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Original Article

High fasting C-peptide levels and insulin resistance in non-lean & non-obese (BMI >19 to < 25 kg/m^2) Asian Indians with type 2 diabetes are independently associated with high intra-abdominal fat and liver span

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ABSTRACT

Background and objectives: We aimed to compare C-peptide levels & measures of insulin resistance in non-lean & non-obese Asian Indian patients with type 2 diabetes (T2DM) *vs.* non-lean, non-diabetic controls and to correlate with anthropometric measures, volumes of abdominal adipose tissue depots, pancreas, & liver span.
Methods: Non-lean, non-obese (BMI >19 and < 25 kg/m^2) Asian Indian patients with T2DM, (cases n, 87), diagnosed within one year, on metformin mono therapy, were compared to BMI-matched non-diabetic subjects (controls, n, 37). Measures of glycaemia, insulin and C-peptide levels (fasting and post-prandial), lipid profile, and hepatic transaminases were analysed. Abdominal adipose tissue volumes [subcutaneous & intra-abdominal], pancreatic volume and liver span were assessed using 1.5 Tesla MRI scan.
Results: In cases, the mean values of HbA1c, fasting and post prandial insulin and C-peptide levels, and 3 measures of insulin resistance were significantly higher than controls, but not for HOMA-B. Higher fasting C-peptide levels correlated significantly with HOMA-IR ($r = 0.42$, $p < 0.001$), Fasting Insulin Resistance Index ($r = 0.42$, $p < 0.001$), Bennett's Index ($r = 0.38$, $p < 0.05$), and volumes of SCAT and IAAT only in cases. The independent predictors of higher fasting C-peptide level were IAAT volume ($\beta = 0.057$; $p = 0.002$), liver span ($\beta = 0.057$, $p = 0.005$) and fasting insulin levels ($\beta = 0.35$, $p = 0.02$).
Conclusion: Higher fasting and post-prandial C-peptide levels and surrogate measures of insulin resistance in non-obese Asian Indian patients with T2DM are independently associated with IAAT volume and liver span.

1. Introduction

The rising prevalence of type 2 diabetes mellitus (T2DM) in India continues to remain unabated. Increasing obesity in urban and suburban areas is a pivotal factor causing insulin resistance and consequent diabetes in south Asia [1]. While most patients with T2DM are obese, nearly 20–30% of Asian Indians with T2DM are non-obese [body mass index (BMI< 25 kg/m^2) [2] and some are lean (BMI <19 kg/m^2) [3]. Patients with diabetes who are non-obese/lean are particularly seen in tropical countries including India and sub-Saharan Africa. The pathogenesis of apparent T2DM in non-obese individuals remains ill-understood. Besides T2DM, other etiological factors of such patients with diabetes include monogenic diabetes, chronic calcific pancreatitis and lipodystrophies. When such etiological factors, admittedly rare, are excluded, most common etiology remains T2DM.

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How non-obese individuals develop diabetes continues to be debated and researched. In particular, the pathogenesis of T2DM in lean/underweight individuals (BMI <19 kg/m^2) is even more intriguing, and has been researched in India to some extent. However, such lean individuals with diabetes are in minority (<5%) when all 'non-obese' patients with diabetes are taken in account [4].

It is possible that non-lean, non-obese (BMI >19 to < 25 kg/m^2) Asian Indians may harbor several physical and metabolic characteristics which may predispose them to develop insulin resistance [5]. Specifically, despite being non-obese, these individuals have ectopic fat deposition. For example, as compared to white Caucasians, non-obese Asian Indians have high body fat, excess fat in liver and skeletal muscles and lesser skeletal muscle mass. Ectopic fat in liver (non-alcoholic fatty liver disease, NAFLD) and pancreas (non-alcoholic fatty pancreas disease, NAFPD) may contribute to insulin resistance leading to T2DM in Asian Indians, even at young age [6,7]. However, such body composition features may not be present in all 'non-obese' individuals, posing a question regarding pathogenesis of hyperglycemia. In particular, such non-lean, non-obese patients with T2DM have not been researched in the context of insulin resistance and its relation to C-peptide levels and body composition.

An easy way to assess insulin resistance is to evaluate if a person has hyperinsulinemia. However, in patients with prediabetes or T2DM, the pulsatile rhythm of pancreatic insulin secretion is deranged and plasma insulin levels in such patients may be deceptive owing to hepatic insulin clearance and/or inter-ethnic variations [8]. In comparison to insulin, the connecting (C) peptide is secreted at a more constant rate across time durations and can be used as a marker of existing pancreatic beta cell function. The C-peptide is composed of 31 amino acids as part of the pro-insulin molecule which links the alpha and beta chains of the insulin molecule to facilitate inter-chain disulphide bond formation. The C-peptide undergoes proteolytic cleavage and enables signal transduction in carboxy terminal of the B chain to the insulin receptor and modulates the glucoregulatory functions of insulin. Importantly, C-peptide levels are more stable (half-life; 20–30 min), than that of insulin (half-life; 3–5 min), which provide a consistent "window period" for testing the beta cell response to glucose/mixed meal challenge test and physiological response to hypoglycaemic agents, especially in patients with young-onset diabetes [9].

