

# Fasting C Peptide Correlated with Fasting Insulin and Post Glucose Load C Peptides in Young Adults but the C Peptides could not be Partitioned Into Groups with and without Family History of Type 2 Diabetes Mellitus

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## ABSTRACT

**Introduction:** Family history of type 2 diabetes (FH) caused hyperinsulinemia. Fasting insulin was found to correlate with post-glucose load oral glucose tolerance test (OGTT) insulin and these insulin groups could be partitioned according to absence or presence FH. In this study, correlation of fasting C peptide with OGTT C peptide, and with fasting insulin and triglycerides in young non diabetic adults were analysed. Influence of FH on fasting and OGTT C peptide groups were also studied.

**Material and Methods:** Participants of this observational cross sectional study, aged 18 to 25 years from rural Central Kerala, have decreased influence of age, growth phase and environment. Clinical and biochemical evaluation of participants were done with cut-off levels of quantitative biochemical variables fixed to include effects of type 2 diabetes mellitus, but excluded other secondary clinical influences (n = 80). When the data had Gaussian distribution with equality of variances in the groups compared, parametric methods were used for analysis. Otherwise, non parametric methods were used.

**Results:** Fasting C peptide correlated well with OGTT C peptide, fasting insulin and triglycerides. Correlations were better with 30 minute OGTT C peptide than with 60 and 120 minute OGTT C peptides. But unlike insulin, fasting and OGTT C peptide groups could not be partitioned according to FH.

**Conclusion:** Difficulty in partitioning C peptide may be due to confounding of increased insulin secretion from insulin resistance by the decreased insulin secretion from beta cell dysfunction, resulting in absence of increase in C peptide in participants with FH.

**Keywords:** Diabetes Mellitus Type 2, C Peptide, Insulin, Triglyceride, Oral Glucose Tolerance Test

(OGTT). Of these, fasting, 60 and 120 minute OGTT insulin could be partitioned according to family history of type 2 diabetes (FH). The most effective partitioning was possible for fasting insulin.<sup>12</sup> These results indicated that hyperinsulinemia of the fasting state was related to hyperinsulinemia in the 30, 60 and 120 minute OGTT insulin. Though fasting insulin did significantly correlate with 30 minute OGTT insulin, the latter could not be partitioned according to FH. The 30 minute OGTT insulin is the most important phase of insulin secretion.

Clinical interpretation of circulating insulin levels as a predictive marker for type 2 diabetes, for risk calculations of type 2 diabetes mellitus and for evaluation of insulin resistance or insulin secretion have not been successful.<sup>13,14</sup> One of the major reasons for the difficulty in the interpretation is the confounding of hyperinsulinemia by insulin secretory deficiency. The multiple factors that cause hyperinsulinemia increase circulating insulin levels and pancreatic beta cell insulin secretory deficiency decreased circulating insulin levels.<sup>1-3,6,15-17</sup> The multiple factors have varying degrees of influence on type 2 diabetes mellitus.

Also, increase in insulin resistance or decreased insulin sensitivity is compensated by increased insulin secretion<sup>15,17</sup> and is responsible for the negative hyperbolic relation between insulin sensitivity and insulin secretion.<sup>1</sup> But insulin secretory deficiency opposed hyperinsulinemia and confounded it.

C peptide may be considered as the secretory component of insulinemia, contributing to hyperinsulinemia. Therefore, C peptide is increased by insulin resistance and decreased by the beta cell dysfunction for insulin secretion.<sup>18</sup> It was shown earlier that hyperinsulinemia could be partitioned according to FH.<sup>6,12</sup> In this study, the relationship of fasting C peptide with fasting insulin and with 30, 60 and 120 minute OGTT C peptides were analysed. The influences of FH on fasting and OGTT C peptide levels were also studied.

## INTRODUCTION

Insulin action on target tissues and insulin secretion by beta cells of pancreas are two fundamental mechanisms altered in type 2 diabetes.<sup>1-3</sup> Hepatic insulin uptake<sup>4</sup>, insulin resistance, insulin secretion<sup>1,3</sup> and rate of insulin degradation<sup>4,5</sup> contribute to circulating insulin levels. It was earlier reported that family history of type 2 diabetes caused hyperinsulinemia<sup>6</sup>, thereby implying genetic and familial contributions to hyperinsulinemia. Genetic factors also contribute to heritability of insulin secretory deficiency by beta cells of pancreas<sup>7,8</sup> and metabolic changes<sup>9</sup>, resulting in higher prevalence of diabetes in families<sup>10</sup> and among twins.<sup>11</sup>

Morning fasting glucose homeostasis is a steady state condition without diurnal influences and C peptide represents insulin secretion.<sup>1-3</sup> Fasting insulin correlated with post glucose load (30, 60 and 120 minute) insulin of oral glucose tolerance test

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## MATERIAL AND METHODS

Healthy participants (n = 80) of Central Kerala state, South India between 18 and 25 years of age, took part in this observational cross sectional study. Samples for this study were collected from relatives and friends of students, research fellows and laboratory staff of Amala from rural population.

