IS BETA CELL DYSFUNCTION RESPONSIBLE FOR FLAT GLUCOSE TOLERANCE CURVE IN PRIMARY HYPOTHYROIDISM? (A HYPOTHESIS)

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ABSTRACT

An intimate relationship between thyroid hormones and carbohydrate metabolism has long been recognised and oral glucose load produces flat glucose tolerance curve in patients with primary hypothyroidism. Although delayed glucose absorption was proposed to explain flat glucose tolerance curve exact mechanism remains to be elucidated. Hence this study was undertaken to assess glucose and insulin response to OGTT and IVGTT in 25 freshly detected cases of hypothyroidism and 25 healthy control. The cases were matched for sex, age, BMI, and waist hip ratio with controls. Cases and controls with past or family history of obesity, diabetes mellitus, or hypertension were excluded from study. The biochemical profile of the cases and controls was also comparable except for haemoglobin (11.2 ± 0.31 vs 12.9 ± 0.22 gm/dl) ($p=0.0004$). Serum cholesterol and triglyceride levels were higher in the cases but difference was not statistically significant. Fasting plasma glucose level was significantly lower in hypothyroid patients (78 ± 2.2 vs 88 ± 4.4 mg/dl, $p=0.049$). The oral glucose tolerance curve was flat with plasma glucose levels significantly lower at 30 minutes. The insulin levels during OGTT were found to be higher in the cases at all stages. There was loss of first phase insulin response to the glucose load during the IVGTT, which was blunted at all stages and the difference was statistically significant at 0 and 3 minutes. Loss of first phase insulin response to IV glucose suggests that there is evidence of beta-cell dysfunction. Patients with hypothyroidism were more insulin sensitive than control and insulin secretion was comparable with controls. Therefore flat glucose tolerance curve can be explained by absence of insulin priming effect leading to decreased glucose absorption followed by increased glucose disposal because of higher insulin levels following OGTT and increased glucose disposal caused by increased insulin sensitivity.

MJAFI 2001; 57 : 120-125

KEY WORDS: Beta-cell function; Hypothyroidism; Insulin resistance; Insulin sensitivity.

Introduction

Hypothyroidism is the clinical and biochemical syndrome of low thyroidal production of thyroxine (T₄) triiodothyronine (T₃) with low serum T₄ and T₃ concentrations and that is ameliorated by administration of thyroid hormones [1]. An intimate relationship between thyroid hormones and carbohydrate metabolism has long been recognised [2]. It is well known that oral glucose load produces flat glucose tolerance curve in patients with primary hypothyroidism [3,4]. There is vast body of information about glucose metabolism in experimental animals [5-10] and man [11-17] in clinical hypothyroidism. Although delayed glucose absorption [4] was proposed to explain flat glucose tolerance curve exact mechanism remains to be elucidated. Hence this study was planned to assess glucose homeostasis, first phase insulin response to intravenous glucose infusion (IVGTT), insulin response to oral glucose (OGTT), calculation of insulin sensitivity and

secretion by HOMA method [18] and its relation to flat glucose tolerance curve.

Material and Methods

In this study the study group comprised 25 freshly detected cases of hypothyroidism and 25 healthy controls at the endocrine center of a large referral hospital. The cases were matched with respect to sex, age, body mass index (BMI), and waist hip ratio with controls. Cases were having symptoms and signs of hypothyroidism, and raised (> 20 mIU/L) level of thyroid stimulating hormone (TSH). Controls were euthyroid. Cases and controls with past or family history of obesity, diabetes mellitus, or hypertension were excluded from study. All had completed their puberty. OGTT was carried out next day after overnight fast with 75 g glucose in sitting position. IVGTT was carried out next day after overnight fast with 0.5 gm/kg glucose (maximum 35 gm) infused over 3 minutes as 25% dextrose solution in lying position. Plasma glucose was measured by glucose oxidase method and samples for insulin were collected and kept at -20°C till assay, which was done by RIA kits supplied by BRIT, Mumbai. Interassay and intraassay coefficient of variation was 13% and 8% respectively. Statistical analysis was done using computer program EP1-INFO 6 and results were expressed as mean $\pm$ SEM.