In ethnic groups other than Asian Indians, elevated C-peptide levels have been shown to be associated with metabolic syndrome [10], T2DM and atherosclerosis [11]. However, the association of C-peptide levels with insulin resistance in non-obese Asian Indians has not been researched earlier. Further, the relationship of C-peptide levels with abdominal fat depots and ectopic fat depots; e.g. NAFLD, is not known in non-lean, non-obese Asian Indians with T2DM. Thus, in this study, we aimed to evaluate C-peptide levels and various measures of insulin resistance in non-lean, non-obese (BMI >19 to < 25 kg/m^2) Asian Indians with T2DM as compared to non-lean, non-obese, non-diabetic controls of similar BMI range.

2. Methodology

The study was reviewed and approved by the institutional review board and conducted according to the declaration of Helsinki 2013 [12]. Details of study subjects have been published previously [6,7]. Briefly, non-lean, non-obese (BMI >19 to < 25 kg/m^2) Asian Indians with T2DM (n, 87), aged between 18 and 40 years and diagnosed within one year from onset of diabetes & non-lean, non-obese (BMI >19 to <25 kg/m^2) non-diabetic subjects (controls; n, 37) were recruited with informed written consent. For truncal adiposity, skinfolds at four sites (abdominal skinfolds; horizontal and vertical, subscapular, supra-iliac & and sum of all four skinfolds), waist circumference, and hip circumference were measured as mentioned previously. Abdominal obesity was defined as waist circumference ≥90 cms in men and ≥80 cms in women [13]. All subjects underwent MR imaging (1.5 Tesla) to assess the volumes of total abdominal & abdominal adipose tissue compartments *viz.* subcutaneous abdominal adipose tissue (SCAT; anterior, posterior, superficial & deep) and intra-abdominal adipose tissue [IAAT; intra-peritoneal (IPAT) & retroperitoneal (RPAT)], liver span and pancreatic volume, using previously published protocols (6,7). Pancreatic volume index was calculated as pancreatic volume (cm^3)/body surface area (m^2) [14]. Liver span was measured using a T2 weighted coronal scan and fat infiltration in the liver was measured using IN/OUT FSPGR sequence as previously described (6, 7). NAFLD was determined based on presence of steatosis on imaging [15] and was graded as normal, grade 1, grade 2 or grade 3 in the absence of significant alcohol intake, viral infections and use of hepatotoxic drugs [16].

Based on fasting insulin and glucose levels, the following indices of insulin resistance were calculated;

1. Homeostasis model of insulin resistance (HOMA-IR): Fasting plasma insulin (mU/l) x fasting plasma glucose (mmol/l)/22.5 [17], HOMA IR value > 2.29 was considered as insulin resistance [18].
2. Fasting Insulin Resistance Index (FIRI): Fasting insulin x fasting glucose/25 [19].
3. Bennet's Index: 1/log (fasting insulin) x log (fasting glucose) [20].
4. Homeostasis model of beta cell function assessment (HOMA-B): 20 × fasting insulin (μIU/ml)/fasting glucose (mmol/ml) – 3.5 [21].

2.1. Biochemical analysis

Fasting and post-prandial (after a standard meal of 265 calories; nutrient composition: proteins 12%, carbohydrates: 77% and fats: 11%) blood samples were analysed for glycaemic and lipid profile and hepatic transaminases [22]. Serum C-peptide levels (fasting and post-prandial) were determined quantitatively by solid phase direct sandwich enzyme linked immunosorbant assay (ELISA) using commercial kits (SIGMA, St Louis, USA). The sensitivity of the assay kit was 0.013 ng/ml while the mean inter-assay coefficient of variation (16 replicates) and intra-assay (20 replicates) were 9.6% and 3.6%, respectively. The detection limit of the C-peptide assay was 1.12–6.7 picomoles/liter (0.5–3.0 ng/ml).

2.2. Statistical analysis

Continuous variables were summarized as mean ± standard deviation or median values as appropriate. Independent sample's *t*-test was applied to test for significance in mean values between groups. Pearson's correlation analysis was applied to test for significant correlations of fasting C-peptide levels with measures of anthropometry, skinfolds, biochemical variables, abdominal adipose tissue depots, pancreatic volume, pancreatic volume index and liver span. Multiple logistic regression (MLR) analysis was applied to determine predictors for higher fasting C- peptide levels in the study cohort. Results were considered statistically significant at P value less than 0.05. STATA 14.2 software (Stata Corp, Texas USA) was used for data analysis.

