Insulin, Glucagon and Growth Hormone and CIMT in Glucose Intolerance

Glukoz İntoleransında İnsulin, Glukagon, Büyüme Hormonu ve Karotis İntima Media Kalınlığı

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ABSTRACT

Objective: There is an increasing evidence that glucagon and growth hormone (GH)-insulin-like growth factor (IGF) axis may play an important role in glucose metabolism since early stages of glucose intolerance. Carotid intima media thickness is a marker for subclinical atherosclerosis. We aimed to evaluate glucagon, GH and IGF-1 in prediabetic states and their relationship with carotid intima media thickness.

Methods: One hundred subjects underwent a 75 gr oral glucose tolerance test and were divided into 4 groups according to their state of glucose tolerance: (i) normal glucose tolerance (NGT)/Controls (n=21), (ii) impaired glucose tolerance (IGT) (n=35), (iii) impaired fasting glucose (IFG) (n=22), (iv) type 2 diabetes mellitus (n=22). Insulin, glucagon and GH were measured at 0, 60 and 120. minutes of OGTT and their area under the curve (AUC) were calculated. Fasting IGF-1 levels and carotid intima media thickness were determined in all participants.

Results: AUC for Glucagon was significantly higher in subjects with IGT, IFG and type 2 diabetes mellitus compared to NGT subjects. AUC for GH was significantly higher in subjects with IFG compared to subjects with IGT, type 2 diabetes mellitus and NGT. Plasma IGF-1 levels were significantly lower in subjects with abnormal glucose tolerance. CIMT was significantly higher in IFG group and CIMT was found to be negatively correlated with IGF-1 levels in subjects with IFG.

Conclusion: There are pathological alterations of glucagon, GH-IGF-1 and insulin in prediabetic stages. Among these alterations insulin resistance and IGF-1 are associated with CIMT. Further studies needed to investigate the role of treatments targeting insulin sensitivity will have an impact on the association between insulin and early atherogenesis

Key Words: Glucose intolerance, Insulin like Growth Factor-1, carotid intima media thickness, insulin resistance

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ÖZET


Yöntem: Çalışmaya dahil edilen 100 hastaya 75 gr oral glukoz tolerans testi yapıldı ve sonuçlara göre hastalar dört gruba ayrıldı: (i) normal glukoz toleransı (NGT)/kontrol (n=21), (ii) Bozulmuş glukoz toleransı (BGT) (n=35), (iii) Bozulmuş açlık Glukozu (BAG) (n=22), (iv) tip 2 diabetes mellitus (n=22). Test sırasında 0,60 ve 120. Dakikalarda insülin, glukagon ve BH düzeyleri ve 0. dakikada IGF-1 düzeyi ölçüldü. İnsülin, glukagon ve BH için Eğri altında kalan alan hesaplandı. Hastaların karotis intima media kalınlıkları değerlendirildi.


Anahtar Sözcükler: Glukoz İntoleransı, İnsülin benzeri Büyüme faktörü-1, Karotis intima media kalınlığı, insülin direnci

INTRODUCTION

Type 2 Diabetes Mellitus (DM) is a heterogeneous syndrome with complex underlying pathophysiology, which involves concomitant impairment of insulin action and insulin secretion (1). There is growing evidence that type 2 diabetes mellitus may not be merely a beta cell dysfunction but also develops as a result of failure of all endocrine pancreas and dysregulation of other associated hormones. Growth hormone (GH), insulin-like growth factor-1 (IGF-1) and glucagon may have roles in glucose homeostasis and in pathogenesis of type 2 diabetes mellitus (2,3). It is suggested that alterations of insulin, glucagon and IGF-1 begin in prediabetic stages.

Type 2 DM is associated with increased cardiovascular morbidity and mortality (4). Impaired fasting Glucose (IFG) and impaired glucose tolerance (IGT) are also risk factors for cardiovascular events (5,6). Carotid intima media thickness (CIMT), a marker of subclinical atherogenesis and a predictor of cardiovascular events is increased in both diabetes and prediabetic stages. Beta cell dysfunction and insulin resistance is related with increased CIMT however little is known about the relationship between CIMT and glucagon and GH-IGF-1 axis.

In this study, we aimed to evaluate the alterations of insulin, glucagon and GH-IGF-1 axis in prediabetes and the relationship between these alterations and CIMT in prediabetes and DM.

