this diet and that to which they are naturally accustomed. It could therefore be expected that they would readily become adapted to this diet. The White volunteers, on the other hand, who are naturally accustomed to high fat caloric intake, have to become adapted to a less familiar dietary environment.

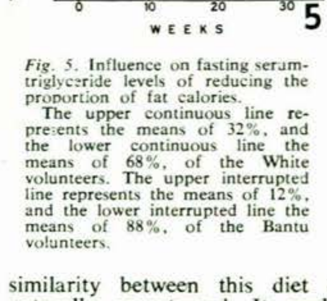


Fig. 5. Influence on fasting serum-triglyceride levels of reducing the proportion of fat calories.

The upper continuous line represents the means of 32%, and the lower continuous line the means of 68%, of the White volunteers. The upper interrupted line represents the means of 12%, and the lower interrupted line the means of 88%, of the Bantu volunteers.

The slow return to their base levels by almost a third of the White volunteers may also be related to the observation in our previous study that the older White male population was not homogeneous — different sections showing different age trends as indicated by the significant increase in the standard deviation with increasing age. This would appear to lend further support to the implication that the older overtly normal population consists of a mixture of true normals with a large proportion of subjects who may represent potential cases of ischaemic heart disease.

Before the increase in the proportion of fat calories, volunteers of both races had subsisted for at least 1 year on a low-fat diet, and their mean fasting serum-triglyceride levels were low. Even after a period of about 2 years on high fat-calorie diets which resulted in elevated serum levels for considerable periods, all levels had returned to the low base values by about 6 months, when the low fat-calorie diet was reinstituted. Since both groups again had low fasting serum-triglyceride levels it is apparent that low-fat diets which may temporarily elevate serum-triglyceride levels eventually reduce values that have been raised through habitual consumption of high-fat diets.

It is clear from this aspect of the trial, however, that it is important to consider the previous dietary history of subjects before changes in dietary habits are recommended, since by curtailing the proportion of dietary fat calories, the possibility exists of temporarily raising previously elevated serum-triglyceride levels even higher before lowered values finally result. In view of recent work which appears to indicate a close relationship between hyperlipaemia on the one hand and accelerated blood clotting and decreased blood fibrinolytic activity on the other, the production of even a temporary lipaemia may be inadvisable in patients with ischaemic heart disease.

Although there is some parallel between cholesterol (or beta-lipoproteins) and triglycerides in the blood, this relationship does not exist in all persons. Thus, a given individual might have normal values for serum cholesterol and yet have very high values for serum triglycerides. This fact becomes especially important when one considers the implication of prescribing special diets. Many investigators have demonstrated that a diet restricted in fat will often result in a decrease in the concentration of cholesterol in the blood. This same diet will, if the calories thus removed are replaced by carbohydrate, cause marked hypertriglyceridaemia.[5,6] Our own work here shows that in the majority of cases this condition returns to normal after a variable period.[7]

INFLUENCE OF DIETARY FAT ON SERUM-LIPID FATTY-ACID COMPOSITION

The influence of dietary fat on the fatty-acid composition of the various lipid classes (cholesterol esters, phospholipids, and triglycerides) is very marked. Changes take place fairly rapidly and after 7 weeks on a particular diet stable patterns are obtained.

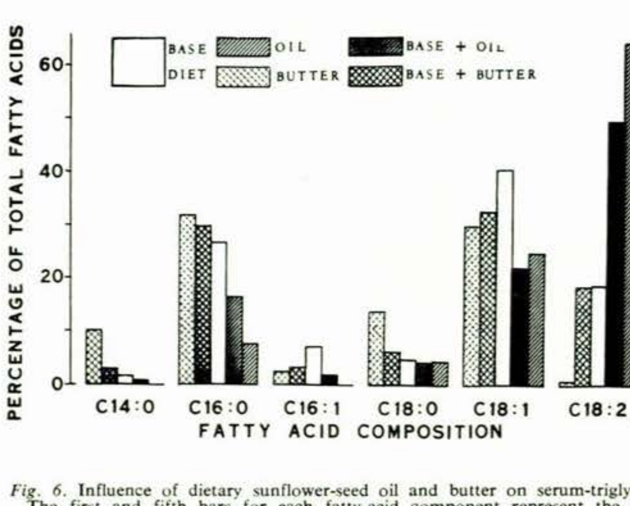
The fatty-acid composition of the various diets was not analysed. However, since 15% of the fat calories (derived mainly from the meat of the diet) were unaltered throughout, the changes in the serum-lipid fatty-acid patterns can be compared in relation to the composition of the dietary fat substituted for carbohydrate; that is, 25% of the total fat calories.