3. Results

In non-lean, non-obese Asian Indians with T2DM (n, 87) (males: n, 67, females: n, 20), the mean age (34.3 ± 3.1 years) was significantly higher (p = 0.000) as compared to controls (males: n, 24, females: n, 13; mean age: 26.2 ± 4.3 years). The following anthropometric parameters were significantly higher in patients with T2DM as compared to controls even after adjustment for age *viz*; waist circumference, skinfolds [biceps, triceps, thighs, calf, supra-iliac (horizontal, vertical and & average of the two] (Table 1).

Importantly, fasting and post-prandial levels of insulin and C-peptide were significantly higher in patients with T2DM as compared to controls, even after adjustment for age. Furthermore, the mean levels of glycosylated haemoglobin, serum triglycerides, total cholesterol, very low-density lipoprotein cholesterol and hepatic transaminases were significantly higher in patients with T2DM (Table 2).

On MRI, significantly higher mean values were seen in patients with T2DM for the following *viz*; total SCAT, IAAT, IPAT, total IAAT, pancreatic volume, pancreatic volume index and liver span (p < 0.05) as compared with controls (Table 3). NAFLD was observed in 44 T2DM patients (50.5%). As for indices of insulin resistance, significantly higher mean values were recorded in T2DM patients for HOMA-IR, Bennet's Index (Fig. 1) and FIRI (Fig. 2) as compared to controls. More importantly, in this study, 59 (67.8%) patients with T2DM and 2 controls (5.4%) showed HOMA-IR value indicative of insulin resistance in Asian Indians. No significant differences were observed between patients with T2DM and non-diabetic subjects, for HOMA-B index (surrogate measure of beta cell function) (Table 4). Significant positive correlation was observed for fasting C-peptide level with biochemical variables namely fasting insulin, post-prandial C-peptide, SGPT and all three surrogate indices of insulin resistance in patients with T2DM (Table 5). Further, fasting C-peptide levels in patients with T2DM correlated significantly with abdominal (horizontal & vertical) and suprailiac skinfolds (horizontal, vertical and average). On MRI, significant positive correlation was observed for fasting C-peptide levels with volumes of total IAAT (Fig. 3), IAAT, IPAT and SCAT compartments (Fig. 4) except posterior and deep SCAT in patients with T2DM. In contrast, no significant correlations of fasting C-peptide levels were observed for liver span, pancreatic volume and pancreatic volume index in patients with T2DM and in controls. On MLR analysis, total intra-abdominal fat volume, liver span and fasting insulin appeared as significant predictors of fasting C-peptide levels in the study cohort. Specifically, the beta coefficient value was highest for fasting insulin but lower and equal for liver span and total abdominal fat volume.

4. Discussion

This is the first study showing high C-peptide levels in non-lean, non-obese patients with T2DM living in north India. In addition, all surrogate markers of insulin resistance calculated in this study were significantly higher in such patients with T2DM, indicating

Table 1
Group-wise comparison of anthropometric profile.