MATERIAL and METHODS

Patient Population

A total of 100 subjects (83 females, 17 males; age range: 35-60 years) were included in the study. Subjects were recruited from outpatient clinics of Ankara University Hospital. All of the subjects were free of cardiovascular, pulmonary, renal, hepatic, or other major organ system disease as determined by history, physical examination, and routine laboratory tests. Patients treated with any drugs that might interfere with glucose metabolism were excluded from the study. The purpose and potential risks involved in the study were carefully explained to all subjects before obtaining their written voluntary consent. The study protocol was reviewed and approved by the Institutional Ethic Committee of the Ankara University. The study was conducted according to the declaration of Helsinki.

The demographic data and the anthropometric measures were obtained from all subjects. Body mass index (BMI) was calculated as weight in kilograms divided by the height in meters squared. Waist and hip circumferences were measured as the subjects standing. Blood pressure was measured twice in a sitting position after 15 minutes rest and the mean of two measurements was considered as the participant’s blood pressure. Mean arterial pressure (MAP) was calculated with the use of the following formula: MAP = (2 ×DBP + SBP) / 3.

Measurement of biochemical parameters

All subjects participated in the study underwent 75 gr oral glucose tolerance test (OGTT). Based on the results of OGTT, the subjects were divided into four groups according to American Diabetes Association (ADA) recommendations 7: (i) Normal Glucose Tolerance (NGT) (served as the control group in this study) (n=21), (ii) Impaired Fasting Glucose (IFG) (n=22), (iii) Impaired Glucose Tolerance (IGT) (n=35) and (iv) type 2 diabetes mellitus (n=22). Before the OGTT, a small polyethylene catheter was placed into an antecubital vein and blood samples were collected at 0, 60, and 120 minutes for the measurement of plasma glucose, insulin, glucagon and GH concentrations. Fasting venous blood samples were also obtained for measurement of serum lipids and IGF-1. Samples for analysis of glucagon, concentrations. Fasting venous blood samples were also obtained for measurement of serum lipids and IGF-1. Samples for analysis of glucagon, insulin-like growth factor-1 (IGF-1) and glucagon may have roles in glucose homeostasis and in pathogenesis of type 2 diabetes mellitus (2,3). It is suggested that alterations of insulin, glucagon and IGF-1 begin in prediabetic stages.

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In this study, we aimed to evaluate the alterations of insulin, glucagon and GH-IGF-1 axis in prediabetes and the relationship between these alterations and CIMT in prediabetes and DM.

Measurement of Carotid Intima Media Thickness

Carotid Intima Media Thickness (CIMT) was evaluated by a single operator using General Electric Logic 400 Doppler Ultrasound machine and a 12 MHz linear probe. Subjects were examined in the supine position. Each scan of the common carotid artery began just above the clavicle, and the transducer was moved cephalad through the bifurcation and along the internal carotid artery. The near and distant walls of the common carotid artery, the carotid bulb, and the internal carotid artery were scanned for the presence of atherosclerotic plaques. Three segments were identified on each side: 1.0 centimeters (cm) distal to the common carotid artery proximal to the bifurcation, the bifurcation itself and 1.0 cm proximal to the internal carotid artery. At each of the three segments, for distant walls in the left and right carotid arteries, intima-media thickness was defined as the distance between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. Maximum thickness of the wall was calculated at each side. The reported CIMT for each subject is the average of these 10 measurements of distant walls (5 measurements from the right and 5 from the left carotid artery). The investigator (RE) performed all the ultrasonographic examinations blinded to the knowledge of the group the subjects belonged.

Statistical Analysis

All analyses were performed using SPSS software program version 11.5 for windows. Continuous variables are expressed as means ± SD, non-normally distributed variables are expressed as median (ranges). The ANOVA test was applied for normally distributed continuous data and the Kruskal Wallis test was applied to non-normally distributed continuous data. In case of p<0.05 between groups, multiple comparison test was used. The Chi Square test was performed for categorical data. The values of the area under the concentration-time curve for insulin, glucagon and GH were calculated by means of trapezoidal rule. For all analyses, a p<0.05 value was considered to be statistically significant.

RESULTS

The groups were similar for age, sex, BMI, waist circumference and mean BP (Table 1). There were no significant differences among groups in terms of mean serum levels of Total Cholesterol, LDL Cholesterol, HDL Cholesterol and triglycerides (Table 1). HOMA-IR was significantly higher in subjects with IFG, IGT and type 2 diabetes mellitus compared to control group (p<0.01) (Table 1).