Study was approved by the Institutional Research and Ethics Committees (AIMSIEC/01/2011 dated 9/3/2011). Informed written consent was obtained from each participant. Volunteers underwent a clinical evaluation for inclusion of individuals without any disease conditions, were on regular diet, exercise, rest, sleep, had no drugs for one week and all female participants were in the pre gestational period. Clinical Biochemistry laboratory evaluation was done for further exclusion of unhealthy individuals at the subclinical level and for evaluating the characteristics of diabetes-related variables. Exclusion criteria for these were: BMI >30 kg/m<sup>2</sup>, family history suggestive of obesity and type 1 diabetes, serum triglyceride >250 mg/dl (2.825 mmol/l), waist circumference ≥100 cm, fasting glucose ≥126 mg/dl (7 mmol/L), 2 hour glucose challenged or postprandial glucose >180 mg/dl (10 mmol/L), BP ≥140/90, serum alanine aminotransferase above 125 U/L, hsCRP >5 mg/l, serum creatinine >1.3 mg/dl (114.9 μmol/L) in males and >1.2 mg/dl (106.1 μmol/L) in females, TSH outside the reference range of 0.5 to 3 mIU/L, cortisol >14 μg/dl (386.3 nmol/L), prolactin in males >380 mIU/L (776.52 pmol/L) and in females >400 mIU/L (817.4 pmol/L). These abnormal cut off levels were designed for this study and permitted inclusion of individuals with consequences of increased insulin resistance.

Blood samples were drawn without anticoagulants, after a 10 to 12 hour overnight fast and after two and half hours of waking up from sleep, between 7.30 and 8.30 in the morning. Samples were centrifuged immediately in plastic tubes to sediment cells before clotting. Plasma was transferred to glass tubes for clotting and clot was separated by a second centrifugation. If clotting was observed after the first centrifugation, then the plasma was allowed to clot in the same tube and then centrifuged. This procedure reduced hemolysis and increased the yield of serum which was preferred over plasma for storage. Cell lysis caused insulin degradation. All assays were done immediately after preparation of serum.

Three autoanalysers used for assays were Liaison, Diasorin, Italy; 5,1 FS (Ortho Clinical Diagnostics, USA), and Access 2 (Beckman Coulter, USA). C peptide and Insulin assays were done with Liaison and Access 2 machine respectively, using their reagents, with immunometric assay on magnetic bead coated antiinsulin or anti C peptide antibody.<sup>19,20</sup> The chemistry autoanalyser used was 5,1 FS and reagents were from Ortho Clinical Diagnostics.

Diagnostic criteria for diabetes laid by the WHO were with fasting plasma glucose ≥126 mg/dl (7mmol/l) or 2 hour postprandial or 2 hour post-glucose load (75g in 300 ml water for adults) value ≥200 mg/dl (11.1mmol/l).<sup>21</sup> Diabetes mellitus type 2 was differentiated from type 1, by the former having a minimum history of 6 months of glycemic control by drugs, diet and exercise before insulin injection.

Body mass index (BMI) and waist circumference were defined by the revised criteria for Asian Indians<sup>21,22</sup> as underweight of <18.5 kg/m<sup>2</sup>, normal range of 18.5 - 22.9 kg/m<sup>2</sup>, overweight of

23 - 24.9 kg/m<sup>2</sup>, obese I of 25 - 29.9 kg/m<sup>2</sup>, obese II ≥30 kg/m<sup>2</sup> for both males and females. A person was considered to have abdominal obesity if waist circumference ≥90 cm for males and ≥80 cm for females.

Daily internal quality control data were analysed according to Westgard rules for acceptance or rejection of analyte data.<sup>23</sup> If there is a rejection, appropriate measures were taken to set right errors in machine functioning, reagents or calibration levels.