Anthropometric variables	Unadjusted	Unadjusted	p value	Adjusted for age	Adjusted for age	p value
	Non-lean, non-obese patients with T2DM (n, 87)	Non-lean, non-obese, non-diabetic controls (n, 37)		Non-lean, non-obese patients with T2DM (n, 87)	Non-lean, non-obese, non-diabetic controls (n, 37)	
Body mass index (kg/m^2)	22.6 ± 2.0	21.3 ± 2.1	0.21	22.8 ± 1.9	22.4 ± 1.8	0.33
Body surface area (cm^3/m^2)	1.69 ± 0.1	1.59 ± 0.2	0.09	1.7 ± 0.09	1.6 ± 0.18	0.97
Waist circumference (cm)	84.7 ± 6.8	81.8 ± 7.7	<0.01	85.8 ± 4.8	84.5 ± 5.6	0.02
Hip circumference (cm)	89.5 ± 4.6	90.8 ± 7.3	0.23	87.3 ± 4.8	91.3 ± 5.0	0.06
Waist-to-hip ratio	0.94 ± 0.0	0.90 ± 0.0	<0.001	0.95 ± 0.0	0.90 ± 0.0	<0.001
Mid arm circumference (cm)	27.1 ± 2.4	27.4 ± 5.4	0.6	27.2 ± 2.8	27.6 ± 3.1	0.53
Mid-thigh circumference (cm)	49.5 ± 4.2	48.7 ± 4.8	0.24	49.7 ± 3.8	48.7 ± 4.4	0.26
Skinfold measurement						
Biceps (mm)	12.6 ± 8.8	8.2 ± 4.1	<0.01	13.8 ± 6.3	8.3 ± 5.7	<0.01
Triceps (mm)	14.4 ± 5.3	17.9 ± 7.4	<0.01	15.5 ± 0.3	19.3 ± 6.3	<0.01
Thigh skinfold (mm)	26.2 ± 9.1	22.4 ± 5.7	<0.01	26.2 ± 7.5	23.5 ± 6.6	<0.01
Calf skinfold (mm)	20.8 ± 7.4	11.3 ± 5.2	<0.01	20.1 ± 5.8	12.4 ± 5.7	<0.01
Subscapular skinfold (mm)	21.1 ± 5.8	21.8 ± 8.5	0.79	21.9 ± 6.7	22.3 ± 6.9	0.8
Supra iliac (horizontal) (mm)	19.7 ± 5.1	15.8 ± 5.4	<0.01	19.7 ± 4.6	16.2 ± 5.8	<0.01
Supra iliac (vertical) (mm)	21.2 ± 4.9	17.2 ± 4.5	<0.05	21 ± 5.0	18.0 ± 9.6	<0.05
Supra iliac(average) (mm)	19.2 ± 5.3	16.8 ± 4.7	<0.05	18.9 ± 5.0	17.8 ± 4.2	<0.05
Abdominal(vertical) (mm)	24.1 ± 6.9	24.7 ± 5.3	0.87	24.6 ± 8.1	23.8 ± 5.7	0.61
Abdominal (horizontal) (mm)	22.9 ± 6.8	23.7 ± 5.4	0.6	24.6 ± 8.1	24.8 ± 5.7	0.91
Abdominal(average) (mm)	23.8 ± 7.5	24.1 ± 5.3	0.71	24.7 ± 8.1	24.0 ± 5.7	0.66
Abdominal(total) (mm)	48.7 ± 10.6	43.8 ± 18.9	0.07	48.7 ± 13.8	43.8 ± 21.2	0.05

Values are presented as means ± SD. $p < 0.05$: Statistically significant.

Table 2
Group-wise comparison of biochemical profile.

	Unadjusted	Unadjusted	p value	Adjusted for age	Adjusted for age	p value
	Non-lean, non-obese patients with T2DM (n = 87)	Non-lean, non-obese, non-diabetic controls (n = 37)		Non-lean, non-obese patients with T2DM (n = 87)	Non-lean, non-obese, non-diabetic controls (n = 37)	
Glycaemic profile (mg/dl)						
Fasting blood glucose	147.8 ± 51. 1	89.4 ± 2.6	<0.000	148.1 ± 12.4	88.8 ± 32	0.92
Post-prandial blood glucose	222.59 ± 82.9	89.36 ± 13.7	<0.01	222.9 ± 70.5	79.8 ± 12.6	<0.01
Glycosylated haemoglobin (%)	9.00 ± 2.5	5.2 ± 0.37	<0.01	9.0 ± 2	5.1 ± 2	<0.001
Serum insulin (mIU/ml)						
Fasting insulin	9.5 ± 0.6	5.5 ± 0.4	<0.001	10.0 ± 9.5	6.8 ± 8.1	<0.05
Post prandial insulin	22.3 ± 3.0	8.0 ± 0.5	<0.001	21. 5 ± 44.6	13.0 ± 28.6	0.07
C-peptide (pmol/litre)						
Fasting C- peptide	931.64 ± 251.7	503. 57 ± 128.6	<0.01	928.58 ± 236.4	485.87 ± 119.6	<0.01
Post prandial C- peptide	1523.4 ± 486. 0	792. 8 ± 185.7	<0.001	1486.3 ± 475.0	785.7 ± 175.7	<0.001
Serum lipids (mg/dl)						
Total cholesterol	175.44 ± 41.50	152.48 ± 29.14	<0.01	175.4 ± 38.4	152.6 ± 42.2	0.34
Serum triglycerides	170.10 ± 99.47	97.15 ± 45.8	<0.01	170.0 ± 87.3	97.4 ± 88.2	<0.001
High-density lipoprotein cholesterol	40.77 ± 8.93	42.47 ± 8.33	0.31	40.8 ± 8.6	42.4 ± 8.1	<0.05
Low-density lipoprotein cholesterol	110.3 ± 31.27	96.81 ± 25.32	<0.05	110.2 ± 29.7	98.0 ± 29.6	<0.001
Very low density lipoprotein cholesterol	33.5 ± 19.32	19.47 ± 9.18	<0.01	19.5 ± 16.3	33.5 ± 17.0	<0.001
Hepatic transaminases (U/L)						
Serum glutamic pyruvic transaminase	57.81 ± 21.76	45.60 ± 18.12	<0.01	57.9 ± 20.1	47.1 ± 21.4	0.17
Serum glutamic oxaloacetic transaminase	27.89 ± 12.84	23.68 ± 9.18	<0.05	27.8 ± 11.5	24.6 ± 11.9	<0.001

Values are presented as means ± SD. $p < 0.05$: Statistically significant.

