

## To Investigate the Impact of Oral Anticholinergics on Insulin Secretion in Subjects with Impaired Glucose Tolerance (IGT): A Retrospective Study

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Conflict of interest: Nil

### Abstract

**Aim:** The aim of the present study was to investigate the impact of oral anticholinergics on insulin secretion in subjects with impaired glucose tolerance (IGT), in comparison with volunteers having normal glucose tolerance (NGT).

**Material & Methods:** This retrospective study was conducted in the Department of Pharmacology, ANMMCH, Gaya, Bihar, India from November 2019 to October 2020, and recruited 30 IGT and 30 NGT subjects. An oral glucose tolerance test (OGTT) was conducted twice in the absence and presence of hyoscine butyl-bromide (HBB). The plasma glucose (PG) and insulin levels were serially estimated at 30-min increments for 2 h after the OGTT. Early ( $\Delta I30/\Delta PG30$ ) & late (insulin/PGAUC 60-120) phase insulin activity were assessed subsequently.

**Results:** In the present study, 60 subjects including 30 IGT (13 male/17 female, BMI: 26.4±2.4) and 30 NGT (15 male/15 female, BMI: 24±0.6) met the study requirements and completed the experimental protocol. In both the groups, a higher proportion belonged to the “overweight” category. The effect of HBB in the IGT group was examined in terms of pharmacodynamic parameters obtained during a 75 g OGTT (0–120 min). The presence of HBB did not have an impact on their fasting PG and PG Cmax values. In the IGT group, the presence of HBB had no effect on fasting insulin levels and insulin Cmax at t = 60 min. The addition of HBB also did not impact on the insulin total AUC 0–120 min. The presence of HBB had no effect on fasting insulin levels ( $6.50 \pm 1.32$  vs.  $5.70 \pm 0.87$  mIU/L) and insulin Cmax at t = 60 min. However, the addition of HBB significantly decreased the insulin total AUC 0-120 min. In the NGT group, similar to the IGT group, the presence of HBB did not impact on the plasma glucose-based parameters, for example, fasting PG.

**Conclusion:** Our study findings indicate that insulin secretion is influenced by cholinergic system and that oral anticholinergics may attenuate the late phase insulin activity in varying degrees of glycemic status.

**Keywords:** Impaired Glucose Tolerance, Incretin, Insulin, Oral Anticholinergic

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### Introduction

Diabetes affects approximately 1 in 11 adults worldwide, and people with diabetes are at a twofold excess risk for cardiovascular disease (CVD). [1,2] A decline in insulin sensitivity is an early sign of susceptibility to type 2 diabetes, typically manifested as elevated levels of fasting insulin. [3] Insulin is a key regulator of glucose metabolism by promoting glucose uptake in peripheral tissues and inhibiting glucose production in the liver. [4] Insufficient insulin action results in increased fasting glucose and eventually leads to overt type 2 diabetes. [4] Insulin resistance (IR) is also linked to the development of cardiometabolic complications, the risk arising already prior to the onset of type 2 diabetes. [5,6] Studies in the fasting state have identified a cluster of biomarkers robustly associated with IR and predisposing to increased risk for CVD. [3,5,6] In the modern society, however, people

spend most of their waking hours at a postprandial state, yet we are not aware of epidemiological studies on non-fasting metabolism in representative cohorts.

An oral glucose tolerance test (OGTT) assesses an individual's ability to clear circulating glucose after an ingestion of a 75-g glucose bolus taken after an overnight fast. An OGTT induces a transition from fasting to feeding, and subsequent changes in various metabolic nutrients occur as the body makes adjustments to achieve glucose homeostasis. [7] It is thus feasible to expect that individuals with impaired insulin action are likely to display a widespread systemic abnormality beyond glucose. Although the dynamics of insulin and glucose during an OGTT in both healthy and insulin-resistant individuals are well studied [8,9], much less is known on other,

particularly emerging cardiometabolic biomarkers, lipoprotein lipid profiles, amino acids, ketone bodies, and inflammatory markers. [10,11]

Instead, a role of the parasympathetic system has been proposed: GLP-1, upon secretion, stimulates the local parasympathetic fibres that, in turn, stimulate the pancreatic beta-cells to secrete insulin. [12-14] The physiological relevance of the results, however, is not clear because GLP-1 infusion is not equivalent to meal-induced GLP-1 secretion. It is only in the latter situation that GLP-1 can be expected to act on local parasympathetic fibres to result in vagally mediated insulin secretion. Carbohydrate-rich meal ingestion is the most important physiological stimulation for both secretion of insulin and incretins. [15,16]

The aim of the present study was to investigate the impact of oral anticholinergics on insulin secretion in subjects with impaired glucose tolerance (IGT), in comparison with volunteers having normal glucose tolerance (NGT).

### Material & Methods

This prospective observational study was conducted in the Department of Pharmacology, ANMMCH, Gaya, Bihar, India from November 2019 to October 2020, and recruited 30 IGT and 30 NGT subjects. An oral glucose tolerance test (OGTT) was conducted twice in the absence and presence of hyoscine butyl-bromide (HBB). The plasma glucose (PG) and insulin levels were serially estimated at 30-min increments for 2 h after the OGTT. Early ( $\Delta I_{30}/\Delta PG_{30}$ ) & late (insulin/PGAUC 60-120) phase insulin activity were assessed subsequently.