HOMA METHOD : The HOMA model was developed and validated against hyperinsulinemic euglycaemic clamp (for insu-

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TABLE 1
Clinical parameters

Parameter	Case	Control	p value
Age (years)	32.87 ± 1.87	35.167 ± 2.18	0.43
Height (cm)	160.83 ± 1.62	163.25 ± 1.77	0.32
Weight (kg)	57.33 ± 1.92	59.41 ± 1.94	0.54
BMI	22.09 ± 0.57	21.99 ± 0.54	0.85
Waist (cm)	82.17 ± 1.65	84.25 ± 1.85	0.50
Hip (cm)	95.26 ± 1.45	94.167 ± 1.46	0.63
WHR	0.86 ± 0.12	0.89 ± 0.12	0.15
BP - Systolic	123.08 ± 3.2	124 ± 2.4	0.86
- Diastolic	80.16 ± 2.4	77.5 ± 2.0	0.60

TABLE 2
Biochemical profile

Parameter	Case	Control	p value
Hb (gm/dl)	11.2 ± 0.31	12.9 ± 0.22	0.0004
Urea (mg/dl)	27.43 ± 1.76	27.147 ± 0.96	0.66
Cholesterol (mg/dl)	197.75 ± 16.44	160.3 ± 7.66	0.27
Triglycerides (mg/dl)	122.5 ± 17.91	101.7 ± 7.46	0.88

lin resistance) and hyperglycemic clamp (for insulin insensitivity) by Matthews et al [19]. The formulae are as follows :

$$\text{Insulin Resistance} = \frac{\text{F1} \times \text{XG}}{22.5} \quad \text{Insulin Secretion} = \frac{20 \times \text{F1}}{\text{G}-3.5}$$

Where F1 = fasting insulin $\mu\text{IU/ml}$, and G = fasting glucose (mmol/l)

Results

The study group comprised 25 freshly detected cases of hypothyroidism at the endocrine center of a large referral hospital. Patients presented to the clinic with a wide array of symptoms and signs related to hypothyroidism. Hormonal profile of the cases confirmed the presence of hypothyroidism with grossly depressed T_3 and T_4 levels. Mean T_3 and T_4 levels were 0.85 ± 0.12 ng/ml and 1.64 ± 0.08 $\mu\text{g/dl}$ respectively, and TSH levels were raised several folds in the study group 206.02 ± 45.9 mIU/L (range 27.54 to 1054). The etiology of hypothyroidism was primary atrophic thyroiditis 52%, Hashimoto's thyroiditis 32%, post-thyroidectomy 12% and post radioablation 4%.

The patients were matched with respect to height, weight, sex, BMI, and waist-hip ratio (WHR) with 25 normal controls. The mean age of the study group was 32.87 ± 1.87 years (15 to 48 years), whereas it was 36.67 ± 2.18 years in the control group (16 to 52 years, $p = 0.43$). The BMI was 22.09 ± 0.57 in cases vs 21.99 ± 0.54 in the control group ($p=0.85$). The two groups were also similar as regards measurement of waist, hip, waist-hip ratio (WHR) and basal blood pressure as depicted in Table-1. The biochemical profile of the cases and controls was also comparable except for haemoglobin which was significantly lower in the hypothyroid group (11.2 ± 0.31 gm/dl) than in the control group (12.9 ± 0.22 gm/dl, $p=0.0004$). Both groups had normal renal and liver function tests. Serum cholesterol and triglyceride levels were higher in the cases than in the control population though the difference was not significant statistically (Table-2). Classically hypothyroid patients have hypercholesterolemia and hypertriglyceridemia, but in present study only 24% of patients (6) had

Fig. 1 : OGTT - Mean glucose (mg/dl)

TABLE 3
OGTT - Glucose levels in mg/dl

Time (minutes)	Cases	Control	p value
0	78 ± 2.2	88 ± 4.4	0.049
30	116 ± 3.4	149 ± 6.0	0.001
60	130 ± 6.4	145 ± 6.0	0.07
90	105 ± 5.6	114 ± 4.2	0.31
120	85 ± 2.8	89 ± 3.2	0.66

serum cholesterol >250 mg/dl and 16% (4) had serum triglyceride >200 mg/dl. None of the controls had hypercholesterolemia or hypertriglyceridemia.