Table 3
Group-wise comparison of volumes of abdominal fat depots, pancreatic volume and liver span.

	Unadjusted	Unadjusted	p value	Adjusted for age	Adjusted for age	p value
	Non-lean, non-obese patients with T2DM (n, 87)	Non-lean, non-obese, non-diabetic controls (n, 37)		Non-lean, non- obese patients with T2DM (n, 87)	Non-lean, non-obese, non-diabetic controls (n, 37)	
Subcutaneous abdominal adipose tissue volumes (cm^3)						
Superficial subcutaneous	83.9 ± 26.7	82.4 ± 30.8	0.77	77.8 ± 27.5	84.8 ± 27.8	0.35
Deep subcutaneous	22.3 ± 10.5	19.6 ± 8.3	0.15	22.1 ± 6.5	20.3 ± 8.4	0.35
Anterior subcutaneous	48.0 ± 20.7	46.7 ± 20.1	0.56	48.3 ± 18.2	48.4 ± 20.7	0.98
Posterior subcutaneous	57.4 ± 19.6	55.8 ± 19.4	0.75	54.2 ± 19.9	58.0 ± 19.8	0.41
Total subcutaneous	101.1 ± 33.3	102.1 ± 38.0	0.86	101.9 ± 31.1	105.1 ± 33.2	0.64
Intra-abdominal adipose tissue volumes (cm^3)						
Retro-peritoneal	31.5 ± 12.5	19.6 ± 9.0	<0.001	33.4 ± 11.3	19.9 ± 10.8	<0.001
Intra-peritoneal	70.3 ± 29.5	47.0 ± 21.0	<0.001	68.2 ± 23.5	44.4 ± 27.5	<0.001
Total Intra-abdominal	102.8 ± 38.8	68.5 ± 28.5	<0.001	69.1 ± 35.0	49.3 ± 36.7	<0.001
Pancreas						
Pancreatic volume (cm^3)	65.4 ± 22.9	53.2 ± 20.8	<0.01	67.1 ± 22.7	53.9 ± 22.8	<0.01
Pancreatic volume index (cm^3)	37.7 ± 12.8	31.9 ± 10.8	<0.01	38.6 ± 10.3	32.2 ± 12.8	<0.05
Liver span (mm)	163 ± 14.5	147.0 ± 18.1	<0.001	165.1 ± 14.1	149.0 ± 16.2	<0.001

Values are presented as means ± SD. $p < 0.05$: Statistically significant.

significant insulin resistance despite apparent absence of obesity based on BMI. Interestingly, no beta cell secretory defect was noted between the two groups. In further detailed evaluation, we also show associations of fasting C-peptide levels with surrogate indices of insulin resistance, abdominal and supra-iliac skinfold thickness and MRI based quantification of SCAT and IAAT volumes. Importantly, higher C- peptide levels independently predicted total intra-abdominal fat volume, increased liver span and higher fasting hyperinsulinaemia.

Elevated C-peptide levels have been consistently used as a marker of insulin resistance, metabolic syndrome and cardiovascular mortality in other ethnic groups [31,32] but no research, till now, has been conducted in Asian Indians. In this study, fasting C-peptide levels correlated significantly with fasting insulin levels in non-lean, non-obese Asian Indians with T2DM. Thus, it can be inferred that C-peptide levels measured in such patients are indicative of endogenous insulin as they were on metformin monotherapy and not exogenous insulin or sulphonylureas at any point of time.

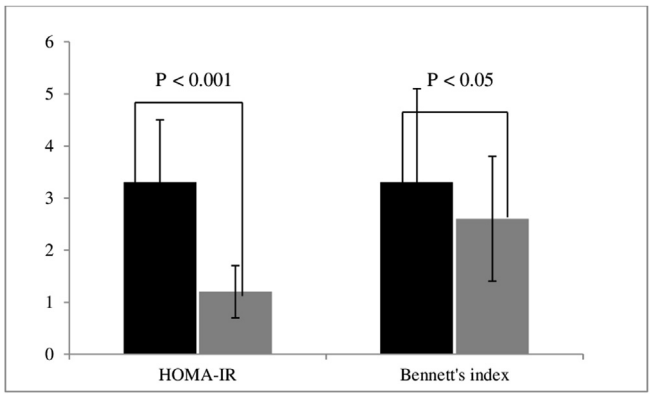

Fig. 1. Showing surrogate indices of insulin resistance in non-lean, non-obese patients with T2DM (n, 87; shown in black histogram) and controls (*n*, 37; shown in grey histogram).
Values are presented as Mean ± SD (shown as error bars above histograms), $p < 0.05$: Statistically significant