Triglyceride Fatty Acids

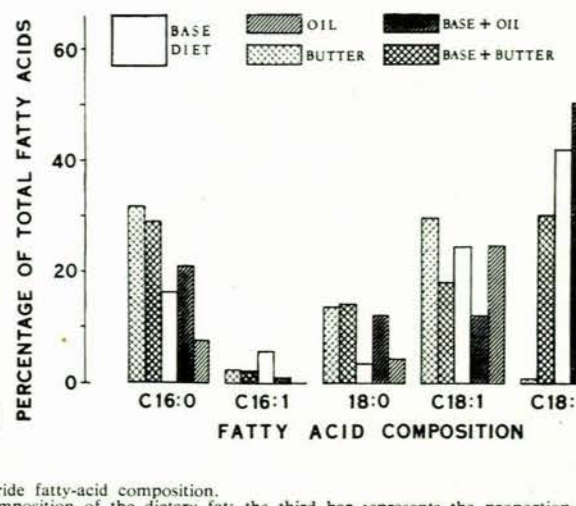
When part of the carbohydrate of the basal diet was changed to butter or hydrogenated sunflower-seed oil, there were no significant changes in the serum pattern of triglyceride polyenoic fatty acid. The similar change by substitution of sunflower-seed oil, however, caused a significant increase in the proportion of dienoic fatty acids. Not only were the polyenoic fatty-acid patterns obtained on the butter and base diets almost identical, but examination of the total fatty-acid patterns (obtained by G/L chromatography) revealed that the proportion and nature of the other fatty-acid constituents were also very similar. The major changes produced in the serum patterns of triglyceride fatty acid of volunteers on the basal diet by an alteration in the nature and proportion of the fat calories (shown graphically in Fig. 6) were as follows:

Substitution of sunflower-seed oil resulted in an increase in the linoleic-acid content (from 18% to approximately 50%) with a proportional decrease in that of palmitic, palmitoleic and oleic acids. The stearic-acid content was unchanged, and the oleic/stearic acid ratio was decreased from 8·75 to 5·58. *Substitution of butter* caused an increase in the content of palmitic and stearic acid, while that of palmitoleic and oleic acids fell, the oleic/stearic acid ratio again decreasing to 5·4.

The results indicate that the total triglyceride fatty-acid composition of the fasting serum bears a strong resemblance to that of the dietary fat substituent, as has been previously reported by Ahrens and his co-workers.[8] In this latter study, when menhaden oil was used as the sole dietary fat the linoleic-acid content of the serum-triglyceride fatty acids fell to 8·4% in one patient. Since the corresponding proportion of serum-triglyceride linoleic acid in the present study showed virtually



6



7

Fig. 6. Influence of dietary sunflower-seed oil and butter on serum-triglyceride fatty-acid composition.
The first and fifth bars for each fatty-acid component represent the composition of the dietary fat; the third bar represents the proportion of fatty acid in the basal serum triglyceride; and the second and fourth bars represent the proportion of fatty acids in the serum triglycerides on the high-fat diets. The serum-triglyceride fatty-acid composition clearly reflects that of the dietary fat.

Fig. 7. Influence of dietary sunflower-seed oil and butter on serum-cholesterol-ester fatty-acid composition.
The first and fifth outer bars for each fatty-acid component represent the composition of the dietary fat; the third bar represents the proportion of fatty acid in the basal serum cholesterol ester; and the second and fourth bars represent the proportion of fatty acids in the serum cholesterol esters on the high-fat diets. The serum-cholesterol-ester fatty-acid composition tends to reflect that of the dietary fat.

no change from 18% on either the basal or high butter-fat diets, it would appear either that the former diet still contained a fair proportion of linoleic acid (probably of vegetable origin), or that the subjects were capable of synthesizing linoleic acid from endogenous sources.