Fasting blood glucose level was significantly lower in hypothyroid patients (78 ± 2.2 vs 88 ± 4.4 mg/dl, $p=0.049$). Glucose response to OGTT was flat (Fig-1). The mean blood glucose levels during the OGTT were found to be lower at all stages in the cases though the difference was not statistically significant except for the value at 30 minute (116 ± 3.4 vs 149 ± 6.0 mg/dl, $p=0.001$) (Table-3). This difference became exaggerated when area under the curve was measured (130 ± 0.84 vs 149 ± 0.76 , $p=0.007$) (Fig-1). However mean plasma glucose was comparable in both groups during IVGTT (Table-4, Fig-2). The insulin levels during OGTT were found to be higher in the cases at all stages, though again there was no statistically significant difference (Table-5, Fig-3). There was loss of first phase insulin response to glucose load during IVGTT, which is well depicted in combined graph showing mean insulin response to IVGTT and OGTT (Fig-4). Mean plasma insulin levels were lower at all stages during IVGTT and the difference was statistically significant at 1 (139.8 ± 29.0 vs 226.8 ± 50.0 , $p=0.001$) and 3 minutes (112.8 ± 14.8 vs 254.0 ± 50.8 , $p=0.02$) (Table-6, Fig-5). Insulin sensitivity and insulin secretion calculated by HOMA model were comparable in both groups (Table-7), however hypothyroid patients secreted more insulin than controls inspite of being more insulin sensitive, which is similar to the findings of insulin response to OGTT. There was no correlation between the severity of the hypothyroidism with beta cell function but it was negatively correlated with duration of hypothyroidism (Table-8).

Discussion

This study showed that there is lower fasting blood glucose in hypothyroid patients compared to normal healthy controls (Fig-1, Table-3). Animal experiments have revealed varied and conflicting results of glucose

TABLE 4
IVGTT - Glucose levels in mg/dl

Time (minutes)	Cases	Control	p Value
0	80 ± 2.2	88 ± 2.4	0.04
01	441 ± 21.6	478 ± 6.8	0.26
03	396 ± 14.2	388 ± 18.4	0.82
05	363 ± 12.4	339 ± 18.4	0.56
10	309 ± 8.4	267 ± 7.8	0.61

TABLE 5
OGTT - Insulin levels in μ U/ml

Time (minutes)	Cases	Control	p Value
0	21.8 ± 2.5	23.9 ± 2.6	0.62
30	123.0 ± 20.8	110.0 ± 10.8	0.65
60	155.2 ± 21.6	121.1 ± 10.4	0.26
90	119.3 ± 10.8	88.7 ± 9.4	0.24
120	77.2 ± 13.0	52.9 ± 6.8	0.18

TABLE 6
IVGTT - Insulin levels in μ U/ml

Time (minutes)	Cases	Control	p Value
0	21.8 ± 2.5	23.9 ± 2.6	0.62
01	139 ± 29.0	226.8 ± 50.0	0.01
03	112.8 ± 14.8	254.0 ± 50.8	0.02
05	110.7 ± 15.4	200.2 ± 40.4	0.06
10	96.9 ± 12.8	130.6 ± 19.8	0.20

homeostasis in hypothyroidism. Fasting blood glucose has been found to be high [6,9] and low [3]. However except one [5] all other studies [4,11,14,16,17] have reported low plasma glucose in human studies. Exact mechanism of this effect is not known. The plasma glucose concentration is usually maintained within a relatively narrow range (70-110 mg/dl) despite wide variation in glucose influx and efflux. Fasting plasma glucose is maintained by hepatic glucose output by glycogenolysis and gluconeogenesis. Glucose release from the adipose tissue is slowed in hypothyroidism and the availability of amino acids and glycerol for gluconeogenesis is decreased, which contribute to decreased hepatic glucose output [14]. However McKenzie et al reported that hypothyroidism had no effect on gluconeogenesis [10]. Hypothyroidism may affect various enzymes involved in gluconeogenesis, and glycogenolysis which had not been studied in literature. This opens up further avenue to study hepatic glucose machinery in hypothyroidism. Low fasting plasma glucose was nega-

Fig. 2 : IVGTT - Mean p Glucose (mg/dl)

Fig. 3 : OGTT - Insulin (μ U/ml)

Fig. 4 : IVGTT - Insulin (μ U/ml)

tively correlated with duration of disease but had no relation with severity of hypothyroidism (Table-8).