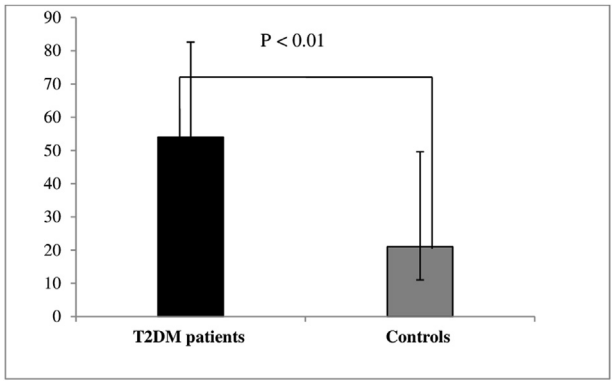

Fig. 2. Showing fasting insulin resistance index (FIRI) in non-lean, non-obese patients with T2DM (n, 87; shown in black histogram) and controls (*n*, 37; shown in pale grey histogram).
Values are presented as Mean ± SD (shown as error bars above histograms).
$p < 0.05$: Statistically significant

From review of literature it appears that non-obese patients with diabetes need further characterization into two groups; lean (BMI: <19 kg/m^2) and non-lean and non-obese (BMI > 19 to < 25 kg/m^2). Diabetes appears to be occurring in these categories of patients possibly due to different pathophysiological pathways. Lean individuals may have diabetes due to various causes; chronic calcific pancreatitis, lipodystrophies, maturity-onset diabetes in young (MODY), late-onset autoimmune diabetes (LADA) etc. The clinical profile of T2DM patients recruited in this study was not befitting these categories. Previous studies in India have deciphered the clinical profile of 'lean' patients with T2DM. Such lean Asian Indians with diabetes generally do not show autoimmune markers and have normal C-peptide levels and low serum insulin levels [4]. In this type of diabetes, seen specifically in the Indian subcontinent, distinctive insulin kinetics, and altered profile and behavior of key enzymes related to carbohydrate metabolism have been described. Interestingly, these individuals are less prone to develop macrovascular disease [3]. On the other hand, non-lean, non-obese Asian Indians with T2DM have features of adiposity such as high body fat, abdominal fat, and NAFLD [6,7] which are prominently seen in obese individuals. In this context, it is important to note that as compared to other ethnic groups, Asian Indians show dysmetabolic state even at BMI levels in the non-obese category [22]. This was elegantly shown in a study by Petersen et al. [23], who compared young, non-obese, apparently healthy, non-smoking adults from five ethnicities including Asian Indians (n, 59, BMI, 22.4 + 2.3 kg/m^2) wherein the prevalence of insulin resistance was 2–3-folds higher in Asian Indians compared with all other ethnic groups. Interestingly, the high magnitude of insulin resistance in Asian Indians was associated with two-fold increase in hepatic triglycerides content and higher levels of pro-inflammatory markers as compared to Caucasian men. In another study performed in Singapore on 59 normoglycaemic lean (BMI < 23 kg/m^2) adult males (14 Chinese, 21 Malays and 24 Asian Indians), Asian Indians showed higher waist circumference and body fat percentage than Chinese and Malays despite similar BMI. More importantly, in this study Asian Indians were the least insulin sensitive as compared to other ethnic groups [24]. These studies show two important observations; as compared to other ethnic groups higher insulin resistance and subclinical inflammation in non-obese Asian Indians, and that Asian Indians have higher body fat, waist circumference and hepatic triglycerides even at BMI level considered non-obese. These factors may lead to high propensity to develop diabetes in this ethnic group as compared to others. In particular, metabolic impact of excess triglyceride accumulation in liver (NAFLD) continues to be researched in Asian Indians; and apart from diet and exercise, may be related to genetic predisposition such as polymorphisms in the *palatin-like phospholipase-3* (*PNPLA* 3) [25] and *peroxisome proliferator-activated receptor gamma* (*PPAR-*γ) genes [26]. Finally, it has been shown that subcutaneous adipocytes are larger in volume than in white Caucasians, and thus increase higher free fatty acid flux and insulin resistance in Asian Indians [27]. Such larger subcutaneous adipocytes also show higher activity of several genes associated with inflammation in Asian Indians as compared to whites [28].

In our study, no significant correlations were observed for fasting C-peptide levels with pancreatic volume and pancreatic volume index despite significantly higher values of these measures in patients with T2DM. Importantly, the observations of the current study are similar to those of a study on 64 obese, elderly Caucasian males with impaired glucose tolerance, wherein no significant

Table 4
Group-wise comparison of indices of insulin resistance and beta cell function.

