



Association of Adipocytokines (Leptin, Adiponectin TNF-alpha), Insulin and Proinsulin with Diabetes - The Mumbai Obesity Project [MOP]

RD Lele*, Shashank R Joshi**, Arpita Gupte***

Abstract

Asian Indians have a unique phenotype characterized by increased abdominal obesity and visceral fat despite low body mass index [BMI]. Though studies have indicated some adipocytokines to be associated with diabetes and obesity in Indians, there are virtually no studies relating adipocytokines and proinsulin with diabetes and obesity in Asian Indians. In this study we looked at adipocytokines- leptin, adiponectin and tumour necrosis factor- α [TNF- α] and insulin and proinsulin in subjects with diabetes and obesity. Thirty five diabetic subjects and 50 healthy controls were recruited for the study. Leptin [$p=0.002$] and adiponectin levels [$p=0.011$] were lower and proinsulin values higher [$p<0.001$] in diabetic subjects compared to non-diabetic subjects. In addition, leptin [$p<0.001$] and proinsulin [$p<0.001$] were higher and adiponectin [$p<0.001$] lower, in obese subjects compared to non-obese subjects. TNF- α failed to show any significant difference between the study groups. Leptin and proinsulin showed a significant and positive correlation with BMI [$p<0.001$] and waist circumference [$p<0.001$]. Adiponectin showed an inverse correlation with BMI [$p=0.050$] and waist circumference [$p=0.002$]. Proinsulin showed a significant negative association with adiponectin [$p=0.002$]. Logistic regression analysis revealed leptin to be negatively associated [Odds ratio [OR]: 0.864, 95% confidence interval [95% CI]: 0.775 -0.963, $p=0.008$] and proinsulin [OR: 1.567, 95% CI: 1.246-1.971, $p<0.001$] to be positively associated with diabetes even after adjusting for age, gender and BMI. Leptin [OR: 1.365, 95% CI: 1.170-1.592, $p<0.001$] and proinsulin [OR: 1.617, 95% CI: 1.218 -2.147, $p=0.001$] showed a significant positive association with obesity, while adiponectin [OR: 0.927, 95% CI: 0.865 - 0.995, $p=0.035$] had a significant inverse association. Linear regression analysis revealed that adiponectin is inversely associated with proinsulin even after the addition of age, gender and diabetes status [$\beta= -0.61$, $p=0.033$] into the model. In conclusion, in urban Asian Indians in western India, proinsulin levels showed a positive association, while leptin and adiponectin showed a negative association with diabetes. With regard to obesity, leptin and proinsulin had a positive association, while adiponectin had a negative association. Proinsulin levels showed an inverse association with adiponectin indicating a possible link between insulin secretion and insulin resistance. ©

INTRODUCTION

The 'Asian Indian Phenotype' refers to a peculiar phenotype observed in Asian Indians characterized by increased waist circumference and increased visceral fat despite low body mass index.¹ This has been associated with metabolic abnormalities inclusive of greater degree of insulin resistance,^{2,3} high prevalence rates of diabetes^{4,5} and cardiovascular disease.⁶ Abdominal obesity and fat modulate the metabolic

derrangements via insulin resistance.⁷ Corroborating this association are the results from a recent study in Indians, that documents a strong relation between intra-abdominal fat and diabetes.⁸

Adipose tissue secretes several adipocytokines, of which adiponectin, resistin, leptin and tumour necrosis factor- α [TNF- α] have been widely recognized to play a contributory role in insulin resistance, diabetes and cardiovascular disease.^{7,9-11} Though studies have documented that adiponectin has protective effect on diabetes,¹² its association with other markers like leptin and TNF- α has not been assessed, particularly in Asian Indians. Similarly, though proinsulin, a marker of beta cell dysfunction has been recorded as a predictor for diabetes,¹³ there is limited data associating proinsulin with adipocytokines. Since type 2 diabetes is an outcome

*Director, Nuclear Medicine and RIA; **Consultant Endocrinologist; ***Research Officer; Lilavati Hospital and Research Centre, Bandra, Mumbai.
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of both insulin resistance and insulin secretory defects, it is worthwhile to assess the relation between adipocytokines representing insulin resistance and proinsulin representing beta cell dysfunction. This study is therefore aimed at determining the association of adiponectin, leptin, TNF- α and insulin and proinsulin with diabetes and also the interrelation between these biomarkers in an urban western Indian population.

METHODS

Sample selection

Type 2 diabetes: Thirty five consecutive type 2 diabetic patients (21 males, 14 females) aged between 18 – 60 years registered at Lilavati Hospital and Research Centre, Mumbai were selected for the study. Pregnant females, subjects with renal diseases [serum creatinine >3 mg/dL] and known cases of liver diseases were excluded from the study. Diagnosis of type 2 diabetes was based on the WHO consulting group criteria i.e. fasting plasma glucose ≥ 126 mg/dl or 2 hr post glucose ≥ 200 mg/dl.¹⁴

Non-diabetic controls: Fifty healthy non-diabetic subjects (19 males and 31 females) were recruited from the OPD clinic of Lilavati Hospital who had come for routine health checkups other than diabetes.