Glucose tolerance curve following oral glucose load was flat in present study. Mean plasma glucose levels at 30 minutes were significantly lower in cases. This difference between cases and controls was further augmented when area under the curve was measured. Glucose level during fed state mainly depends upon influx from intestinal absorption but a small contribu-

TABLE 7

Insulin resistance / secretion

	Cases	Control	p value
Insulin resistance (IR)	4.02 ± 0.48	5.42 ± 0.72	0.21
Insulin secretion = β cell function	872 ± 136	827 ± 157	0.82
IR/IS	0.008 ± 0.001	0.019 ± 0.004	0.01

TABLE 8

Correlation with severity and duration

(r value and 95% confidence interval)

	Severity	Duration
FP. Glucose	0.00 (-0.31 to 0.32)	-0.30 (-0.62 to 0.10)
AUC Glucose	-0.15 (-0.44 to 0.17)	-0.35 (-0.65 to 0.06)
IVGTT - Insulin (1+3+5)	-0.06 (-0.37 to 0.26)	-0.25 (-0.60 to 0.17)

tion is also made by glycogenolysis and gluconeogenesis and efflux by way of glycogenesis, glucose oxidation and lipogenesis. Muller and Seitz [3] found decreased glucose absorption whereas Scow et al [6] reported normal glucose absorption in animal studies. Holdsworth [4] proposed delayed gastric emptying as a mechanism for flat glucose curve among hypothyroid patients. Scow et al [6] and DeRuyter et al [9] postulated that there was decreased peripheral utilisation of glucose, probably due to decreased rate of basal glucose oxidation [1,10]. Thus patients with hypothyroidism may have a decreased rate of glucose absorption and flattened oral glucose tolerance curves despite the concomitant reduction in peripheral glucose assimilation, which is contradictory in itself. These studies had several confounders to affect results. Most of these studies were small, controls were not well matched and many studies have not measured insulin levels which is integral part of glucose homeostasis. In this study cases and controls were well matched. The biochemical profile of the cases and controls was also comparable except for haemoglobin, which was significantly lower in the hypothyroid group. Low haemoglobin level in hypothyroid patients is contributed by decreased production of erythropoietin, deficiency of vitamin B₁₂ and folic acid, and menorrhagia [20]. However low haemoglobin level is not known to affect either insulin secretion or insulin resistance.

There was loss of first phase insulin response during IVGTT (Fig-5). Plasma insulin levels at 1 and 3 minutes were significantly lower in hypothyroid patients than control. Plasma insulin values at 5 minutes (110.7±15.4 vs 200.2±40.4, p=0.06) were also lower

Fig. 5 : Insulin level early and late response

than control, however could not reach statistical significance level. Shah and Cerchio had reported delayed peak of early phase insulin secretion during IVGTT [5]. Loss of first phase insulin response to IV glucose suggests that there is evidence of beta-cell dysfunction [21,22]. This aspect has not been studied well in literature. Defective first phase insulin response to IVGTT is associated with islet cell or insulin antibodies in relatives of insulin dependent diabetes mellitus [23]. Whether such antibodies are associated in hypothyroid patient with defective insulin response to OVGTT requires further study. Furthermore, patients with non insulin dependant diabetes mellitus also have an attenuated or absent first phase insulin response to intravenous glucose load, but in contrast to hypothyroid patients they have reduced second phase insulin response [24,25]. The first phase response depends upon rise in cytoplasmic calcium concentration produced by glucose [26]. Diminished rise in intracytoplasmic calcium concentration in hypothyroidism can also attribute to sluggish end organ response [16]. However further study is required to document its reversibility with treatment, because persistence of it will require monitoring of patients for development of diabetes mellitus. The ability of beta cells to respond rapidly to glucose is thought to be essential for optimal glucose homeostasis [27]. First phase insulin response which also exists with oral glucose load [28,29] which may be essential for rapid glucose absorption from the intestinal tract during OGTT is known to modify number of glucose transporters in the gut - GLUT5 [30]. Hence defective first phase response may also contribute to lower rate of absorption of glucose resulting in delayed peak with flat glucose tolerance curve.