## STATISTICAL ANALYSIS

Normality of distribution was estimated by Shapiro-Wilk test. Equality of variances of the groups compared was analysed by Levene's test. Statistical analysis and calculations were done with SPSS software or manually. When log<sub>10</sub> transformations converted most of the positively skewed groups to Gaussian distribution. When variables had Gaussian distribution (before or after transformation) and when there was equality of variance in the groups compared, parametric methods of analysis were used. Otherwise, non parametric methods were used. The significant variation of fasting insulin of different groups were analysed by 95% confidence interval of means, two-tailed Student's t test and Mann Whitney U test.<sup>24-26</sup>

## RESULTS

Characteristics of some common diabetes-related quantitative variables in the total sample (n = 80) are given (Table 1). Mean of all the variables are within their reference intervals and upper limit of ranges are below the specified cut off levels of inclusion criteria. Distribution of most of the type 2 diabetes related variables in the serum samples were positively skewed.

### Fasting C peptide correlated directly with OGTT C peptide, and with fasting insulin and triglycerides

X-Y scatter diagram of fasting C peptide showed a significant and good positive correlation with 30 minute (r = 0.584; P = <0.001) and 60 minute (r = 0.441; P < 0.001) post glucose load OGTT (Figure 1 and Table 2). There was a moderate correlation of fasting C peptide with 120 minute C peptide (0.248; P = 0.027). As C peptide represents insulin secretion, these results indicated that when fasting insulin secretion increased, post glucose load OGTT insulin secretion also increased. Also, the correlation was maximum with 30 minute C peptide and progressively decreased at 60 and 120 minute.

The relationship of type 2 diabetes related parameters, fasting C peptide, fasting insulin and fasting triglycerides, with each other were also examined (Table 2). There was a very good and significant correlation of fasting C peptide with fasting insulin (r = 0.648; P <0.001) and with fasting triglycerides (r = 0.409; P <0.001). Fasting insulin also correlated well with fasting triglycerides (r = 0.390; P <0.001). There were direct and significant relationships between fasting type 2 diabetes related parameters, insulin (fasting hyperinsulinemia), C peptide (insulin secretion) and triglycerides (insulin resistance).

### Influence of FH on the correlations of fasting C peptide

The above correlations were also examined separately in the groups without and with FH (Table 3). There were good and significant correlations of fasting C peptide with 30 minute OGTT C peptide in groups without and with FH. But 120 minute and fasting C peptide showed a significant correlation

Quantitative variables n = 80 (Standard Units) (Conventional Units)	Mean±SD in Standard Units (Conventional Units)	Range in Standard Units (Conventional Units)
Age (years)	21.80±1.91	18 – 25
BMI (kg/m <sup>2</sup> )	21.54±3.43	15.47 – 29.09
Waist circumference (cm)	78.38±7.89	64.50 – 99.00
Fasting Glucose (mmol/L) (mg/dL)	4.89±0.523 (88.07±9.43)	3.97 - 6.70 (71.50 – 120.70)
2 hour OGTT, Glucose (mmol/L) (mg/dL)	5.47±1.34 (98.55±24.11)	2.77 – 9.76 (49.91 – 175.80)
Fasting Insulin (pmol/L) (μIU/ml)	37.16±18.37 (6.19±3.06)	12.24 – 106.14 (2.04 – 17.69)
Fasting C peptide (nmol/L) (ng/mL)	0.596±0.191 (1.79±0.575)	0.33 – 1.19 (0.98 - 3.57)
30 minute C peptide (nmol/L) (ng/mL)	2.44±0.842 (7.32±2.53)	1.00 – 5.66 (3.01– 17)
60 minute C peptide (nmol/L) (ng/mL)	2.79±0.886 (8.38±2.66)	1.33 – 5.19 (3.98 – 15.60)
120 minute C peptide (nmol/L) (ng/mL)	2.22±1.03 (6.67±3.08)	0.47 – 5.73 (1.41 – 17.20)
Total Cholesterol (mmol/L) (mg/dL)	4.62 ±0.820 (178.51±31.68)	3.42 - 6.89 (132 – 266.00)
Triglycerides (mmol/L) (mg/dL)	0.893±0.396 (79.00±35.06)	0.42 – 2.72 (37 – 241)
LDL Cholesterol (mmol/L) (mg/dL)	2.87±0.735 (110.86±28.36)	1.48 – 5.05 (57 - 195)
HDL Cholesterol (mmol/L) (mg/dL)	1.34±0.360 (51.61±13.89)	0.65 – 2.25 (25 – 87)