The values of area under the concentration-time curve for insulin, glucagon and GH between time 0 and 120 were calculated. Plasma insulin excursion after glucose load in subjects and area under the curve (AUC) values are shown in Figure 1. Area under the curve calculated for insulin was significantly higher in subjects with IFG compared to IGT (p=0.03), type 2 diabetes mellitus (p=0.01) and control group (p=0.002). There was a significant difference between subjects with IGT and NGT (p=0.03) while there was no difference between diabetic subjects and NGT (p=0.515). Area under the curve calculated for glucagon was significantly higher in subjects with IGT (p<0.001), IFG (p<0.001) and type 2 diabetes mellitus (p<0.003) when compared with NGT subjects. There was no difference between IGT, IFG and diabetic group (Figure 1). Area under curve calculated for GH was significantly higher in subjects with IFG compared to subjects with IGT (p=0.018), type 2 diabetes mellitus (p=0.014) and NGT (p=0.004) (Figure 1).

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Table 1 Anthropometric and biochemical characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>IGT (n=35)</th>
<th>IFG (n=22)</th>
<th>T2DM (n=22)</th>
<th>Control (n=21)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Age (yrs) †</td>
<td>51.3±6.9</td>
<td>53.9±8.4</td>
<td>53.7±7.2</td>
<td>48.8±7.6</td>
<td>0.065</td>
</tr>
<tr>
<td>Sex(F/M)</td>
<td>30/5</td>
<td>19/3</td>
<td>16/6</td>
<td>18/3</td>
<td>0.927</td>
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<tr>
<td>BMI (kg/m²) †</td>
<td>31.0±0.6</td>
<td>33.9±1.9</td>
<td>31.3±1.2</td>
<td>30.5±1.2</td>
<td>0.271</td>
</tr>
<tr>
<td>Waist Circumference(cm) †</td>
<td>101.6±10.7</td>
<td>107.6±16.6</td>
<td>102.5±11.1</td>
<td>99.5±15.4</td>
<td>0.215</td>
</tr>
<tr>
<td>Mean BP (mmHg) †</td>
<td>99.0±12.0</td>
<td>101.3±13.8</td>
<td>93.0±9.0</td>
<td>94.0±13.3</td>
<td>0.137</td>
</tr>
<tr>
<td>Glucose (mg/dl) ‡</td>
<td>93(19)</td>
<td>107(12)</td>
<td>198.7±42.2</td>
<td>193.3±31.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>T. Cholesterol (mg/dl) †</td>
<td>202.4±33.0</td>
<td>211.7±48.1</td>
<td>121.1±32.5</td>
<td>116.2±32.5</td>
<td>0.821</td>
</tr>
<tr>
<td>LDL (mg/dl) †</td>
<td>121.6±24.9</td>
<td>128.6±40.9</td>
<td>45.6±9.8</td>
<td>50.3±9.7</td>
<td>0.313</td>
</tr>
<tr>
<td>HDL (mg/dl) †</td>
<td>179.9±90.8</td>
<td>166.6±74.7</td>
<td>47.7±9.8</td>
<td>50.9±14.3</td>
<td>0.091</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) †</td>
<td>3.4(3.1)</td>
<td>4.1(2.7)</td>
<td>3.6(3.5)</td>
<td>2.1(2.0)</td>
<td>0.002*</td>
</tr>
<tr>
<td>IGF-1 (ng/ml) †</td>
<td>0.73(0.11)</td>
<td>0.81(0.14)</td>
<td>0.76(0.14)</td>
<td>0.69(0.10)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Insulin (µU/ml) ‡</td>
<td>14.5 (13.4)</td>
<td>16.1 (12.8)</td>
<td>12.7 (12.4)</td>
<td>9.3 (10.3)</td>
<td>0.065</td>
</tr>
<tr>
<td>Glukagon (pg/ml) ‡</td>
<td>74.5 (26.5)</td>
<td>69.0 (19.0)</td>
<td>64.5 (11.5)</td>
<td>52 (12.0)</td>
<td>0.005*</td>
</tr>
<tr>
<td>GH (ng/ml) ‡</td>
<td>0.13 (0.21)</td>
<td>0.27 (0.28)</td>
<td>0.13 (0.15)</td>
<td>0.10 (0.34)</td>
<td>0.061</td>
</tr>
</tbody>
</table>

† Data are mean±SD
‡ Date are median (Interquartil range)

*Control vs IGT, IFG and T2DM


![Graphs](image_url)  
**Figure 1.** Plasma Insulin (A), Glucagon (B) and Growth Hormone (C) excursion after oral glucose load and area under curve graphics. *p<0.05
DISCUSSION