In present study plasma insulin levels in cases were comparable with controls during OGTT. Though plasma insulin levels among cases were higher than controls, these were not statistically significant. But

in presence of lower plasma glucose we expected lower plasma insulin levels, but these were higher leading to relative hyperinsulinemia. This was further confirmed by HOMA analysis of insulin sensitivity and secretion. Patients with primary hypothyroidism were relatively insulin sensitive compared to normal controls, however this was not significantly different between two groups. But IR/IS ratio was significantly lower in hypothyroid patients indicating significantly higher basal insulin secretion in comparison to prevalent insulin sensitive state. Higher plasma insulin levels in presence of low plasma glucose level can be explained on the basis of reduced degradation of insulin [16]. Reduced degradation of insulin with resultant prolonged action can be postulated to explain flat oral glucose tolerance curve. This will lead to more glucose utilisation in presence of decreased and delayed glucose absorption, hence glucose tolerance curve will be flat. Contrary to OGTT, plasma glucose concentration during IVGTT was comparable between cases and controls. This further indirectly confirms delayed intestinal glucose absorption in flat glucose curve during OGTT.

Other possible way to explain relative hyperinsulinemia is possibly insulin resistance [4], however insulin resistance measured by HOMA Model was not significantly different in hypothyroid patients compared to normal healthy individuals. Animal studies have reported increased plasma insulin levels [9], probably due to decreased uptake by tissues [7]. Insulin levels were reported as either high [4,11] or normal [5] in human studies. Holdsworth and Besser reported some degree of insulin resistance in hypothyroid subjects [4], whereas Shah and co-workers in their study reported a prolonged hypoglycaemic action of exogenously administered insulin in hypothyroidism, suggesting an increased sensitivity to insulin [11]. Using fat cells from normoglycemic hypothyroid patients, Arner and co-workers demonstrated that the insulin receptor number was increased by 70% and simultaneously 4 fold increased sensitivity to insulin [13].

There is considerable overlap between two groups. This may be related to either severity of hypothyroidism or duration of disease. There was no correlation between severity of hypothyroidism and insulin levels during IVGTT in this study. Duration of disease in hypothyroid patients is difficult to determine because of its insidious nature. However plasma insulin levels 1,3 and 5 minutes were negatively correlated with known duration of symptoms. Monzani et al [31] have also demonstrated in patients of postablative hypothyroidism that development of muscle cell dys-

Fig. 6: Insulin hypothesis

function is related with duration of disease. Hence this may explain considerable overlap seen among hypothyroid patients.

Furthermore relative hyperinsulinemia may contribute to accelerated atherosclerosis [32]. Diabetes mellitus is known to exist with increased prevalence in patients with thyroid disease [33]. Hence low fasting plasma glucose and flat glucose tolerance curve in hypothyroid patients necessitates applying different criteria for diagnosis of diabetes mellitus, otherwise diabetes mellitus will be underdiagnosed on the basis of fasting blood glucose level and OGTT.

This study revealed that there is lower fasting blood glucose and flat glucose tolerance curve. Flat glucose tolerance curve is contributed by decreased and delayed glucose absorption of glucose in intestinal tract, which can be due to absence of first phase insulin response. Relative increase in insulin sensitivity and relative hyperinsulinemia during OGTT will lead to increased glucose utilisation and explains flat glucose tolerance curve. Low fasting plasma glucose requires study of hepatic glucose homeostasis. This also necessitates further discussion for applying different criteria for diagnosis of diabetes mellitus in hypothyroid patients, otherwise diabetes mellitus will be underdiagnosed on the basis of fasting and postprandial plasma glucose level. Loss of first phase insulin response to IV glucose indicates that there is evidence of beta-cell dysfunction. Further study is required to document its association with autoimmunity to islet cell, and reversibility with treatment because persistence of it will require monitoring of patients for development of diabetes mellitus (Fig. 6).

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