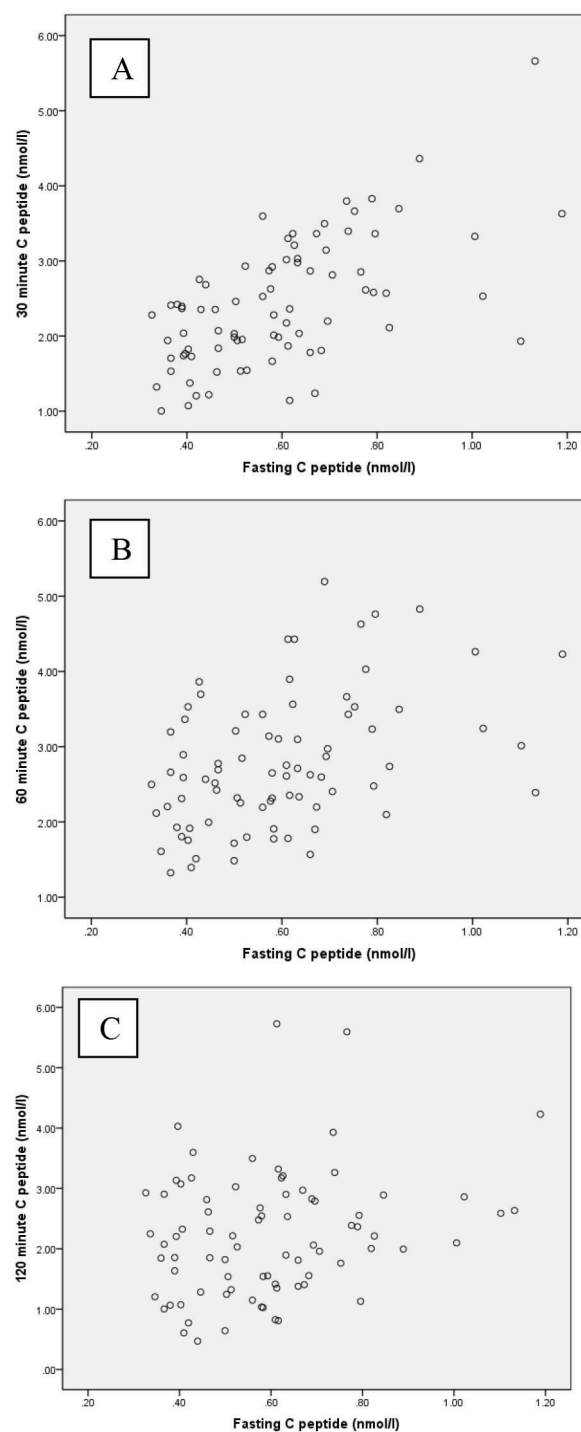
**Table-1:** Characteristics of type 2 diabetes mellitus-related parameters in the sample. OGTT is oral glucose tolerance test.

in the group without family history of type 2 diabetes, but no correlation in the group with family history. These results may indicate that in the group with family history of type 2 diabetes, increase in insulin secretion at 120 minute is confounded by insulin secretory dysfunction.

The fasting hyperinsulinemia and insulin resistance related parameters, fasting insulin, triglycerides and C peptide were analysed for their correlation with each other (Table 3). Fasting insulin and fasting C peptide correlated well in both the groups without ( $r = 0.601$ ;  $P < 0.001$ ) and with ( $r = 0.710$ ;  $P = < 0.001$ ) FH indicating that fasting hyperinsulinemia is contributed by increased insulin secretion. But fasting triglyceride correlated better with fasting insulin and fasting C peptide in the groups with FH than in the group without family history. These results indicated that triacylglycerol, which is related to insulin resistance, increased more with FH.

#### Fasting and OGTT C peptides could not be partitioned into groups without and with FH

The above results on correlations showed differences in the groups without and with FH. Therefore, fasting and OGTT C peptide groups were each split into those without and with FH (Table 4). It was observed that there was no significant difference in the groups without and with FH in fasting, 30, 60 and 120 minute C peptides. These surprising results indicated that increase in insulin secretion resulting from insulin resistance is confounded by decrease in insulin secretion resulting from beta cell dysfunction.



**Figure-1:** X-Y scatter diagram of fasting C peptide with 30 (A), 60 (B) or 120 (C) minute post glucose load OGTT C peptide levels.