While the basal diet in the present study and the natural urban Bantu diet have a similar fat-calorie content (15%), the nature of the fat constituents in these diets differs considerably. The former diet, which contains a considerably greater proportion of meat and lesser proportion of whole maize than the latter,[9] is considerably richer in its content of linoleic acid, and this may account for the lower proportion of linoleic acid in the serum triglycerides of the volunteers on the basal diet as compared to that of the urban Bantu in a previous study (18·3% as against 30·7%). This would also appear to be confirmed by the changes in the triglyceride fatty-acid patterns produced by the different high fat-calorie diets.

Cholesterol-Ester Fatty Acids

When the proportion of fat calories was raised from 15% to 40% by isocaloric substitution of butter or hydrogenated sunflower-seed oil for carbohydrate, the linoleic-acid content of the cholesterol-ester fatty acids fell from 45% to about 35%, while that of arachidonic and hexaenoic fatty acids was also slightly reduced. Substitution by sunflower-seed oil caused an increase in the linoleic-acid content of the cholesterol-ester fatty acids from 45% to approximately 55%, but the content of more highly unsaturated fatty acids was again slightly reduced. These results were obtained by the alkali isomerization procedure. Comparison of the total fatty-acid patterns on butter, sunflower-seed oil or low fat-calorie diets, obtained by the gas-liquid chromatography technique and shown graphically in Fig. 7, revealed significant changes in the proportion of the more saturated cholesterol-ester fatty-acid components. When butter constituted the additional 25% dietary fat calories the proportion of palmitic and stearic acids tended to increase, while that of palmitoleic, oleic and linoleic acids fell. With the sunflower-seed-oil diet, the proportions of palmitic, stearic and linoleic acids all rose, while that of palmitoleic and oleic acids fell. The general pattern of changes produced in the cholesterol-ester fatty acids by the two dietary fats was similar in character to those produced in the triglyceride fatty acids. It is clearly apparent that the cholesterol-ester fatty-acid composition may also be varied by the dietary fat constituents, although to a much lesser extent than that of the serum triglycerides. A notable difference is that the stearic- and palmitic-acid proportions have risen on either of the two high-fat diets. Since sunflower-seed oil has a very low proportion of these acids, it is apparent that their increased proportion on the high-fat diets represents an endogenous rather than an exogenous source for these fatty acids.

Phospholipid Fatty Acids

The composition of the phospholipid fatty acids showed the least change on alteration of the proportion and nature of the dietary fat calories. On the hydrogenated-fat or butter diets there was a tendency for the proportion of dienoic acid to fall, from about 22% of the total fatty acids to about 16%, while on the sunflower-seed-oil diets it rose slightly by approximately 3%, a slight rise also being produced in the proportion of the hexaenoic acid. The changes in the proportion of more saturated fatty acids were also very slight.

DISCUSSION

Effects of Dietary Fats on Serum-Lipid Levels

The dietary studies outlined above have shown the manner in which the concentration of the various serum-lipid classes responds to manipulation of dietary fats and oils. When these results are viewed in relation to epidemiological studies on 'normal' populations, a number of important associations are revealed. The broad picture shows that when the volunteers subsisted on diets like those of normal population groups their serum-lipid levels were also alike. For example, when the volunteers were fed on the low-fat diet similar to that of the urban Bantu, their serum-lipid levels were all low — Bantu and White serum-lipid levels being almost identical. On the high butter or hydrogenated sunflower-seed-oil diets of the White population not living in prison, serum-lipid levels were all elevated, resembling those of the 'normal' White population. In the experiments where the fat calories were contributed to mainly by sunflower-seed oil, the tendency was to produce even lower serum-lipid levels than those caused by low-fat diets. This would correspond to the low serum-lipid levels of populations who subsist on high-vegetable diets or diets with high marine-animal and fish-oil calories, such as rural Greeks and Italians, Yemenite Jews, and Eskimos.

