Circulating Lipids and Cardiovascular Risk in Newly Diagnosed Non-insulin-dependent Diabetic Subjects in India

K.M. Shelgikar, S.S. Naik, M. Khopkar, D.S. Bhat, K.N. Raut, C.V. Joglekar, M.E. Gerard, C.S. Yajnik*

Diabetes Unit, King Edward Memorial Hospital and Research Centre, Sardar Mudliar Road, Rasta Peth, Pune 411011, India

Circulating concentrations of total cholesterol, triglycerides, non-esterified fatty acids (NEFA), glycerol, and 3-hydroxybutyrate (3-HB) were measured in 133 subjects with normal glucose tolerance (NGT), 78 with impaired-glucose-tolerance (IGT) and 189 non-insulin dependent (Type 2) diabetic (NIDDM) patients. Plasma cholesterol concentration was similar in the three groups; NGT (4.2 (2.3–7.5) mmol l^{-1}, median (range)), IGT (4.7 (2.7–6.3)) and NIDDM (4.3 (2.3–6.9)). Plasma triglycerides (NGT 0.88 (0.37–2.80), IGT 1.26 (0.43–3.82) and NIDDM 1.38 (0.62–3.91) mmol l^{-1}) and NEFA (NGT 0.81 (0.29–1.58), IGT 1.02 (0.33–1.87) and NIDDM 1.02 (0.48–2.77) mmol l^{-1}) were higher in the two hyperglycaemic groups, but blood 3-HB concentration was similar in the three groups. Plasma cholesterol concentration in these subjects is lower than that reported in white Caucasians in the UK and USA and migrant Indian NIDDM patients in the UK. In NIDDM patients plasma cholesterol concentration was related to age, body mass index (BMI), and plasma glucose concentration while plasma triglyceride concentration was related to plasma NEFA and insulin (IRI) concentration. Evidence of ischaemia on electrocardiography in patients with diabetes was associated with higher age, blood pressure, plasma triglyceride, glucose, and IRI concentrations. © 1997 by John Wiley & Sons, Ltd.

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Introduction

Clinical observations and epidemiological studies suggest that the prevalence of diabetes is increasing in India.[1] Clinical observations suggest that ischaemic heart disease is also on the rise. The reasons for the rise in these two disorders are not clear but genetic as well as lifestyle factors are thought to be important. Migrant Indians have a higher prevalence of ischaemic heart disease than do many of the native populations of the regions to which they have moved.[2,3] Unfavourable dietary fat intake and associated abnormalities in circulating lipids, reduced physical activity, central obesity, diabetes, insulin resistance and its associated compensatory hyperinsulinaemia are thought to contribute to this.[4–6] Few reliable data are available on the risk factors of ischaemic heart disease in native Indians but clinical observations suggest that diabetes has a significant role to play.

We have set up a prospective study of the clinical, biochemical, and endocrine characteristics of newly diagnosed, non-insulin-dependent (Type 2) diabetic patients at the King Edward Memorial (KEM) Hospital, Pune. These patients provide unique data on native Indians. We have reported an association of central obesity with hyperglycaemia[7] and of blood pressure with obesity and circulating insulin concentrations[8] in our NIDDM patients. We now describe circulating lipid concentrations in subjects with different degrees of glucose tolerance and their associations with different clinical and biochemical measures and with ischaemic electrocardiographic changes.

Patients and Methods

We studied 189 newly diagnosd NIDDM patients, 78 with impaired glucose tolerance (IGT) and 133 with normal glucose tolerance (NGT) from the outpatients clinics and wards of the KEM Hospital over a two and a half year period, excluding those aged more than 65 years, those who were pregnant, those with history of recent (within 6 months) myocardial infarction or stroke,

Abbreviations: BMI body mass index, ECG electrocardiogram, 3-HB 3-hydroxybutyrate, IGT impaired glucose tolerance, IRI plasma immunoreactive insulin, NEFA non-esterified fatty acids, NGT normal glucose tolerance, NIDDM non-insulin-dependent diabetes mellitus, UAER urinary albumin excretion rate, WHR waist–hip ratio

* Correspondence to: Dr. C.S. Yajnik, Diabetes Unit, King Edward Memorial Hospital and Research Centre, Sardar Mudliar Road, Rasta Peth, Pune 411011, India

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those with other severe illness (such as cancer or renal failure), and those on steroid treatment. Approximately 10 % of the eligible subjects declined. The majority of the NIDDM and 36 IGT patients had been referred to us for diagnosis and/or management. We advertised in the hospital outpatient department for control volunteers (people without family history of diabetes). One hundred and seventy-five subjects agreed (143 attending outpatient department for minor illnesses, 16 spouses of NIDDM patients, and 16 hospital staff). Forty-two of these turned out to have IGT.

All subjcts underwent an oral glucose tolerance test (75 g anhydrous glucose load) after an overnight fast and were classified according to WHO 1985 criteria.[9] Plasma glucose was measured by a glucose oxidase method, lipids (total cholesterol and triglycerides) were measured on a fasting blood sample using enzymatic kits on an Abbott VP Super autoanalyser (Irwing, Texas, USA). Glycated haemoglobin (HbA_1) was measured by a colorimetric method.[10] Plasma immunoreactive insulin (IRI) was measued by a double antibody radioimmunoassay[11] using anti-porcine insulin antibody raised in guinea pig (65–104, ICN Immunobiologicals, Lisle, Illinois, USA) and human insulin standards. This antibody cross-reacts with proinsulin. The detection limit of the assay was 2 mU l^{-1} with intra- and inter-batch CV <6 % and <9 %, respectively. 3-Hydroxybutyrate (3-HB) and glycerol were measured on perchloric acid extract of whole blood by enzymatic assays[12] and plasma non-esterified fatty acids (NEFA) by an enzymatic kit (Wako Chemicals GmbH, West Germany). For glucose, IRI, NEFA, 3-HB, and glycerol a mean of two fasting values was used in the analysis. Blood pressure was measured in supine position after a 15-minute rest. Urinary albumin excretion rate (UAER) was measured on an overnight (8 h) urine collection by radial immunodiffusion.[13] Resting 12 lead ECGs were made and coded by the Minnesota method (1982) into normal, coronary probable (codes 1.1, 1.2, 7.1) and coronary possible (codes 1.3, 4.1, 4.2, 4.3, 5.1, 5.2, 5.3).

Statistical Analysis

The data were normalized whenever necessary (log transformation for IRI, NEFA, 3-HB, and glycerol). Statistical significance of the difference between groups was tested by Student's *t*-test and significance of correlations by Spearman's method. Multivariate analysis was by multiple linear regression and multiple logistic regression, as appropriate. All statistical analysis was performed using SPSS/PC + (3.1) package.

Results

As reported previously,[7] NIDDM and IGT subjects were older and more obese and had higher waist-hip ratio (WHR) than the NGT controls (Table 1). IGT subjects were more obese than those with NIDDM. HbA_1 concentrations were higher in women with IGT, compared with NGT women and in NIDDM compared with both NGT and IGT subjects.

Lipids and Ketone Levels (Table 1)

There were no significant differences in the plasma cholesterol concentrations between NGT, IGT, and NIDDM subjects. Six (4.7 %) NGT subjects, 3 (4 %) IGT, and 11 (5.9 %) NIDDM patients had elevated plasma cholesterol concentration (>6.2 mmol l^{-1}).

Plasma triglyceride concentration was higher in IGT (men) and NIDDM (men and women) patients compared to the NGT subjects, with no significant difference between the IGT and NIDDM patients. Plasma NEFA concentration was higher in IGT and NIDDM than that in NGT subjects (men and women), and in NIDDM women compared to IGT women and NIDDM men.

Blood glycerol concentration was available in 72 (39 men) NGT, 34 (13 men) IGT, and 105 (68 men) NIDDM subjects; it was higher in NGT women than in men, in NIDDM men compared to NGT men, and in NIDDM women compared to IGT women. There was no significant difference in blood 3-HB concentration in NGT, IGT and NIDDM subjects.

Significant correlations of plasma lipids and lipid metabolites are shown in Table 2. Multiple linear regression analysis in NIDDM patients revealed the following associations of plasma lipids and lipid metabolites: cholesterol with age ($r = 0.18$, $p < 0.05$), BMI ($r = 0.17$, $p < 0.05$), and fasting glucose ($r = 0.25$, $p < 0.001$); triglyceride with NEFA ($r = 0.31$, $p < 0.01$); NEFA with fasting IRI ($r = 0.25$, $P < 0.01$); and 3-HB with fasting glucose ($r = 0.24$, $p < 0.001$). There were no significant associations with plasma glycerol.

Blood Pressure and UAER (Table 1)

Blood pressure was significantly higher in IGT and NIDDM compared to NGT subjects. NIDDM men showed higher UAER as compared to NGT men and NIDDM women. Eight per cent NGT, 19 % IGT, and 23 % NIDDM subjects showed 'microalbuminuria' range excretion (UAER 20–200 $\mu g\ min^{-1}$).

Angina and Electrocardiogram

Possible ischaemic changes on ECG were seen in 8 %. NGT men and 12 % NGT women, in 21 % IGT men ($p < 0.05$ compared to NGT men) and 17 % IGT women, and in 12 % NIDDM men and 23 % NIDDM women. A history of angina was present in 5 NIDDM men, 4 of these also showed ischaemic changes on ECG. NIDDM men with ischaemic changes on ECG had higher plasma triglyceride concentration than that in those with normal ECG (1.83 vs 1.37 mmol l^{-1}, $p < 0.01$) but plasma cholesterol (4.30 vs 4.20 mmol l^{-1}) and NEFA concentrations (1.30 vs 1.11 mmol l^{-1}) were similar; in NIDDM women

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Table 1. Basic information on subjects studied

	NGT		IGT		NIDDM	
	Men	Women	Men	Women	Men	Women
n	76	57	44	34	123	66
Age (yr)	40 (23–61)	40 (24–55)	49 (28–65)[a]	47 (30–59)[a]	43 (29–60)[b]	45 (28–60)[a]
BMI (kg m^{-2})	23.3 (17.7–29.8)	23.6 (16.9–31.7)	25.5 (19.6–33.0)[a]	27.1 (19.5–38.2)[a]	24.0 (18.7–31.0)	24.9 (19.7–35.3)[a,b,c]
WHR	0.88 (0.78–0.99)	0.77 (0.68–0.87)[a]	0.93 (0.83–0.99)[a]	0.79 (0.74–0.91)[a,c]	0.92 (0.83–1.00)[a]	0.80 (0.72–0.89)[a,c]
Fasting plasma glucose (mmol l^{-1})	4.6 (3.8–5.5)	4.6 (3.7–5.4)	5.1 (3.8–6.9)[a]	5.1 (4.4–6.2)[a]	8.8 (5.1–17.5)[a,b]	8.8 (5.0–14.9)[a,b]
HbA_1 (%)	6.3 (5.5–7.3)	6.3 (5.4–7.3)	6.4 (5.3–9.1)	6.7 (5.1–8.9)[a]	7.0 (6.4–14.8)[a,b]	8.6 (6.1–13.9)[a,b]
Plasma cholesterol (mmol l^{-1})	4.2 (2.3–7.5)	4.3 (2.8–6.5)	4.6 (3.5–6.3)	4.8 (2.7–5.9)	4.2 (2.9–6.0)	4.5 (2.3–6.9)
Plasma triglycerides (mmol l^{-1})	0.91 (0.38–2.60)	0.85 (0.37–2.80)	1.25 (0.67–3.82)[a]	1.26 (0.43–3.30)	1.45 (0.70–3.40)[a]	1.26 (0.62–3.91)[a]
Plasma NEFA (mmol l^{-1})	0.76 (0.29–1.33)[a]	0.82 (0.35–1.58)[a]	0.98 (0.33–1.76)[a]	1.04 (0.40–1.87)[a,b,c]	0.95 (0.48–2.43)	1.20 (0.59–2.77)
Blood glycerol (mmol l^{-1})	0.11 (0.08–0.19)	0.14 (0.05–0.36)[c]	0.17 (0.08–0.24)	0.14 (0.04–0.29)	0.14 (0.06–0.46)[a]	0.19 (0.04–0.71)[b]
Blood 3-HB (mmol l^{-1})	0.05 (0.01–0.20)	0.04 (0.01–0.19)	0.06 (0.01–0.31)	0.05 (0.01–0.24)	0.05 (0.01–0.52)	0.06 (0.01–0.22)
Fasting plasma IRI (mU l^{-1})	8.5 (1.3–32.3)[b,c]	8.6 (1.0–37.0)	11.3 (2.0–66.0)	10.0 (1.0–39.2)	16.0 (4.0–39.0)[a]	16.0 (3.0–42.0)[a,b]
2 h Plasma IRI (mU l^{-1})	84 (15–225)	78 (21–234)	162 (48–371)[a]	134 (21–247)[a]	51 (9–208)[a,b]	65 (18–262)[b,c]
Blood pressure (mmHg)						
Systolic	120 (98–164)	122 (96–160)	126 (106–174)[a]	128 (92–160)[c]	130 (90–180)[a]	130 (100–172)[a]
Diastolic	80 (58–96)	76 (58–98)	80 (70–102)[a]	78 (60–98)	86 (58–118)[a]	80 (60–120)[a]
UAER (μg min^{-1})	3.0 (1.0–116.7)	4.3 (0.8–190.2)	5.6 (1.2–113)	4.1 (0.6–146.0)	10.1 (0.8–208.5)[a]	5.1 (0.6–235.6)[c]

Median (range).
[a]Different from NGT;
[b]Different from IGT (of same sex).
[c]Women different from men (of same group), $p < 0.05$.

Table 2. Significant correlations of blood lipids and their metabolites

	NGT	IGT	NIDDM
Cholesterol	Age (0.31)[c] BMI (0.33)[c] Glycerol (−0.23)[a] Diastolic BP (0.22)[b]	NEFA (0.40)[b] 3-HB (0.16)[a]	F glucose (0.19)[a] 2h IRI (−0.18)[a]
Triglycerides	BMI (0.22)[a] HbA_1 (−0.17)[a] F IRI (0.26)[b] 2h IRI (0.24)[a] NEFA (0.21)[a]	WHR (0.28)[a]	Age (−0.18)[a] F IRI (0.16)[a] NEFA (0.25)[b] Diastolic BP (0.16)[a]
NEFA	BMI (0.27)[b] 2h IRI (0.21)[a] Systolic BP (0.22)[b]	HbA_1 (0.26)[a] Systolic BP (−0.30)[c]	F IRI (0.24)[b]
3-HB	UAER (0.21)[a]		F glucose (0.28)[c]
Glycerol	UAER (0.27)[a]	UAER (0.37)[a]	

F, fasting; 2h = 2 hour value during oral glucose tolerance test.
Values represent Spearman's correlation coefficient (r_s): [a] = $p < 0.05$; [b] = $p < 0.01$; [c] = $p < 0.001$.

plasma triglyceride (1.50 vs 1.29 mmol l^{-1}), cholesterol (4.50 vs 4.60 mmol l^{-1}) and NEFA concentrations (1.29 vs 1.38 mmol l^{-1}) were similar in the two groups. There was no difference in circulating lipid concentrations in those with normal and ischaemic ECG in the NGT and IGT groups. Multiple logistic regression analysis revealed association of ischaemic ECG with age >50 years ($p < 0.001$), blood pressure ($p < 0.01$), plasma triglyceride ($p < 0.001$), and fasting plasma glucose ($p < 0.01$) and IRI ($p < 0.01$) concentrations in NIDDM men, and with age >50 years ($p < 0.05$) and fasting plasma IRI concentration ($p < 0.05$) in NIDDM women.

Discussion

Ours is a clinic-based study of newly diagnosed, untreated NIDDM patients in urban Pune. Even though it does not represent the general population, it provides novel data in native Indians not hitherto available.

Plasma cholesterol concentration in our study subjects is lower than that reported in Americans,[14] in white Caucasian and in migrant Asian NIDDM patients in the UK.[15] Less than 6% of our study subjects showed hypercholesterolaemia (>6.2 mmol l^{-1}), much lower than the reported figures of approximately 25% in NGT, approximately 35% in IGT and approximately 35% in NIDDM subjects in the NHANES II study from the USA.[14] Most of our subjects are vegetarian, less than 20% eating non-vegetarian food (and that usually not more than twice a week), which could be a major contributor to their lower plasma cholesterol. A comparative study of native and migrant Punjabi Indian subjects[6] suggested that dietary and other environmental factors rather than genetics contribute to the lower plasma cholesterol concentration in native Indians. Plasma cholesterol concentration in NIDDM, IGT, and NGT subjects was similar, suggesting that diabetes is not associated with hypercholesterolaemia in native Indians. Similar findings have been reported before.[16] Plasma cholesterol concentration was related to age, obesity (BMI), and plasma glucose concentration in NIDDM patients.

Unlike cholesterol, plasma triglyceride concentration was higher in both IGT and NIDDM compared to NGT subjects, with no significant difference between IGT and NIDDM patients. Our findings are similar to those in many other studies of NIDDM patients in different populations.[17,18] Plasma triglyceride concentration is thought to be an important risk factor for ischaemic heart disease[17,19] and is a component of the so-called 'insulin resistance syndrome' (syndrome X).[20] The exact mechanism of the increased cardiovascular risk of elevated plasma triglyceride concentration is not clear but is at least partly through its inverse relationship with the concentration of cardioprotective HDL-cholesterol.[21] Our results highlight that plasma triglyceride concentration is elevated even in those with relatively mild hyperglycaemia (IGT), so in NIDDM patients this risk was probably present for many years before diagnosis. In men it was associated with ischaemic change on the electrocardiogram, highlighting its importance as a risk factor for ischaemic heart disease. The positive association between plasma triglyceride and IRI concentration would suggest that it is a part of the 'insulin resistance syndrome' in native Indian NIDDM patients.

Elevated circulating NEFA and glycerol concentrations in hyperglycaemic subjects suggest accelerated adipose tissue lipolysis. The rather unexpected direct relation between plasma IRI and NEFA concentration suggests an 'insensitivity' of the adipose tissue lipolysis to the suppressive action of circulating IRI. The elevated triglycerides with normal 3-HB concentrations in the hyperglycaemic subjects suggests that the NEFA was promoting lipogenesis rather than ketogenesis (probably under the influence of circulating IRI). Similar observations have been made by Modan[22] and Reaven[23] in populations which are more obese than ours. Despite their low BMI, our NIDDM patients had a high WHR, suggestive of excess intra-abdominal fat which could cause insulin

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resistance by presenting the liver with excess NEFA through the portal circulation.[24]

We have used an internationally accepted system of diagnosing ischaemic heart disease (history and Minnesota coding of the resting ECG). This has not been validated in the developing countries but there is no other better accepted method. Ischaemic changes in the ECG were more common in women compared to men, a finding that is similar to other epidemiologic studies from India.[25,26] The proportion of false positives (especially in women) is difficult to ascertain in the absence of invasive investigations and in one study these changes have been interpreted as 'non-specific'.[25] Multivariate analysis showed that in NIDDM patients, ischaemic change on ECG was associated with age greater than 50 years, higher blood pressure, and higher plasma triglyceride, glucose, and IRI concentrations, but not with obesity or UAER.

In summary, circulating triglyceride and NEFA concentrations were elevated in native Indian subjects with IGT and NIDDM, while cholesterol concentrations were relatively low and similar to NGT subjects. Ischaemic change on electrocardiograms in NIDDM patients were associated with higher age, blood pressure, triglyceride, glucose, and IRI concentrations. These data provide useful comparison with data from other populations, especially migrant Indian populations, and shed light on possible modifiable factors in the genesis of diabetes and ischaemic heart disease in Indian people.

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