#### DISCUSSION

It was earlier observed that hyperinsulinemia is influenced by FH<sup>6,12</sup> and that fasting, and post glucose load 60 and 120 minute OGTT insulin could be partitioned according to family history of type 2 diabetes mellitus.<sup>12</sup> It was also observed that fasting insulin correlated with post glucose load OGTT insulin and that fasting hyperinsulinemia could be better partitioned than post glucose load insulin.<sup>12</sup> Fasting C peptide is the secretory component of fasting hyperinsulinemia or hyperinsulinemia. Insulin resistance contributes to fasting hyperinsulinemia and increased insulin secretion.<sup>1-3</sup> If so, then fasting C peptide would also



Row No.	Correlating parameters in fasting and OGTT samples (n = 80)	95% Confidence Interval of mean	Shapiro Wilk test (after log <sub>10</sub> transformation) P	Correlation coefficient (r)	Significance of correlation P
1.	Fasting C peptide (nmol/L)	0.553 – 0.638	0.125	--	--
2.	30 minute C peptide (nmol/L)	2.25 – 2.62	0.689	0.584	0.000
3.	60 minute C peptide (nmol/L)	2.59 – 2.99	0.774	0.441	0.000
4.	120 minute C peptide (nmol/L)	1.99 – 2.45	0.075	0.248	0.027
5.	Fasting Insulin (pmol/l)	33.08 – 41.25	0.171	0.648	0.000
6.	Fasting Triglyceride (mmol/l)	0.805 – 0.981	0.013	0.409	0.000
7.	Fasting Insulin vs Triglyceride	--	--	0.390	0.000

**Table-2:** Pearson's correlation of fasting serum C peptide (Row No. 1) with 30, 60 and 120 minute OGTT C peptide, or with fasting insulin and triglyceride (Rows Nos. 2 – 6) or between fasting insulin and triglyceride (Row 7) in the total sample. All quantitative variables were log<sub>10</sub> transformed. At least one of the two correlating quantitative variables should have Gaussian distribution by Shapiro Wilk test for parametric calculation of correlation.

Row No.	Correlating parameters in groups without and with FH	Groups without and with FH (correlation calculated after log <sub>10</sub> transformation)					
		Without FH (n = 51)			With FH (n = 29)		
		Shapiro- Wilk test P	Correlation coefficient (r)	Significance of correlation P	Shapiro Wilk test P	Correlation coefficient (r)	Significance of correlation P
1.	Fasting C peptide (nmol/L)	0.619	--	--	0.174	--	--
2.	30 minute C peptide (nmol/L)	0.532	0.595	0.000	0.878	0.576	0.001
3.	60 minute C peptide (nmol/L)	0.638	0.556	0.000	0.757	0.286	0.132
4.	120 minute C peptide (nmol/L)	0.632	0.279	0.047	0.139	0.206	0.283
5.	Fasting Insulin (pmol/l)	0.591	0.601	0.000	0.250	0.710	0.000
6.	Triglyceride (mmol/l)	0.004	0.296	0.035	0.811	0.553	0.002
7.	Fasting Insulin vs Triglyceride	-	0.195	0.170	--	0.590	0.001

**Table-3:** Pearson's correlation of fasting C peptide (Row No. 1) with 30, 60 and 120 minute OGTT C peptide or with fasting insulin and triglyceride (Rows Nos. 2 – 6) or between fasting insulin and triglyceride (Row No. 7) in the groups without and with family history of type 2 diabetes in parents (FH). At least one of the two correlating quantitative variables should have Gaussian distribution by Shapiro Wilk test.

correlate with post glucose load C peptide; the secretory component of hyperinsulinemia, C peptide, may also be partitioned according to FH.

#### Correlation of fasting and post glucose load C peptide may be explained by increased insulin secretion from insulin resistance in offspring with FH

Decreased insulin sensitivity increased insulin secretion giving a negative hyperbolic relationship between them.<sup>1</sup> Therefore, increase in insulin resistance increases insulin secretion and C peptide levels. The 30 minute C peptide is the most important post glucose load insulin secretion.

The results of this study showed that fasting C peptide did correlate well with post glucose load OGTT C peptide (Table 2) but there was a major difference when compared with the correlations of fasting and OGTT insulin. The correlation of fasting C peptide was better with 30 minute than with 60 and 120 minute OGTT C peptides (Table 2). On the other hand, the correlation of fasting insulin was better with 60 and 120 minute OGTT insulin than with 30 minute insulin.<sup>12</sup> It is important to note that 30 minute C peptide is the major secretory component and this explains the better correlation of fasting C peptide to 30 minute OGTT C peptide. The decreased control of 90 and 120 minute OGTT blood glucose by insulin is important as a risk factor detrimental to health and for mortality.<sup>27-29</sup> Therefore, hyperinsulinemia at 60 and 120 minute OGTT sample may be more confounded by beta cell dysfunction than the early 30 minute hyperinsulinemia in young non diabetic individuals.

#### Decreased correlation of fasting C peptide with later OGTT C peptides in the group with FH may be due to increased secretory dysfunction of insulin

The correlation of fasting C peptide with OGTT C peptide was also examined after partitioning the sample into groups without and with FH (Table 3). As 30 minute C peptide represents the most important secretory phase of insulin, the correlations with fasting C peptide with 30 minute C peptide were highest in both groups. There was least correlation of fasting C peptide with 120 minute C peptide in both groups. But there was a major difference in the group with FH: fasting C peptide did not correlate with 90 and 120 minute C peptides. The increased pancreatic beta cell dysfunction in the group with FH may be confounding the increase in insulin secretion from insulin resistance, and this may explain the lack of correlation of 60 and 120 minute C peptide with fasting C peptide (Table 3).

But as insulin resistance was high in the group with FH, fasting insulin correlated well with fasting triglycerides in that group, but they correlated poorly in the group without FH. Fasting triglycerides, which is related to fasting insulin resistance cannot be confounded by insulin secretory dysfunction.

#### In the group with FH, increased insulin secretion from insulin resistance is opposed by decreased insulin secretion from beta cell dysfunction, resulting in no difference of C peptide without and with FH

This study also showed that none of the C peptide groups, fasting, 30, 60 or 120 minute C peptide could be partitioned according to FH (Table 4). This result was despite the fact that fasting C peptide correlated well with fasting insulin and post

Serum C peptide (pmol/L)	Comparison of groups without and with FH					C peptide (after log <sub>10</sub> transformation)			
	Without FH (n = 51)		With FH (n = 29)		95% CI of mean	Shapiro-Wilk test for normality P	Levene's test for equality of variances P		Student's t-test P
	Mean±SD (Range)	%CV	Mean±SD (Range)	%CV			Without FH	With FH	
Fasting C peptide	0.584±0.163 (0.33 – 1.02)	27.91	0.617±0.235 (0.34 – 1.19)	38.09	0.54 – 0.630	0.619	0.174	0.062	0.696
30 minute C peptide	2.42±0.787 (1.00 – 4.36)	32.52	2.47±0.945 (1.07 – 5.66)	38.26	2.20 – 2.64	0.532	0.878	0.803	0.907
60 minute C peptide	2.77±0.868 (1.33 – 4.83)	31.34	2.84±0.930 (1.51 – 5.19)	32.75	2.52 – 3.01	0.638	0.757	0.665	0.736
120 minute C peptide	2.17±1.14 (0.47 – 5.73)	52.53	2.31±0.805 (0.77 – 4.23)	34.85	1.85 – 2.49	0.632	0.139	0.081	0.257

**Table-4:** Comparison of fasting C peptide in groups without and with family history of type 2 diabetes mellitus in parents (FH). As groups were positively skewed, log<sub>10</sub> transformation was done, followed by Student's t test for Gaussian distributed insulin data or the non parametric Mann Whitney U test. % CV is percent coefficient of variation.

glucose load C peptides (Table 2). It has been observed earlier that insulin secretion also has a genetic and familial component, and is decreased in individuals with family history of type 2 diabetes.<sup>7,8</sup> Therefore, the reason for the lack of significant difference in fasting and post glucose load OGTT C peptide in groups without and with family history of type 2, may be due to the confounding of increased insulin secretion from insulin resistance by the decreased insulin secretion from beta cell dysfunction.

Fasting hyperinsulinemia is influenced by insulin resistance<sup>1,3</sup>, insulin uptake especially by liver<sup>4</sup>, insulin secretion by beta cells of pancreas<sup>1,3</sup>, regulation of insulin degradation<sup>5</sup> and gender differences in insulin sensitivity.<sup>30</sup> Fasting C peptide may be considered as the secretory component of fasting insulin level and is influenced by the ability of insulin secretion by beta cells of pancreas and insulin resistance. It is related to postprandial- and glucose-induced insulin secretion, which can have additional influences by incretins.

It may be concluded that the complexities and differences, in the correlations and partitioning of fasting insulin and fasting C peptide are related to the confounding of hyperinsulinemia by insulin secretory deficiency in group with FH, and heterogeneity of the influences on insulin secretion, insulin action and insulin metabolism.

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