It is not clear why, although the low serum-lipid concentrations produced by the high fat-calorie sunflower-seed-oil diet are clearly associated with a significant increase in the proportion of linoleic acid in the fasting serum lipids, it is that on saturated-fat diets the proportion of linoleic acid in the raised serum-lipid levels is considerably lower. On the high-oil diet the lowered concentrations of cholesterol esters are rich in their proportion of linoleic acid, while on the butter diets the raised cholesterol-ester concentrations have a lower proportion of linoleic acid. It is perhaps significant that the total concentration of cholesterol esterified with linoleic acid (± 100 mg. per 100 ml. serum as cholesterol linoleate) is approximately the same on all the high- or low-fat calorie diets. This relative constancy would appear to indicate a definite role for cholesterol linoleate in lipid metabolism and possibly in fat transport, and would appear to be independent of the diet. The value of 100 mg. per 100 ml. of serum is the mean for the group as a whole, however, since different individuals within the group may have lower or higher basal levels. It may be rather that there is a constant value for a particular individual and it is this value that tends to remain constant. If the pool of fatty acids available for esterification of cholesterol is dependent on and governed by the composition of the dietary fat (probably through alteration of the composition of the depot fats by different dietary fats) then this may offer a reason for the stabilization of cholesterol levels on a particular diet. When the proportion of linoleic acid is higher in the pool available for esterification of cholesterol, then an increased proportion of the cholesterol esters will be combined with linoleic acid. Conversely, with low proportions of available linoleic acid there would be a decreased proportion of cholesterol esters combined with linoleic acid. In order to maintain a constant proportion of cholesterol linoleate in the serum the total serum-cholesterol level would be higher or lower depending on the composition of the available fatty-acid pool, and therefore on the composition of the dietary fat. Although linoleic acid has been considered here, it is probable that the more highly unsaturated fatty acids are also part of this mechanism, and it is probably the total amount of highly unsaturated fatty acids that is significant, since highly unsaturated fish oils, poor in their content of linoleic acid, have the same effect.

The composition of the triglyceride fatty acids has also been shown to be dependent on that of the dietary fat. As in the case of the cholesterol esters, the low serum-triglyceride concentrations produced by the sunflower-seed-oil diet are clearly associated with an increased proportion of linoleic acid in serum-triglyceride fatty acids. The comparatively similar fashion in which the composition and concentration of the cholesterol esters and triglycerides respond to dietary manipulation would suggest a common fatty-acid pool for both classes of esters. On the saturated-fat diets, however, there is a significant difference in their concentration patterns. While the serum-cholesterol concentration reaches its elevated level fairly rapidly, the fasting serum-triglyceride concentration rises at a slower but equally significant rate, the end-result being raised levels of both of these lipid classes.

Although the serum-phospholipid fatty-acid composition does not appear to vary significantly, its concentration is also dependent on the dietary fat. All three classes of serum lipids would therefore appear to be closely related in the mechanisms of fat utilization. Since all the changes in the serum lipids are closely reflected by corresponding changes in the beta-lipoproteins, this would indicate a major role for the beta-lipoproteins in fat metabolic processes.

A recent study carried out by Mead and Fillerup[10] has shown that dietary fatty acids are incorporated at different rates into the various blood-lipid components. They found that, when carboxy-labelled stearate, oleate and linoleate were given to rats, a distinctively large proportion of the labelled linoleate appeared very rapidly in the plasma phospholipids, whereas the oleate and stearate were incorporated into the phospholipids at a very much slower rate. Since all these fatty acids enter the blood almost entirely as triglycerides in lymph chylomicrons, they suggested that this indicated rapid preferential incorporation of linoleate into higher-density lipoproteins of the blood. In our present study, therefore, the possibility exists that triglycerides of different fatty-acid composition also have different plasma turnover rates (e.g. trilinolein may be transported away from the plasma at a faster rate than triolein or tristearin), which may then influence other triglyceride metabolic processes of fat clearance and utilization. An effect of habituation to diets rich in their content of unsaturated fatty acids may therefore be that increased rates of triglyceride removal from the blood stream result in lower residual serum-triglyceride levels. Conversely, habituation to high saturated-fat diets may decrease fat-clearance rates, the net result being elevated residual serum-triglyceride levels.

Our own studies with ^{131}I-triolein in White and Bantu subjects on their natural diets would appear to lend strong support to this concept.[11] We found that the ingestion of ^{131}I-triolein led to lower initial levels of plasma radioactivity in Bantu subjects than in White subjects, both with and without ischaemic heart disease. Furthermore, in the Bantu subjects the peak values tended to occur at an earlier period after the ingestion of the fat load. The measured levels of radioactivity represent a resultant of absorption into, and clearance from, the plasma, and since absorption rates appeared to be similar in all the subjects, it was inferred that the rates of removal of fat from the plasma were different, and that this may have accounted for the raised fasting serum-triglyceride levels in the older White subjects and patients with ischaemic heart disease.

Although similar investigations were not carried out on the volunteers in this study, it could be inferred by analogy that the raised serum-triglyceride levels produced by the high saturated-fat diets may also be due to altered fat-clearance rates.

SUMMARY

On a prison diet similar to that consumed by the urban Bantu population (15% fat calories), serum-lipid patterns of White and Bantu prisoners were almost identical and agreed with that of the urban Bantu population. On the high fat-calorie (40%) diets of the White population, serum-lipid patterns of both races were again similar but resembled that of the White population. These patterns were maintained throughout the whole period of the long-term dietary change, and varied only when diets were changed again.

After one year on the high fat-calorie diets:

1. Irrespective of the nature of the dietary fat, serum-cholesterol/phospholipid ratios had risen significantly. Dietary fats, which raised the cholesterol level, led to a smaller rise in the phospholipid level, while oils, which lowered the cholesterol level, produced a more pronounced lowering of the phospholipid level.

2. On butter or hydrogenated-fat diets, fasting serum-triglyceride levels rose significantly, while on oil diets the levels either fell or were maintained at the initial low level.

In short-term studies cholesterol/phospholipid ratios rose only on saturated-fat diets; while triglyceride levels were not materially influenced by the nature of the fat consumed.

We wish to thank the Director and Deputy Director of the South African Institute for Medical Research, Dr. J. H. S. Gear and Prof. J. F. Murray, for their interest in this study.

Figs. 1, 2, 3 and 7 have already appeared in the *American Journal of Clinical Nutrition* and Figs. 4, 5 and 6 in the *Lancet*. They are reproduced with the approval of the respective Editors.

REFERENCES

1. Smit, Z. M. (1957): S.Afr. J. Lab. Clin. Med., **3**, 29.
2. Antonis, A. and Bersohn, I. (1962): Amer. J. Clin. Nutr., **11**, 142.
3. Ahrens, E. H. jnr., Insull, W. jnr., Blomstrand, R., Hirsch, J., Tsaltas, F. F. and Peterson, M. L. (1957): Lancet, **1**, 943.
4. Antonis, A. and Bersohn, I. (1960): *Ibid.*, **1**, 998.
5. Albrink, M. J. and Man, E. B. (1959): Arch. Intern. Med., **103**, 4.
6. Albrink, M. J. (1962): Nutr. Rev., **20**, 233.
7. Antonis, A. and Bersohn, I. (1961): Lancet, **1**, 3.
8. Ahrens, E. H. jnr., Hirsch, J., Peterson, M. L., Insull, W. jnr., Stoffel, W., Farquhar, J. W., Miller, T. and Thomasson, H. J. (1959): *Ibid.*, **1**, 115.
9. Higginson, J. and Oettlé, A. G. (1960): J. Nat. Cancer Inst., **24**, 589.
10. Mead, J. F. and Fillerup, D. L. (1957): J. Biol. Chem., **227**, 1009.
11. Metz, J., Antonis, A., Bersohn, I. and Hart, D. (1960): Brit. Med. J., **2**, 1270.