Indices of insulin resistance	Unadjusted	Unadjusted	p value	Adjusted for age	Adjusted for age	p value
	Non-lean, non-obese patients with T2DM (n, 87)	Non-lean, non-obese, non-diabetic controls (n, 37)		Non-lean, non- obese patients with T2DM (n, 87)	Non-lean, non-obese, non-diabetic controls (n, 37)	
HOMA -IR	3.3 ± 1.2	1.2 ± 0.56	<0.001	3.35 ± 0.37	1.2 ± 0.54	<0.001
Fasting Insulin Resistance Index	53.9 ± 28.6	20.8 ± 9.1	<0.01	52.8 ± 27.2	19.6 ± 8.6	<0.001
Benett's Index	3.3 ± 1.8	2.6 ± 1.2	<0.05	3.3 ± 1.2	2.6 ± 1.2	<0.01
HOMA -B	21.3 ± 14.6 *19.7 (0.3, 82.7)	20 ± 11.4 * 21.3 (2.7,53.7)	0.62	20.2 ± 13.8 *18.4(0.3, 82.7)	19.5 ± 10.8 *20.8 (0.2,78.4)	0.55

Values are presented as means ± SD. $p < 0.05$: Statistically significant.Values indicated in asterisk are median values, with the maximum and minimum values shown parentheses.

Table 5
Correlations of fasting C-peptide levels with anthropometric variables, volumes of abdominal adipose tissue compartments, biochemical profile and surrogate indices of insulin resistance.

Skinfolds (mm)	Non-lean, non-obese patients with T2DM (n, 87)	p value	Non-lean, non-obese, non-diabetic controls (n, 37)	p value
	r value		r value	
Supra-iliac (horizontal)	0.28	**0.01**	0.25	0.18
Supra-iliac (vertical)	0.34	**< 0.01**	0.27	0.11
Supra-iliac (average)	0.32	**<0.01**	0.23	0.15
Abdominal (vertical)	0.22	**0.02**	0.25	0.14
Abdominal (horizontal)	0.26	**0.02**	0.32	0.07
Volumes of abdominal adipose tissue compartments (cm^3)				
Anterior subcutaneous	0.25	**0.01**	0.12	0.44
Superficial subcutaneous	0.23	**0.02**	0.05	0.71
Total subcutaneous	0.25	**0.04**	0.04	0.77
Retroperitoneal	0.21	**0.03**	0.25	0.12
Intra-peritoneal	0.27	**0.003**	0.34	0.06
Total intra-abdominal	0.28	**0.001**	0.32	0.08
Biochemical variables				
Fasting insulin (mIU/dl)	0.44	**0.0001**	−0.17	0.28
2-hr post prandial C-peptide (pmol/l)	0.63	**0.001**	0.32	**< 0.04**
Serum glutamic pyruvic transaminase (U/L)	0.24	**0.01**	0.1	0.5
Indices of Insulin resistance				
Homeostatic Model of Assessment of Insulin Resistance (HOMA -IR)	0.42	**< 0.001**	−0.09	0.54
Fasting Insulin Resistance Index (FIRI)	0.42	**< 0.001**	−0.11	0.47
Benett's Index	0.38	**< 0.05**	−0.14	0.38

$p < 0.05$: Statistically significant.
PS: Only significant correlations shown here.