In this study, subjects with both impaired glucose tolerance, impaired fasting glucose and type 2 diabetes mellitus were characterized by hyperglycaemogenic compared to normal glucose tolerant subjects. Plasma glucose concentrations are inappropriately elevated in diabetic individuals, and α-cell suppression by hyperglycaemia is blunted (10). Although dysregulated glucagon secretion and elevated glucagon levels are known to be associated with high blood glucose in type 2 diabetes mellitus (11), these conditions are usually considered a consequence instead of a cause of diabetes, and it remains controversial whether glucagon, plays an active role in diabetes development (12). Our data suggest that combined decreased insulin secretion and increased insulin resistance may not be sufficient to induce hyperglycaemia without dysregulated glucagon secretion, and thus reveal a previously underappreciated role of the regulation of glucagon secretion in the development of diabetes. Increased glucagon secretion may also promote diabetes development at prediabetic stages such as impaired glucose tolerance and impaired fasting glucose. There was no significant correlation between AUC glucagon and CIMT in our study. There is little known about the relationship between glucagon and atherosclerosis in diabetic patients. However studies suggest that Glucagon like peptide-1 analogs which suppresses glucagon secretion have cardioprotective role independent of their glucose lowering effects (13). Further studies needed in this era.

In our study, we observed a negative correlation between serum IGF1 levels and CIMT and positive correlation between HOMA-IR and CIMT among all participants. Observational studies investigating associations between IGF1 and its binding proteins (IGFBPs) with CIMT have yielded inconsistent results. Both increased and decreased levels of IGF1 was found to be associated with thickening of carotid intima media (14-18). Previous analysis by Martin et al. revealed no association between IGF-1 and IMT (19). There are several insulin receptors located predominantly in endothelial cells. IGF-1, is a potent mitogenic and antiapoptotic factor for vascular smooth muscles (20). In early stages of atherosclerosis decrease of IGF-1 levels might be beneficial but in advanced stages IGF-1 has an important role on plaque stability (21). This dual role of IGF-1 on atherosclerosis may explain the negative correlation between CIMT and IGF-1 in our study. All participants in our study were obese. Obesity is characterized with low IGF-1 levels (22). This might be another explanation of low IGF-1 levels of our patients.

One important result of our study was positive correlation between HOMA-IR and IGF1. This is an agreement with a recent study that has shown that patients with higher CIMT had lower insulin sensitivity (23). Rousell et al. showed that insulin secretion is associated with early atherosclerosis in non diabetic individuals (24). Insulin receptors are expressed both on endothelial cells and vascular smooth muscle. Insulin signaling pathways in vascular endothelium stimulates vasodilator NO and also stimulates vasoconstrictor ET-1 (25). Inappropriate insulin secretion and action may triggers an imbalance between these signaling pathways in the endothelium and may cause subclinical atherosclerosis.

Plasma IGF-1 levels were significantly lower in subjects those with abnormal glucose tolerance compared to subjects with NGT. Our data is consistent with a previous study by Sesti et al. who found a significant positive correlation between insulin sensitivity and endogenous IGF-1 concentration among patients with varying degrees of glucose intolerance (26). There is considerable evidence suggesting that IGF-1 has insulin-like effects on peripheral uptake of glucose and fatty acids (27). Obesity, an insulin resistance state and a strong risk factor for type 2 DM, was associated with altered IGF-1 levels. Taken together with our present data suggests that IGF-1 may have role in pathogenesis of type 2 DM. Area under curve calculated for GH was significantly higher in subjects with IFG compared to subjects with IGT, type 2 diabetes mellitus and NGT. We also found that the patients with IGT had the lowest IGF-1 levels compared to other groups. This may be the result of inadequate negative feedback of low plasma IGF-1 concentrations at the level of the hypothalamus and/or pituitary, thus resulting in growth hormone (GH) hypersecretion and a decrease in insulin sensitivity.

This study has some limitations. We only measured total IGF-1 and not the free fraction, which is the form that interacts with IGF-1 receptor and is responsible for the peripheral effect. Relativly small sample size and cross sectional design are also the limitations of the study.

As a conclusion, there are pathological alterations of glucagon, GH-IGF-1 and insulin in prediabetic stages. Among these alterations insulin resistance and IGF-1 are associated with CIMT. Further studies needed to investigate the role of treatments targeting insulin sensitivity will have an impact on this association between insulin and early atherosgeresis.

ACKNOWLEDGEMENTS

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Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES


