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SHORT COMMUNICATIONS

Plasma Lipids and Glucose in Normal Health, Diabetes and Cardiovascular Disease

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Summary. Information and specimens of blood collected during a diabetic survey of the population of Arbroath have been used to study plasma cholesterol, NEFA and β-lipoprotein in diabetes and cardiovascular disease. The mean levels of post-prandial plasma cholesterol, β-lipoprotein and blood glucose are significantly raised in persons with histories of cardiovascular disease compared with those without. In the family history of patients with cardiovascular disease, diabetes was found more often than in the family history of normal persons. This difference was statistically significant.

Lipides et glucose plasmatiques chez des sujets normaux, des diabétiques et des sujets atteints de maladie cardiovasculaire

Résumé. Les observations et les prélèvements de sang, recueillis au cours d'une enquête sur le diabète dans la population d'Arbroath, ont servi à étudier la teneur du plasma en cholestérol, NEFA et β-lipoprotéine dans le diabète et les maladies cardio-vasculaires. Les taux moyens post-prandiaux de cholestérol et β-lipoprotéine plasmatiques et de glucose sanguin sont significativement augmentés chez les personnes présentant une histoire d'atteinte cardiovasculaire par comparaison avec celles qui n'en présentaient pas. Le diabète était trouvé plus souvent dans l'histoire familiale des patients atteints de maladie cardiovasculaire que dans celle des sujets normaux. Cette différence est statistiquement significative.

Plasmalipide und Glucose bei Gesunden, Diabetikern und Personen mit cardiovasculären Erkrankungen

Zusammenfassung. Bei einer epidemiologischen Untersuchung über das Vorkommen von Diabetes in der Bevölkerung von Arbroath wurden Blutproben auf ihren Gehalt an Cholesterin, NEFA und β-Lipoproteine im Hinblick auf Diabetes und Krankheiten der Arterien untersucht. Die postprandialen Durchschnittswerte des Plasma-Cholesterin, der β-Lipoproteine und der Glucose sind bei Personen, bei denen in der Anamnese kardiovasculäre Störungen vorkommen, statistisch höher als bei denen, in deren Anamnese dies nicht der Fall ist. In den Familien von Patienten mit kardiovasculären Krankheiten liegt Diabetes öfter vor als in gesunden Familien. Dieser Unterschied ist statistisch gesichert.

Key-words: Diabetic survey, plasmalipids, NEFA, GTT, cardiovascular disease.

It is now accepted that a disturbance of both fat and carbohydrate metabolism is implicated in the complex syndrome of essential diabetes. Serum lipid levels may be elevated in diabetes (NEW et al., 1963) and adipose tissue is a predominant site of insulin action (REYNOLD et al., 1965). Atherosclerotic disease of the peripheral arteries and coronary arteries occurs more commonly in diabetics than in those persons not predisposed to the disorder, though MITCHELL and SCHWARTZ (1965) doubt the validity of much of the evidence. Conversely, disturbances in carbohydrate and lipid metabolism occur more frequently in patients with atherosclerotic vascular disease than in otherwise normal individuals (OSTRANDER et al., 1965).

In a diabetic survey of the population of Arbroath, blood levels of lipids and glucose were studied and the opportunity was also taken to select groups of persons with and without vascular disease, and with and without family histories of diabetes. The results of the assays used have been statistically analysed to show what changes in blood, taken post-prandially and during a glucose tolerance test (GTT), can be detected with reference to age and sex, diabetes and cardiovascular disease, and whether any hereditary cross-tendencies are shown by the two diseases or by the biochemical findings.

Methods

Selection of material. The method of detection of 387 glucosuric individuals, the selection of 349 "control" non-glucosurics and the full details of their subsequent grouping have been described previously (MITCHELL and STRAUSS, 1964). Briefly, a specimen of blood was removed from each person at a known time (about 1½ h) after a substantial meal (including at least five slices of toast). On the basis of the blood glucose level found, each glucosuric together with a non-glucosuric control person matched for age and sex was classified into diabetic, "doubtful" and non-diabetic. Individuals in the "doubtful" group (228 glucosurics, 228 age- and sex-matched non-glucosurics) were then asked to attend for a GTT. Blood specimens taken both after the meal and during the GTT were assayed for NEFA, β-lipoprotein and cholesterol (β-lipoprotein assays were not carried out on the GTT samples).

Each person completed a form giving information as to his own, or his relative's, history of diabetes, coronary disease and peripheral vascular disease. The information was checked during a subsequent interview between the person concerned and his own general practitioner.

Biochemical techniques. The method of DOLE and MINERTZ (1960) was used to measure plasma NEFA. The 95% confidence limits within the range 300—1000 μeq./l were found to be ±105 μeq./l. Plasma cholesterol was assayed by the AutoAnalyzer using method 24 provided by Technicon Instruments Co. Ltd., Chertsey, London. The immunological "Beta L" test of Hyland Laboratories, Los Angeles was used to assay β-lipoprotein, the results being obtained in arbitrary units. Blood glucose was estimated by the glucose-oxidase method of DISCOMBE (1963) using the AutoAnalyzer.

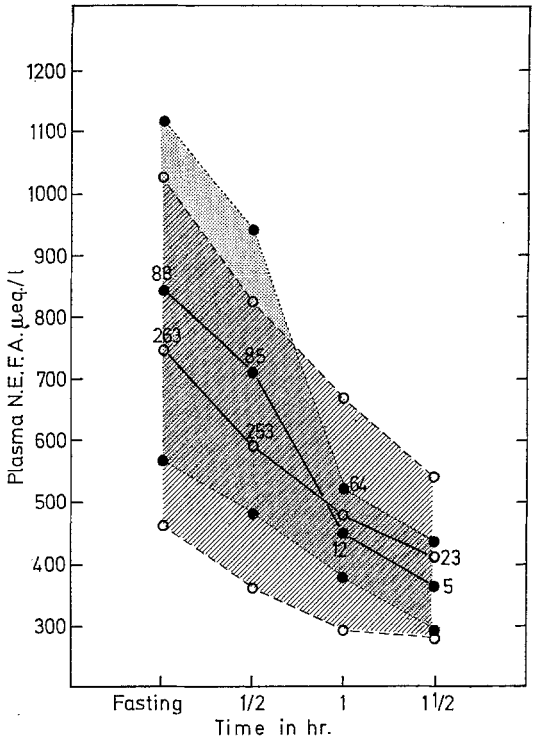

Fig. 1. Comparison of plasma NEFA during GTT's on diabetic and non-diabetic individuals. The number of individuals used to determine each spot is shown

●——● Mean for diabetics

o——o Mean for non-diabetics

Dotted area denotes the range of S.D. for diabetics

Lined area denotes the range of S.D. for non-diabetics

The biochemical findings together with the clinical details from each patient were punched on a card, and the information so collected was analysed on an 80 column I.B.M. data-processing machine. Individuals were categorised as to age, sex and carbohydrate tolerance, and also on the basis of the presence or absence of vascular disease and a family history of diabetes. All groups were closely matched with normals.

Results

NEFA, cholesterol and β-lipoprotein. The level of plasma N.E.F.A. does not appear to be influenced by increasing age. No significant difference was shown between male diabetics, female diabetics and normals. The decline with time after a meal showed no difference between age groups, and was so slight that all values were pooled and plotted against age. The normal range then given by 480 individuals showed little variation from 588 μeq./l ± 256 S.D. from aged 15 to 85 yr. The decrease during a GTT was most evident in diabetic subjects, the slope was markedly less for non-diabetics (Fig. 1).

For cholesterol and β-lipoprotein neither sex showed a significant change after the predominantly carbohydrate meal nor in cholesterol during a GTT

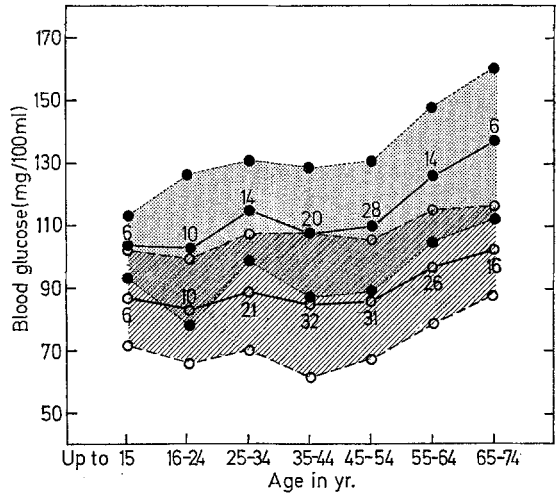

Fig. 2. Blood glucose in non-glucosuric individuals assayed 60—90 min (●——●, dotted area denotes range of S.D.) and 90—120 min (o——o, lined area denotes range of S.D.) after a carbohydrate meal and plotted against age. The number of individuals used to determine each spot is shown

(β-lipoprotein was not assayed during GTT's); as no other significant difference amongst the groups of individuals studied was evident, all results were averaged and plotted against age. The resulting graphs showing the standard deviation of each average value have been published previously (MITCHELL et al., 1966). They indicate a steady rise in both cholesterol and β-lipoprotein up to the age of 70 yr. when a sharp fall is evident.

Glucose. Figs. 2 and 3 show the increase in postprandial blood glucose with age in non-glucosuric and glucosuric individuals.

Biochemical findings in persons with a history of vascular disease. Two groups of individuals were selected from the glucosurics and controls. Group 1 contained 91 persons with either peripheral vascular or car-

diovascular disease, and Group 2, 649 persons with no such disease. (Cerebrovascular disease was considered to be a complaint mainly of the elderly and not clearly implicated with diabetes. Persons with histories of cerebrovascular disease were therefore excluded.) Known diabetics were also excluded. Except where subsequently stated, the assays were carried out on blood taken post-prandially.

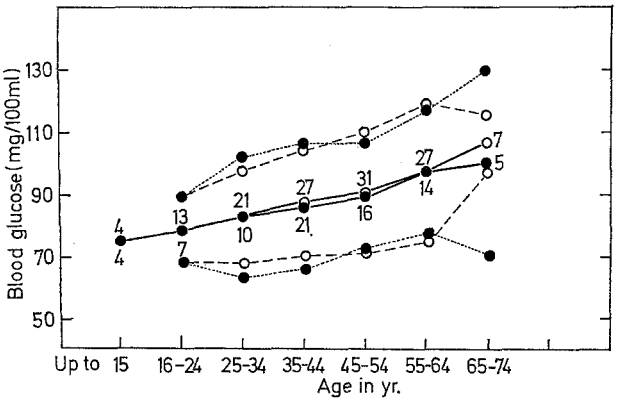

Fig. 3. Blood glucose in glucosuric individuals assayed 60—90 min (●——●) and 90—120 min (○——○) after a carbohydrate meal and plotted against age. The S.D. and the number of individuals used to determine each spot is shown

more relatives with diabetes: i.e. at least 19.8% of the group with vascular disease came from families with a diabetic trait.

Each of the 649 persons without vascular disease had on average 14.7 relatives; and 78 of these persons (12.0%) knew of 1 or more relatives with diabetes: i.e. at least 12.0% of the group without vascular disease came from families with a diabetic trait. This difference between the two groups is significant at the 5% level.

Discussion

A steady rise in the level of plasma cholesterol with age is known to occur. SCHILLING et al. (1964) assayed the serum cholesterol in 4000 persons but did not extend their survey beyond the age of 65 yr., and thus did not detect the sharp fall shown after this age. Only 14 persons over the age of 75 yr. were studied in the survey reported here, but the drop is just significant at the 5% level (observed value for $t=2.3$; critical level $t=2.0$). It is interesting to note that the plasma β-lipoprotein follows a similar pattern. The falls after the age of 65 yr. could be due to natural selection by death. The relationship between the two assays has been discussed elsewhere (MITCHELL et al., 1966).

Figs. 2 and 3 show a rise in the post-prandial blood glucose level with age. A similar rise with age has been

Table. *Biochemical findings in persons with and without vascular disease*

	Mean value. Persons with vascular disease (Group 1)	Mean value. Persons without vascular disease (Group 2)	Difference	„*t*" test of significance
No. of persons	91	649		
Mean age (yr.)	58.1	44.7	13.4	
Plasma cholesterol (mg/100 ml)	265.1	245.7	19.4 13.9[a]	Sig. at 0.1% level Sig. at 1% level[a]
Plasma β-lipoprotein (arbitrary units)	3.09	2.77	0.32 0.23[a]	Sig. at 0.1% level Sig. at 1% level[a]
Plasma NEFA, post-prandial	549.5	571.5	22.0	Not sig.
Plasma NEFA fasting μeq./l)	722.5	780.1	57.6	Not sig.
Blood glucose post-prandial	106.7	92.5	14.2 10.0[a]	Sig. at 0.1% level Sig. at 0.5% level[a]
Blood glucose fasting	88.1	86.9	1.2	Not sig.

[a]Corrected for normal difference between the two groups due to age.

Except where stated, assays were completed on post-prandial specimens.

The Table shows the biochemical findings together with the results of the "t" test of significance.

The occurrence of diabetes was studied in the relatives (parents, siblings, aunts, uncles, children, grandchildren) of persons in the two groups. On average, each of the 91 patients with vascular disease had 17.3 relatives; 18 of these patients (19.8%) knew of one or

shown for the 1 h GTT blood glucose (College of General Practitioners Working Party, 1963). One reason for the similarity of the 60—90 min and the 90—120 min graphs in the glucosurics may be a contribution by the glucosuria itself.

BIERMAN et al. (1957) showed that the decrease in plasma NEFA following glucose administration was

not so rapid in diabetics as it was in normal subjects, but Shafrir and Gutman (1965) have shown that this is true only for the insulin-dependent diabetic showing a grossly abnormal GTT. They showed that "mild" diabetics and patients with "impaired" GTT's tended to show a steeper rate of decrease of plasma NEFA from a higher original level to a lower level at 1.5–2 h than did the normal. The "early or chemical" diabetics studied in the survey reported here show a similar pattern (Fig. 1). In Fig. 1 the rate of fall of NEFA up to 30 min is less than that for non-diabetics, but during the subsequent 30 min the rate of fall in diabetics overtakes the normal. Shafrir and Gutman (1965) and Hales et al. (1968) have indicated that in the "mild diabetic" there is a delay before the release of insulin is sufficient to allow tissue-glucose utilization.

To shut down the supply of NEFA to the plasma, adipose tissue has to utilise glucose to make glycerophosphate. This then acts as the acceptor for esterification and retention of NEFA. In the "mild diabetic" the process apparently takes place satisfactorily during a GTT but not in the fasting state, as the NEFA level is then some 100 μeq./l higher than in the normal (Fig. 1). It is interesting to note that of the substances measured in the post-prandial specimens, only plasma NEFA shows no rise in concentration with age. Decreasing intestinal absorption could be having an effect, but it is possible that the increase in the level of glucose with age would tend to decrease the release of NEFA.

The mean blood glucose levels shown in the Table, for persons with or without vascular changes, support the work of Cohen and Shafrir (1965) and Ostrander et al. (1965). They found a reduced glucose tolerance in patients with myocardial infarction. A difference is shown in the post-prandial blood glucose which is significant at the 0.5% level. The finding of no significant difference in the fasting blood glucose is not surprising in view of the relative insensitivity of the fasting level for the detection of mild glucose intolerance (Mitchell and Strauss, 1964). There is no significant difference in the post-prandial plasma NEFA levels between the two groups.

The fact that patients with vascular disease have more diabetic relatives than do a group of controls has not been shown before, though the reverse (that diabetics tend to have a family history of vascular disease) is known.

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