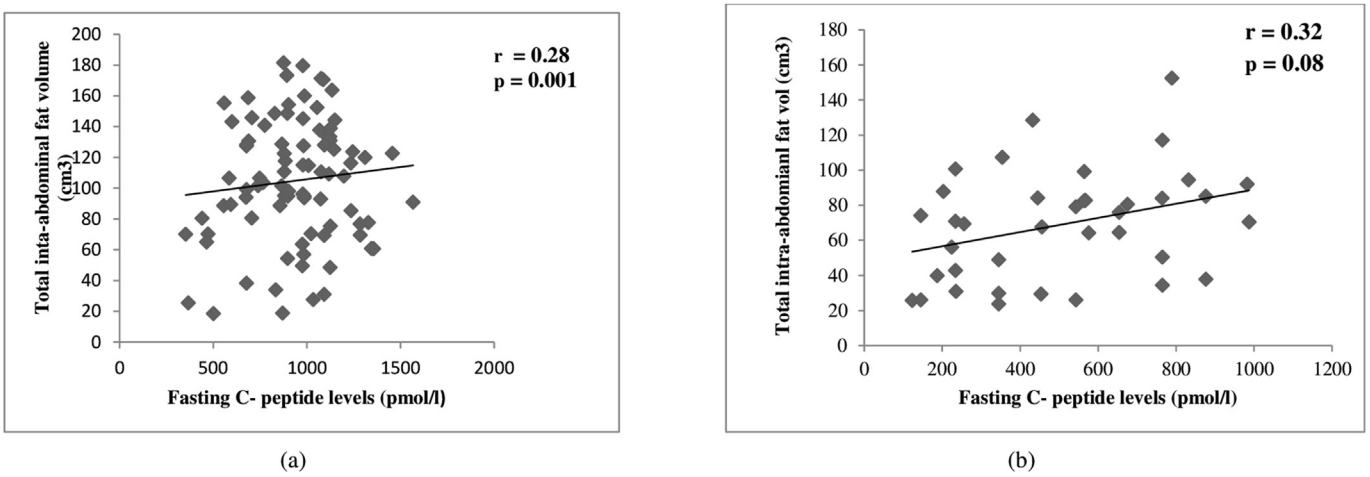

Fig. 3. Showing correlation of fasting C-peptide levels with total intra-peritoneal fat volume in non-lean, non-obese cases with T2DM (a) and lack of significant correlation of fasting C- peptide levels with total intra-peritoneal fat volume in controls (b).
$p < 0.05$: Statistically significant

correlations were observed for C- peptide levels with pancreatic volume [29]. In the present study, increased liver span appeared a significant predictor of higher fasting C- peptide levels in non-obese T2DM patients. These observations can be compared to those of an MRI based study in a multiethnic cohort of 503 obese adolescents with young-onset NAFLD, wherein Caucasian and Hispanic adolescents had significantly higher fasting glucose and C-peptide levels at similar total body fat mass. Importantly, these authors reported that increased hepatic fat and weight gain were significant predictors of higher C- peptide levels [30]. In a magnetic resonance spectroscopy (MRS) based study on 52 obese Chinese T2DM patients with and without NAFLD, higher fasting, early phase and post prandial C-peptide levels were observed in response to a standard oral glucose tolerance test in T2DM patients with NAFLD indicative of beta cell hypersecretion and insulin resistance [31].

In summary, our observations show that insulin resistance is predominant as compared to beta cell secretory defect, in non-lean, non-obese Asian Indian patients with T2DM. A prominent feature is the significant association of elevated fasting and post-prandial insulin and C-peptide levels with higher intra-abdominal and total abdominal adiposity. The marked feature of increased intra-abdominal adiposity, elevated insulin and C-peptide levels and concomitant insulin resistance in non-lean, non-obese (BMI < 23 kg/m^2) Asian Indian patients with T2DM show that such patients would benefit better from pharmacotherapy using insulin sensitizers (thiazolidinediones) or weight loss therapy (by

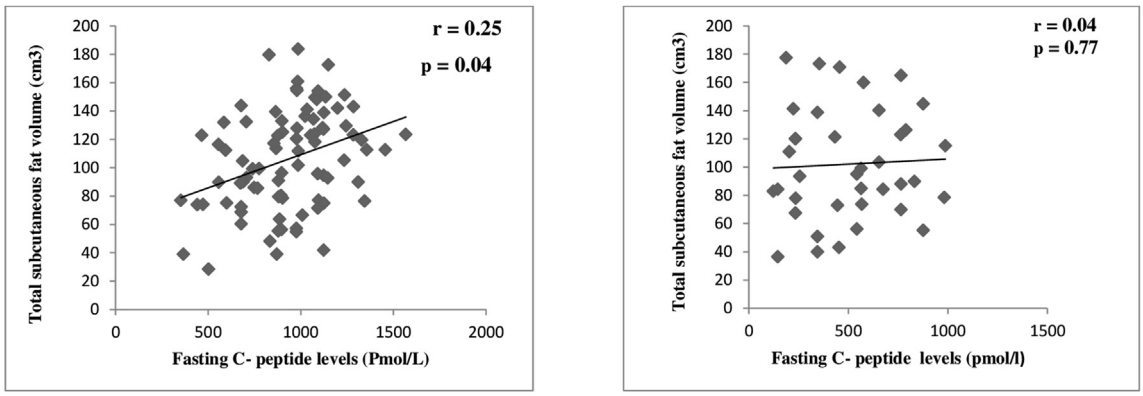

Fig. 4. Showing correlation of fasting C-peptide levels with total abdominal subcutaneous fat volume in non-lean, non-obese cases with T2DM (a) and lack of significant correlation of fasting C-peptide levels with total abdominal subcutaneous fat volume in controls (b).
$p < 0.05$: Statistically significant

metformin or sodium glucose co-transporter 2) as mentioned in the clinical algorithm for management of T2DM specifically in Asian Indians [32].

Conflicts of interest

The authors declare that no competing interests exist in the publication of this manuscript. The authors whose names are listed certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2018.11.041.

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