### Participants

Subjects of either sex aged between 18 and 55 years were recruited. Subjects were excluded if they had Type-2 diabetes mellitus, body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>, had contraindications for anticholinergic agents, preexisting gastrointestinal disorders, derangement of renal or hepatic functions, history of substance abuse, or were being treated with medicines that could not be paused for 12 h.

Pregnant or breastfeeding women or immunocompromised subjects were also excluded from the study. We recruited a total of 20 subjects, 10 who had NGT and 10 who had IGT (as per the World Health Organization definition of IGT [2 h PG <11.1 mmol/L, but >7.8 mmol/L]).

### Experimental Protocol

Subjects underwent an OGTT (glucose 75 g; t = 0 min) without (day 1) and with HBB 20 mg (day 3). Two tablets of Hyocimax<sup>®</sup> 10 mg was prescribed to all study participants on day 4. The drug had to be taken 30 min before start of 75 g OGTT (t = -30 min) as single dose. Blood samples were collected at 30-min interval (0–120 min) for serial PG and insulin estimations.

### Statistical Analysis

Baseline and biochemical characteristics were analysed by descriptive statistics and presented as mean  $\pm$  standard deviation or standard error of mean as indicated. Normality was tested using Kolmogorov–Smirnov Goodness-of-Fit. Selected data were described with a 95% confidence interval.

Student's t-test (for parametric data) and Mann–Whitney U-test (nonparametric data) were used to compare mean values. Data at individual time points were compared using one-way repeated measures analysis of variance, followed by Tukey–Kramer *post hoc* analysis. Area under the concentration–time curve (AUC) over 0–120 min was calculated using the linear trapezoidal rule. Inferences were made on the estimated treatment contrast with HBB (20 mg) versus baseline data. Correlation between beta-cell function (insulinogenic index;  $\Delta I_{30}/\Delta PG_{30}$ ) and mean insulin levels was analyzed in relation to outcome. Simple regression was performed between pairs of indices in each group. Fisher's test was used to compare categorical data. Two-tailed  $P < 0.05$  was significant. GraphPad Prism version 6.0 for Windows (GraphPad Software, La Jolla, CA) was used for statistical analysis.

### Results

**Table 1: Baseline characteristics**

Characteristics	IGT subjects (n=30)	NGT subjects (n=30)
Age (years)		
Range	27-77	25-65
Age, mean $\pm$ SD	49.4 $\pm$ 15.5	43.7 $\pm$ 11.9
Median (IQR)	45.5 (35-72)	44.5 (35-55)
Sex, n		
Male	13	15
Female	17	15
BMI (kg/m <sup>2</sup> ), n		
<18	0	0
18-22.9	5	11
23-24.9	18	19

≥25	7	0
Mean±SD	26.4±2.4	24±0.6
Family history of diabetes, <i>n</i>	16	17
Systolic BP (mmHg)±SD	129±4	115±5
Diastolic BP (mm Hg)±SD	78±2	76±4
Resting heart rate (bpm)±SD	69±2	73±7
Body temperature (°C)±SD	37.2±0.3	37.1±0.2

In the present study, 60 subjects including 30 IGT (13 male/17 female, BMI: 26.4±2.4) and 30 NGT (15 male/15 female, BMI: 24±0.6) met the study requirements and completed the experimental protocol. In both the groups, a higher proportion belonged to the “overweight” category.

**Table 2: Evaluation of pharmacodynamic parameters during oral glucose tolerance test**

Parameter	NGT		P Value	IGT		P Value
	Hyoscine (-)	Hyoscine (+)		Hyoscine (-)	Hyoscine (+)	
<b>PG</b>						
Fasting PG (mM/L)	4.3±0.14	4.6±0.34	0.713	6.2±0.07	6.6±0.12	0.412
PG Cmax (mM/L; t=60 min)	6.2±0.32	6.6±0.66	0.540	11.2±0.78	11.5±0.75	0.735
PG AUC (0-120 min, mM/L ×120 min)	696.4±22.58	704.6±22.3	0.931	1334.6±46.64	1370.0±62.18	0.820
<b>PI</b>						
Fasting PI (mIU/L)	6.2±1.35	5.6±0.84	0.634	11.7±1.88	12.6±2.24	0.560
PI Cmax (mIU/L; t=60 min)	31.6±14.46	32.0±7.13	0.931	38.2±8.42	34.6±6.24	0.660
PI AUC (0-120 min, mIU/L ×120 min)	3374±190.60	2755.5±162.76	0.036	3999.2±228.42	3864.0±212.00	0.690

The effect of HBB in the IGT group was examined in terms of pharmacodynamic parameters obtained during a 75 g OGTT (0–120 min). The presence of HBB did not have an impact on their fasting PG and PG Cmax values. In the IGT group, the presence of HBB had no effect on fasting insulin levels and insulin Cmax at t = 60 min. The addition of HBB

also did not impact on the insulin total AUC 0–120 min. The presence of HBB had no effect on fasting insulin levels (6.50 ± 1.32 vs. 5.70 ± 0.87 mIU/L) and insulin Cmax at t = 60 min. However, the addition of HBB significantly decreased the insulin total AUC 0-120 min.

**Table 3: Indices of early (insulinogenic index 0-30) and late (I/glucose area under the curve 60-120) beta-cell function calculated from oral glucose tolerance test**

Arms	IGI0-30 (pM/mM)		P Value	I/GAUC 60-120 (pM/mM)		P Value
	Hyoscine (-)	Hyoscine (+)		Hyoscine (-)	Hyoscine (+)	
IGT	63.2±22.24	66.8±31.79	0.840	23.0±1.17	21.69±0.75	0.025
NGT	100.5±33.07	125.5±77.03	0.760	38.2±1.78	30.28±2.32	0.007

In the NGT group, similar to the IGT group, the presence of HBB did not impact on the plasma glucose-based parameters, for example, fasting PG.

### Discussion

Derangement of the entero-insulin axis is an early determinant of the development of glucose intolerance in type 2 diabetes mellitus. [17] Yet, the interaction between insulin, incretins, and vagal

stimulation is complex and poorly understood. [17-20] In the pancreatic islets, recent experimental data suggest that endogenous acetylcholine not only stimulates  $\beta$ -cell function by activation of M3 and M5 receptors but also mediates recruitment of  $\delta$ -cells (by activating M1 receptors) and somatostatin secretion (that results in inhibition of  $\beta$ -cell function). [19] On the other hand, the L-cells of terminal ileum possess M1 receptors, which upon

receiving vagal stimulation increase glucagon-like peptide 1 (GLP-1) secretion. [20] While both the cells of the islets and the L-cells of terminal ileum possess muscarinic receptors, the net result of vagal stimulation (or blockade) on the entero-insulin axis after ingestion of carbohydrate-rich meal in subjects with impaired glucose tolerance (IGT) remains unexplored.

In the present study, 60 subjects including 30 IGT (13 male/17 female, BMI: 26.4±2.4) and 30 NGT (15 male/15 female, BMI: 24±0.6) met the study requirements and completed the experimental protocol. In both the groups, a higher proportion belonged to the “overweight” category. The effect of HBB in the IGT group was examined in terms of pharmacodynamic parameters obtained during a 75 g OGTT (0–120 min). The effect of intravenous atropinisation has been examined in euglycemic subjects with conflicting findings. Ahrén B and Holst found that atropinisation results in decreased early-phase postprandial insulin secretion; [13] Plamboeck et al. in 2015, on the other hand, observed a decrease in insulin and C-peptide levels after infusion of atropine, glucose, and GLP-1.[14] Our research with administration of an oral anticholinergic shows that the effect is modified depending on whether the subject is euglycemic or has IGT. In the euglycemic, the attenuation of insulin secretion by HBB strengthens the hypothesis that the postprandial insulin secretion is vagally mediated. In IGT subjects, on the other hand, diminution of the incretin effect is a well-known early feature. [17] It is likely that since it is already attenuated in the IGT subjects, an anticholinergic agent did result in further changes. It could be also argued that with a heightened parasympathetic tone in IGT, as opposed to euglycemic subjects, the attenuation of entero-insular axis may be difficult to attain at therapeutic doses of muscarinic antagonists. Taken together, the response to the anticholinergic effect in both the euglycemic and the IGT subjects fits the hypothesis of vagal mediation of the incretin effect.

The presence of HBB did not have an impact on their fasting PG and PG Cmax values. In the IGT group, the presence of HBB had no effect on fasting insulin levels and insulin Cmax at t = 60 min. The addition of HBB also did not impact on the insulin total AUC 0–120 min. The presence of HBB had no effect on fasting insulin levels (6.50 ± 1.32 vs. 5.70 ± 0.87 mIU/L) and insulin Cmax at t = 60 min. However, the addition of HBB significantly decreased the insulin total AUC 0-120 min. In the NGT group, similar to the IGT group, the presence of HBB did not impact on the plasma glucose-based parameters, for example, fasting PG. An important difference from Ahrén and Holst in our results is that the anticholinergic attenuation of insulin secretion in their experiment is limited to the early-phase and

does not extend to the late-phase insulin secretion. This can be explained by the relatively late onset of action of an oral agent like HBB as opposed to the IV atropine Ahrén and Holst use.<sup>13</sup> Importantly, however, it highlights that at least part of the postprandial late-phase secretion of insulin is affected by incretin and that this effect is vagally mediated as well. However, this may be due to delayed gastric emptying induced by hyoscine as incretin hormone release is dependent on the rate of entry of nutrients into the small intestine and may result in a deferred beginning of the incretin effect.

### Conclusion

Our study findings indicate that insulin secretion is influenced by cholinergic system and that oral anticholinergics may attenuate the late phase insulin activity in varying degrees of glycaemic status.

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