Informed consent was obtained from all study subjects and the institutional review board approved the study.

Physical examination included height and weight measurements and the body mass index (BMI) was calculated using the formula: weight (kg) divided by height in meters squared. Obesity was defined as body mass index ≥ 25 kg/m². Waist measurement was made in the standing position using standard techniques.

Table 1 : Clinical characteristics of study subjects

Parameters	Non-diabetic subjects [n = 50]	Diabetic subjects [n = 35]	p value
Age [years]	33 \pm 12	46 \pm 8	< 0.001
Male n [%]	19 (38.0%)	21 (60.0%)	0.046
Body mass index [kg/m ²]	28.3 \pm 7.8	27.6 \pm 5.8	0.633
Waist circumference [cm]	89 \pm 18	93 \pm 12	0.193

A fasting blood sample was taken and the serum was separated and used for the assay. Adiponectin, leptin insulin and Proinsulin were estimated using radioimmunoassay [RIA kit, Linco Research Inc., Missouri, USA]. TNF- α was estimated by immunoradiometric assay [Biosource International, Inc, CA, USA]. The inter and intra assay coefficients of variation of these assays were less than 8%.

Observations and Results

Table 1 shows the characteristics of the study population. Table 2 shows the data on age, BMI, waist and hip circumference, WHR, along with fasting insulin, proinsulin, proinsulin to insulin ratio, leptin, adiponectin and TNF- α serum levels in (A) 21 male diabetics and (B) 14 female diabetics.

Table 3 shows the same data in (A)19 male non-diabetics and (B)31 female non-diabetics. Ranking in each group was according to rising BMI (18 to 55).

Fasting proinsulin was measured as pmol/l and specific insulin as mU/ml. To derive proinsulin to insulin ratio, the insulin values were multiplied by

Table 2A : 21 Male Diabetic

Sr. No.	Age	BMI	Waist (cm)	Hip (cm)	WHR	Insulin (microIU/ml)	Insulin (pM)	Proinsulin (pM)	PI:I%	Leptin (ng/ml)	Adiponectin (ng/ml)	TNF-alpha (pg/ml)
1	32	19.27	77	87	0.89	10.26	71.82	17.9	25.00	2.47	2.25	37.8
2	37	20.05	74	85	0.87	9.585	67.09	17.4	26.00	2.43	0.325	9.609
3	52	20.24	86	90	0.96	7.397	51.78	13.8	27.00	2.81	12.95	34.34
4	40	21.46	83	90	0.92	2.467	17.27	19.7	114.00	4.1	3.96	15.07
5	54	22.27	85	88	0.97	4.372	30.60	20.4	67.00	2.59	20.6	83.53
6	35	22.86	82	89	0.92	14.7	102.90	27.4	27.00	2.89	4.8	33.88
7	65	24.87	93	99	0.94	9.322	65.25	16.5	25.00	4.73	13.78	26.66
8	52	25.32	87	92	0.95	18.81	131.67	39.4	30.00	4.51	1.885	49.24
9	60	25.37	92	90	1.02	13.43	94.01	10.7	11.00	6.78	6.92	159.6
10	48	25.99	99	96	1.03	7.004	49.03	19.4	40.00	4.62	10.95	110
11	39	26	87.5	100	0.88	15.81	110.67	32.9	30.00	7.5	0.54	146.4
12	33	26.23	87	99	0.88	8.814	61.70	15.9	30.00	4.64	19.9	84.93
13	52	26.66	98	102	0.96	32.33	226.31	70.2	31.00	7.27	10.35	131
14	52	27.34	90	95	0.95	15.57	108.99	26.4	24.00	11.9	2.085	49.43
15	40	27.76	90	95	0.95	12	84.00	29.8	35.00	6.06	11.05	256
16	45	28.57	96.5	103	0.94	23.27	162.89	60.9	37.00	6.97	7.7	20.81
17	35	28.93	89	98	0.91	28	196.00	77.4	39.00	8.44	2.14	23.25
18	50	30.12	102	96	1.06	11.91	83.37	20.9	25.00	8.98	2.545	60.01
19	33	34.16	106	113	0.94	11.93	83.51	136	163.00	11	7.35	44.06
20	47	38.14	107	130	0.82	17.38	121.66	37.1	30.00	23.2	20.3	149.1
21	42	48.06	141	164	0.86	28.62	200.34	81.3	41.00	37	6.4	47.04

Table 2B : 14 Female Diabetic

Sr. No.	Age (Yrs)	BMI	Waist (cm)	Hip (cm)	WHR	Insulin (microIU/ml)	I*7	Proinsulin (pM)	PI:I%	Leptin (ng/ml)	Adiponectin (ng/ml)	TNF-alpha (pg/ml)
1	45	21.49	93	91	1.02	14.09	98.63	22.1	22.41	6.43	2.45	42.73
2	43	22.14	86	95	0.91	12.8	89.60	39.1	43.64	19.3	10.05	53.9
3	46	22.22	82	89	0.92	8.603	60.22	34.1	56.62	12	15.7	50.97
4	42	24.45	89	97	0.92	8.099	56.69	13.2	23.28	7.45	2.8	31.52
5	48	27.15	85	103.5	0.82	14.38	100.66	10.7	10.63	30.4	3.63	48.15
6	39	27.41	94	104	0.90	6.433	45.03	9.8	21.76	21	1.26	166.6
7	52	28.62	92	106	0.87	18.39	128.73	14.7	11.42	19.6	4.77	123.1
8	48	29.11	89	102	0.87	7.978	55.85	10	17.91	13.3	9.25	56.28
9	57	29.67	91	106	0.86	14.89	104.23	18.6	17.85	16.8	18.55	13.34
10	48	29.69	103	118	0.87	4.285	30.00	12.8	42.67	13.4	15.8	36.13
11	32	30.53	99	103	0.96	10.77	75.39	24.6	32.63	10.1	2.335	60.91
12	60	30.57	89	113	0.79	8.148	57.04	13.7	24.02	19.5	4	78.58
13	49	35.11	97	113	0.86	36.6	256.20	41.4	16.16	25.1	3.4	43.78
14	47	37.11	107	124	0.86	24.84	173.88	59.6	34.28	17.7	5.15	33.71

Table 3A : 19 Male Non-Diabetic

Sr. No.	Age	BMI	Waist (cm)	Hip (cm)	WHR	Insulin (microIU/ml)	Insulin (pM)	Proinsulin (pM)	PI:I%	Leptin (ng/ml)	Adiponectin (ng/ml)	TNF-alpha (pg/ml)
1	69	17.99	81	86	0.94	6.943	48.60	8.65	18.00	3.13	9.35	133.8
2	28	19.31	76	85	0.89	13.56	94.92	17.9	19.00	3.85	5.8	80.47
3	30	21.3	81	87.5	0.93	6.861	48.03	10.4	22.00	3.63	9.35	65.22
4	26	22	75.5	94	0.80	7.03	49.21	13.6	28.00	1.53	16.2	41.1
5	27	22.01	71	92	0.77	9.473	66.31	12.6	19.00	7.27	12.1	70.51
6	31	22.69	87	100	0.87	8.699	60.89	9.63	16.00	10.4	19.3	54.52
7	28	23.7	83.5	93	0.90	11.5	80.50	16.7	21.00	7.13	6.1	42.49
8	29	24.27	75.5	94	0.80	17	119.00	15.1	13.00	7.86	8.5	94.44
9	35	25.24	92	100	0.92	18.11	126.77	11.3	9.00	11.3	31.9	58.15
10	41	25.43	87.5	96.5	0.91	9.224	64.57	19.2	30.00	4.08	2.615	55.08
11	30	25.54	87	102	0.85	16.08	112.56	13.5	12.00	5.74	11.25	69.57
12	26	25.6	80	94	0.85	1.325	9.28	10.3	111.00	8.91	5.55	13.94
13	26	25.81	85	95.5	0.89	11.4	79.80	12	15.00	6.24	11.8	58.09
14	22	26.43	78.5	90	0.87	7.727	54.09	24	44.00	4.09	6.3	455.1
15	18	28.08	85	91	0.93	11.24	78.68	21.1	27.00	24.3	15.9	67.2
16	37	29	92	98	0.94	28	196.00	31.8	16.00	25.7	0.965	91.49
17	18	35.29	121	133	0.91	21.31	149.17	20.4	14.00	11.6	3.96	97.46
18	42	36	125	130	0.96	26.65	186.55	22.5	12.00	12.6	7.45	119.3
19	52	42	130	139	0.94	27.48	192.36	25	13.00	24.38	1.87	56

seven. Normal range of proinsulin is 5 – 12.4 pmol/l mean 6.2 ± 1.12 . Normal range of fasting insulin is 1 - 10 mU/ml (7 –10 pmol/l). The published range of 3-25 mU/ml is erroneous as it masks a significant number of subjects with hyperinsulinemia as an indicator of insulin resistance. Normal PrI/I ratio is 1 : 6, but always less than 20 per cent.

All 21 male diabetics had proinsulin > 13 pmol/l with ratio > 20%. Normal insulin was seen in 8, high in 11 and low in 2. In these two patients the ratio was 67% and 114% suggestive of a genetic mutant in proinsulin converting enzyme.

Out of 14 female diabetics, 12 had proinsulin > 13 pmol/ and ratio > 20%. Fasting insulin was normal in 7, high in seven.

Out of 19 non-diabetic males, proinsulin was high in eleven (6 out of which had a ratio > 20%). Insulin was normal in eight, higher than normal in ten. In one subject the ratio was 111 suggestive of a genetic mutant in proinsulin converting enzyme.

Out of 31 non-diabetic females, proinsulin was high in 17 with a ratio of > 20% in 8 out of them. Insulin was higher in 17 (in 6 of whom it was > 20 mU/ml).

Leptin normal range in males is 3-7 ng/ml and in females 15-20 ng/ml. Our female subjects had a higher range than the normal female range in western population.

There was a positive correlation between insulin, proinsulin and leptin levels, and between these variables and BMI.

Table 3B : 31 Female Non-Diabetic

Sr. No.	Age (Yrs)	BMI	Waist (cm)	Hip (cm)	WHR	Insulin (microIU/ml)	I*7	Proinsulin (pM)	PI:I%	Leptin (ng/ml)	Adiponectin (ng/ml)	TNF-alpha (pg/ml)
1	26	20.27	62	88	0.70	9.74	68.18	11.4	16.72	10	15.55	46.96
2	23	20.45	83	92.5	0.90	5.016	35.11	10.4	29.62	7.57	18.75	43.39
3	25	20.83	64	92	0.70	4.274	29.92	2.07	6.92	16.5	25	44.14
4	63	21.05	78	93	0.84	6.838	47.87	4.07	8.50	10.1	10.85	97.58
5	34	21.08	74	93.5	0.79	4.588	32.12	7.63	23.76	8.04	20.75	339
6	31	21.57	75	94	0.80	6.75	47.25	8.75	18.52	15.2	20.25	27.83
7	30	22.27	65	90	0.72	41.5	290.50	17	5.85	17.8	30.85	79.5
8	25	23.16	73	94	0.78	11.85	82.95	8.18	9.86	13.2	10.8	33.1
9	32	23.31	76	97	0.78	8.061	56.43	9.58	16.98	22.4	11.4	51.23
10	19	23.51	72	95	0.76	9.877	69.14	7.83	11.33	13.3	13.75	94.84
11	26	23.59	69.5	93.5	0.74	11.48	80.36	7.77	9.67	21.6	14.95	34.64
12	37	23.83	75	95	0.79	7.255	50.78	9.74	19.18	9.73	16	39.49
13	31	24.44	72	89	0.81	10.96	76.72	12.4	16.16	22	7.1	48.09
14	58	25.94	107	95	1.13	12.8	89.60	17	18.97	13.6	3.45	49.43
15	50	26.61	86	100	0.86	12.94	90.58	14.9	16.45	39.5	6.9	87.52
16	28	26.61	86	100	0.86	8.586	60.10	10.9	18.14	20.3	7.6	27.87
17	31	27.21	79	100	0.79	15.71	109.97	13.5	12.28	24.1	14.25	58.21
18	24	29.21	87	107	0.81	9.469	66.28	17	25.65	25.4	11.45	39.91
19	28	31.01	88	104	0.85	16.45	115.15	21.6	18.76	23.5	25.4	123.7
20	19	31.11	88.5	99	0.89	39.15	274.05	47.1	17.19	14.3	8.45	113.2
21	34	32.51	100	120	0.83	9.74	68.18	13.7	20.09	21.4	4.04	113.8
22	55	32.85	98	116	0.84	17.35	121.45	14.6	12.02	27.3	16.6	32.45
23	23	34.96	88	111	0.79	15.62	109.34	17.6	16.10	44.1	5.5	23.3
24	27	35.33	88	117	0.75	12.21	85.47	18.7	21.88	52.5	11.45	31.09
25	50	35.98	105	117	0.90	50.15	351.05	55	15.67	25.2	14.35	82.79
26	30	36.63	98	119	0.82	21.33	149.31	17.1	11.45	51.4	8.7	7.687
27	57	38.57	118	144	0.82	16.29	114.03	23.4	20.52	46.6	0.301	104.1
28	31	39.13	109	120	0.91	18.03	126.21	15.5	12.28	59.2	16.7	65.08
29	36	42.95	124	127	0.98	11.51	80.57	9.99	12.40	32.7	6.35	57.93
30	20	45	125	128	0.98	25.93	181.51	39.1	21.54	42.6	9.1	92.15
31	35	55.23	125	170	0.74	46.02	322.14	36.7	11.39	62.2	4.615	51.57

Adiponectin : Normal mean value in males is 9.8 ± 2.9 mg/l and in females 16.6 ± 5 mg/l. 9 out of 21 male diabetics and 9 out of 14 female diabetics had low values (< 6 mg/ml).

TNF-α: The normal range of *TNF-α* is 0 – 20 pg/ml. *TNF-α* values showed a wide scatter with values 40 - 100 in 6/21 male and 13/19 female diabetics; more than 100 in 5 male and 3 female diabetics. In the non-diabetic population 14 females and 13 males showed values 40-100; 5 females and 3 males showed values > 100 pg/ml.

Out of 21 male diabetics only 2 had low values (9 and 16 pg/ml). Out of 14 female diabetics only one had low (13.34 pg/ml) all others ranged from 31 to 166 pg/ml). The lower range of *TNF-α* in females inspite of higher adipose mass indicates that apart from adipocytes there are other sources of production of *TNF-α*. *TNF-α* does not seem to correlate with BMI, both in males and females.

Statistical analysis

TNF-α and proinsulin were log transformed to obtain normal distribution. Students “t” test was used to compare the mean of the continuous variables. Pearson’s correlation coefficient was used to look for

association among the risk factors. All the groups were combined for Pearson’s correlation analysis. Logistic regression analysis was carried out using either diabetes or obesity as dependent variable and biomarkers as independent variables. For logistic regression analysis logged proinsulin values were categorized into deciles for better prediction. Linear regression analysis was done to determine the association between biomarkers. All analysis was performed with the SPSSPC+ statistical software package (Version 10.0. SPSS Chicago) and p values <0.05 were considered as the level of significance.

Non-availability of a kit to measure human resistin was a major handicap in this study in 2003. Diabetic subjects were older than the non-diabetic subjects [p<0.001]. Male preponderance was observed among diabetic group [p=0.046]. There is no significant difference between the study groups with regard to body mass index and waist circumference.

Fig. 1 presents the values of the biomarkers in the study groups. Leptin [p=0.002] and adiponectin levels were significantly lower [p=0.011] and proinsulin [p<0.001] values were significantly higher in diabetic subjects compared to non-diabetic subjects. *TNF-α* failed

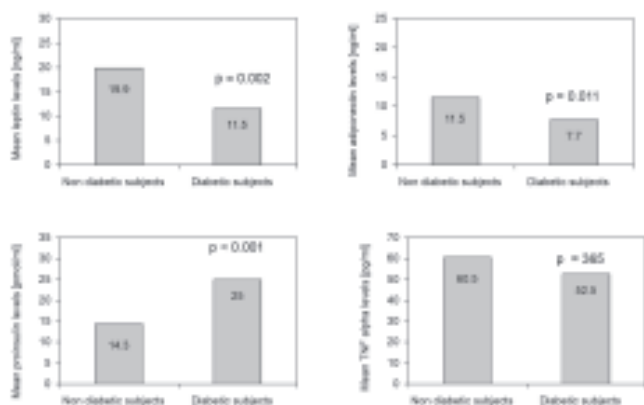


Fig. 1 : Mean values of the biomarkers in the study groups

to show any significant difference between the study groups. When categorized based on gender, in men, proinsulin maintained its significance [p=0.001], while in women, only adiponectin showed significant difference [p=0.006].

Obese subjects had significantly higher levels of leptin [obese: 20.7 ng/ml vs non-obese: 9.4 ng/ml, p<0.001], and proinsulin [obese: 22.4 pmol/ml vs non-obese: 12.5 pmol/ml, p<0.001] compared to non-obese subjects. Adiponectin levels were lower in subjects with obesity [obese: 12.3 ng/ml vs non-obese: 8.5 ng/ml, p<0.001]. When categorized based on diabetes status, the significance in difference was maintained in the non-diabetic group but not in diabetic group. TNF- α showed a significant difference in the diabetic subjects [p=0.013] but not in non-diabetic group.

Leptin and proinsulin showed a significant and positive correlation with body mass index [p<0.001] and waist circumference [p<0.001]. Adiponectin showed an inverse correlation with body mass index [p=0.050] and waist circumference [p=0.002]. Proinsulin showed a significant negative association with adiponectin [p=0.002]. None of the parameters showed any association with TNF- α (Table 4).

When categorized based on gender, leptin and proinsulin levels maintained the positive correlation with body mass index and waist circumference in men [p<0.001], while adiponectin showed no correlation. Proinsulin had a positive correlation with leptin levels (p = 0.014). In women, all the biomarkers showed similar correlation results as observed in the total population.

Logistic regression analysis using diabetes as the dependent variable revealed that leptin had a strong association with diabetes, which was maintained even after adding age, gender and BMI into the model [p=0.008]. Proinsulin increased the risk for diabetes significantly even after adjusting for age, gender and BMI [p=0.001]. Adiponectin showed a significant association with diabetes, which was abolished when age, gender and BMI was introduced into the model [p=0.088] [Table 5].

Regression analysis using obesity as the dependent variable showed that leptin [p<0.001] and proinsulin [p=0.001] had a strong significant positive association with obesity even after adjusting for age, gender and diabetes status. Adiponectin [p=0.035] showed a significant inverse association even after adjusting for age, gender and diabetes status [Table 6].

Linear regression analysis revealed that the association of adiponectin with proinsulin [β =-7.8, p=0.002] was not affected by the addition of age and gender [β =-7.0, p=0.009] and diabetes status [β =-6.1, p=0.033] into the model.

DISCUSSION

The study was performed from July 2002 to August 2003. The main findings of the study are the following :

Table 5 : Multiple logistic regression analysis using diabetes as the dependent variable

Parameters	OR	95% CI	p value
Leptin			
Unadjusted	0.941	0.899 – 0.985	0.009
Adjusted for age and gender	0.931	0.873 – 0.993	0.030
Adjusted for age, gender and BMI	0.864	0.775 – 0.963	0.008
Adiponectin			
Unadjusted	0.912	0.847 – 0.982	0.015
Adjusted for age and gender	0.933	0.858 – 1.014	0.103
Adjusted for age, gender and BMI	0.929	0.854 – 1.011	0.088
Proinsulin			
Unadjusted	1.444	1.193 – 1.749	< 0.001
Adjusted for age and gender	1.493	1.212 – 1.838	< 0.001
Adjusted for age, gender and BMI	1.567	1.246 – 1.971	< 0.001

Table 4 : Correlation of leptin, adiponectin, proinsulin and TNF- α with other study parameters

Variables	Leptin		Adiponectin		Proinsulin		TNF- α	
	r value	p value	r value	p value	r value	p value	r value	p value
Age	- 0.097	0.376	- 0.189	0.083	0.120	0.272	0.051	0.646
Body mass index	0.735	< 0.001	- 0.214	0.050	0.469	< 0.001	0.031	0.776
Waist circumference	0.432	< 0.001	- 0.332	0.002	0.531	< 0.001	0.088	0.423
Leptin	-	-	- 0.032	0.774	0.103	0.349	- 0.096	0.384
Adiponectin	-	-	-	-	- 0.324	0.002	0.074	0.500
Proinsulin	-	-	-	-	-	-	0.010	0.929

Table 6 : Multiple logistic regression analysis using obesity as the dependent variable

Parameters	OR	95% CI	p value
Leptin			
Unadjusted	1.119	1.048 – 1.194	0.001
Adjusted for age and gender	1.330	1.148 – 1.540	< 0.001
Adjusted for age, gender and diabetes status	1.365	1.170 – 1.592	< 0.001
Adiponectin			
Unadjusted	0.925	0.865 – 0.989	0.015
Adjusted for age and gender	0.927	0.865 – 0.993	0.032
Adjusted for age, gender and diabetes status	0.927	0.865 – 0.995	0.035
Proinsulin			
Unadjusted	1.368	1.144 – 1.636	0.001
Adjusted for age and gender	1.364	1.098 – 1.696	0.005
Adjusted for age, gender and diabetes status	1.617	1.218 – 2.147	0.001

1) Leptin and adiponectin levels were lower in diabetic subjects 2) Proinsulin levels were higher in diabetic subjects compared to non-diabetic subjects 3) Leptin and proinsulin are higher while adiponectin lower in obese subjects compared to non-obese subjects 4) Proinsulin showed a significant inverse association with adiponectin even after adjusting for age, gender and diabetes status 5) TNF- α showed no association with diabetes or with other risk markers.

This, to our knowledge, is the first study which reports on the association of adiponectin, leptin, TNF- α , insulin and proinsulin with diabetes and obesity in an Asian Indian population and is significant because of the high risk for diabetes observed in this population.^{4,5} Earlier studies have shown that hypoadiponectinemia and increased high sensitive C-reactive protein are seen in Asian Indians compared to Caucasians.^{15,16} Thus it is clear that Asian Indians have unique biochemical and hormone features which may be considered as additional features of the so called 'Asian Indian Phenotype'. This is particularly significant since the increased predisposition to diabetes among Indians is not explained by conventional factors. Thus identifying newer or novel risk markers could facilitate early detection and thereby lead to risk reduction of diabetes. In this study we report that the leptin, adiponectin and proinsulin show changes in urban Asian Indian diabetic subjects studied in western India.

Adiponectin is a 244 amino acid protein, which has been shown to be associated with insulin sensitivity and better lipid profile, decreased inflammation and improved glycemic control.^{7,9} In this study we observe that diabetic subjects had low levels of adiponectin similar to that reported in a south Indian study.¹² Since adiponectin circulates in multimeric forms, recent reports have focused on the high molecular weight

[HMW] adiponectin and have documented that Asian Indian pregnant women have lower adiponectin values compared to Caucasians.¹⁷ Adiponectin also showed an inverse association with obesity and its indices (BMI and waist circumference) which is strongly associated with insulin resistance. Indeed, intervention studies have shown increase in adiponectin values with weight loss. Our results therefore confirm the protective role of adiponectin in diabetes reported earlier.

Increased TNF- α levels inhibit expression of adipose tissue adiponectin mRNA by > 50 percent and lower plasma levels of adiponectin.¹⁸

The low adiponectin levels indicate the need and scope for therapeutic interventions aimed at raising the level. Intake of diets rich in essential fatty acids EPA/DHA leads to elevated systemic concentrations of adiponectin, largely independent of food intake and adiposity and explain to some extent their antidiabetic effects. EPA/DHA protect against insulin resistance and obesity in rodents and increase insulin sensitivity in human.¹⁹

Leptin is another adipocytokine which correlates with body fat, body mass index and waist circumference.¹⁰ Leptin is considered to be the missing link between obesity and diabetes, as it has been shown to regulate blood sugar via its control on appetite and fat storage. Studies have also shown that leptin levels are higher in obese subjects but lower in diabetic individuals.²⁰ The present study supports these findings as type 2 diabetic subjects had lower leptin levels, despite its positive correlation with obesity indices. This could probably be due to differences in the association of body fat compartments with diabetes and obesity as higher leptin secretion occurs in the subcutaneous fat, whereas it is visceral fat that is more strongly associated with diabetes.⁸

TNF- α is a pro-inflammatory cytokine which has been shown to be associated with obesity. Some studies have documented increased TNF- α levels in subjects with diabetes. This has also been suggested as the link between obesity, diabetes and atherosclerosis.¹¹ However, in the present study we did not observe any association between TNF- α and diabetes. High values were found in both diabetics and non-diabetics. This merits further studies on TNF- α in Asian Indians, and also indicates the need and scope for preventive interventions.

The higher range of TNF- α in males independent of BMI has important implications, in view of the greater muscle component in males. TNF- α causes insulin resistance in muscle via serine phosphorylation of IRS-1 which in this modified form inhibits insulin receptor tyrosine kinase activity.

Our study brings out the importance of routinely measuring both specific insulin and proinsulin in clinical and epidemiological studies of IRS/CDS in India

where the population is at high risk.²¹

Studies in Mexican Americans, a population at high risk for diabetes, showed a stepwise increase in fasting insulin level in healthy subjects: neither parent diabetic - < 70 pmol/l; one parent diabetic - 77.8 pmol/l; both parents diabetic 94.6 pmol/l. Healthy subjects with a diabetic sibling had higher fasting insulin (83.2 pmol/l) than healthy subjects without a diabetic sibling (69.9 pmol/l).²²

In our study 8/19 non-diabetic males had fasting insulin > 90 pmol/l and fasting pro-insulin > 13 pmol/l. Fasting proinsulin rise predicts the development of Type 2 diabetes mellitus.²³ Increased fasting proinsulin to insulin ratio, as seen in our subjects indicates genetics predisposition to Type 2 diabetes mellitus. Absolute hyperproinsulinemia occurs with development of Type 2 diabetes mellitus.²⁴

The cause of disproportionate hyper pro-insulinemia in Type 2 diabetes mellitus is probably an increase in the demand on islet β cells. A prospective study of clinically healthy volunteers undergoing hemipancreatectomy for organ donation showed pre-donation baseline pro-insulin 6.24 ± 1.14 pmol/l; at one year post-donation 34.63 ± 17.47 pmol/l. Normal controls (cross-sectional group) showed levels (5.78 ± 1.12 pmol/l).²⁵

An alternative mechanism of increased proinsulin is a defect in prohormone convertase enzyme (PC1, PC2) which may be a mutant gene. In our study 2 diabetic males and one non-diabetic male had high proinsulin and very low insulin with ratio > 100 which suggests this defect. In that situation we should expect higher levels in other prohormone (pro-glucagon, pro-somatostatin). Recombinant human interleukin 1 b inhibits insulin biosynthesis by preferential attenuation of the rate of conversion of proinsulin 2 in rat islets.²⁶ This possibility remains to be explored in human studies. Whether TNF- α has similar effect remains to be studied.

Another polypeptide secreted by the islet β cells is amylin, which deserves to be studied along with pro-insulin since it is a normal participant in the process of pro-insulin processing and storage.²⁷ We could not obtain the kit for measuring amylin along with proinsulin.

Proinsulin is a predictor of diabetes and cardiovascular disease. Subjects with increased proinsulin could be a target group for risk reduction of both. An interesting observation in this study is that adiponectin showed a significant negative association with proinsulin even after adjusting for diabetes status, indicating a link between adiposity and insulin secretion. The IRIS II study has explored this association in a western population and has indicated proinsulin to be a good marker for diabetes.²⁸

Effects of dietary treatment on the disproportionately

elevated serum proinsulin both fasting and post-glucose load in subject with diabetes showed decrease in proinsulin from 31 ± 18 to 13 ± 5 pmol/l, insulin decreased from $15 + 8$ to $10 + 4$ mU/ml and the molar ratio of proinsulin to insulin decreased from 0.321 ± 0.08 to 0.24 ± 0.1 .²⁹ Other studies found persistence of hyperproinsulinemia in Type 2 diabetes mellitus despite reduction of hyperglycaemia with insulin and sulphonylurea therapy.³⁰

In conclusion, we report that in Asian Indians with diabetes, leptin and adiponectin levels are lower, while proinsulin levels are higher, compared to non-diabetic subjects. Moreover, leptin and proinsulin levels were higher, while adiponectin was lower in obese subjects compared to their non-obese counterparts. Proinsulin levels showed an inverse association with adiponectin indicating a link between insulin resistance and insulin secretion, TNF- α did not show any association with diabetes in this population. Further studies are needed to confirm these observations in other groups of Asian Indians.

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REFERENCES

1. Joshi SR. Metabolic syndrome - Emerging clusters of the Indian Phenotype. *J Assoc Physicians India* 2003;51: 445-46.
2. Mohan V, Sharp PS, Cloke HR, Burrin JM, Schumer B, Kohner EM. Serum immunoreactive insulin responses to a glucose load in Asian Indian and European type 2 (non-insulin-dependent) diabetic patients and control subjects. *Diabetologia* 1986;29:235-37.
3. Sharp PS, Mohan V, Levy JC, Mather HM, Kohner EM. Insulin resistance in patients of Asian Indian and European origin with non-insulin dependent diabetes. *Horm Metab Res* 1987;19: 84-85.
4. Mohan V, Deepa M, Deepa R, et al. Secular trends in the prevalence of diabetes and impaired glucose tolerance in urban South India-the Chennai Urban Rural Epidemiology Study (CURES-17). *Diabetologia* 2006;49:1175-78.
5. Ramachandran A, Snehalatha C, Kapur A, et al. Diabetes Epidemiology Study Group in India (DESI). High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia* 2001;44:1094-1101.
6. Gupta R, Gupta VP, Sarna M, et al. Prevalence of coronary heart disease and risk factors in an urban Indian population: Jaipur Heart Watch-2. *Indian Heart J* 2002;54:59-66.
7. Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. *Diabetes* 2006;55:1537-45.
8. Anjana M, Sandeep S, Deepa R, Vimaleswaran KS, Farooq S, Mohan V. Visceral and central abdominal fat and anthropometry in relation to diabetes in Asian Indians. *Diabetes Care* 2004;27:2948-53.

9. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome *J Clin Invest* 2006;116:1784-92.
10. Abdella NA, Mojiminiyi OA, Moussa MA, Zaki M, Al Mohammedi H, Al Ozairi ES, Al Jebely S. Plasma leptin concentration in patients with Type 2 diabetes: relationship to cardiovascular disease risk factors and insulin resistance. *Diabet Med* 2005;22:278-85.
11. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995;95: 2409-15.
12. Mohan V, Deepa R, Pradeepa R, et al. Association of low adiponectin levels with the metabolic syndrome - The Chennai Urban Rural Epidemiology Study (CURES - 4). *Metabolism* 2005;54:476-81.
13. Pflutzner A, Pflutzner AH, Larbig M, Forst T. Role of intact proinsulin in diagnosis and treatment of type 2 diabetes mellitus. *Diabetes Technol Ther* 2004;6:405-12.
14. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1 : Diagnosis and classification of diabetes mellitus, provisional report of a WHO Consultation *Diabet Med* 1998;15:539-53.
15. Abate N, Chandalia M, Snell PG, Grundy SM. Adipose tissue metabolites and insulin resistance in nondiabetic Asian Indian men *J Clin Endocrinol Metab* 2004;89:2750-55.
16. Chambers JC, Eda S, Bassett P, et al. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104:145-50.
17. Retnakaran R, Hanley AJ, Connelly PW, Maguire G, Sermer M, Zinman B. Low serum levels of high-molecular weight adiponectin in Indo-Asian women during pregnancy: evidence of ethnic variation in adiponectin isoform distribution. *Diabetes Care* 2006;29:1377-79.
18. Lihn AS, Richelsen B, Pedersen SB, et al. Increased expression of TNF α , IL-6 and IL-8 in HALS. Implications for reduced adiponectin expression and plasma levels. *Amer. J. Physiol. Endocr. Metab* 2003;285:E1072-1080.
19. Flachs P, Mohmed Ali V, Hora Kova O, et al. Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high fat diet. *Diabetologia* 2006; 394-7.
20. Tatti P, Masselli L, Buonanno A, Di Mauro P, Strollo F. Leptin levels in diabetic and nondiabetic subjects. *Endocrine* 2001;15:305-58.
21. Haffner SM, Mykkanen L, Stern MP, Valdez RA, Heisserman JA, Bowsher RR. Relationship of proinsulin and insulin to cardiovascular risk factor in non-diabetic subjects. *Diabetes* 1993; 42: 1297-302.
22. Haffner SM, Stern MP, Hazuda HP, Mitchell BD and Patterson JK. Increased insulin concentrations in non-diabetic offspring of diabetic parents. *N Engl J Med* 1988;319:1297-301.
23. Wareham NJ, Byrne CD, Williams R, Day NE, Itals CN. Fasting proinsulin concentrations predict the development of type 2 Diabetes Mellitus. *Diabetes Care* 1999;22:262-70.
24. Ramachandran A, Snehalata C, Satyavani K, Vijay V. Effect of genetic predisposition on proinsulin response in Asian Indians. *Diabetes Res Clin Pract* 1998;41:71-7.
25. Seaquist ER, Kahn SE, Clark PM, Hales CN, Porte D, Roertson RP. Hyperproinsulinemia is associated with increased β cell demand after hemiparacreatectomy in humans. *JCI* 1996;97:455-60.
26. Hansen BS, Nielsen JH, Linde S, et al. Effect of interleukin-1 on the biosynthesis of proinsulin and insulin in isolated rat pancreatic islets : Biomed. *Biochim Acta* 1988;47:305-09.
27. Porte D Jr., Kahn SE. Hyperproinsulinemia and amyloid in NIDDM. Clue to etiology of islet β cell dysfunction? *Diabetes* 1989;38:1336-38.
28. Langenfeld MR, Forst T, Standl E, et al. IRIS II study. IRIS II Study: Sensitivity and specificity of intact proinsulin, adiponectin, and the proinsulin/adiponectin ratio as markers for insulin resistance. *Diabetes Technol Ther* 2004;6:836-43.
29. Yoshioka N, Kuzuya T, Matsuda A, Iwamoto Y. Effects of dietary treatment on serum insulin and proinsulin response in newly diagnosed NIDDM Diabetes 1989;38:262-66.
30. Rachman J, Levy JC, Barrow BL, et al. Relative hyperproinsulinemia of T2DM persists despite reduction of hyperglycaemia with insulin and sulphonylurea therapy. *Diabetes* 1997;96:1557-62.

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For further details contact : **Dr. Kaleem Ahmad**, Bajoria Road, Saharanpur (U.P.) – 247001.
Tel: 0132-2716408 (C); 0132-2716050 (R); Cell: 9837072447