Introduction

The concentration of glucose in the blood is regulated by a complex network of various biochemical processes including glycolysis, glycogenogenesis, glycogenesis, glycogenolysis and many others. These metabolic pathways are controlled and modulated by a number of hormones, in order to maintain the concentration of glucose within relatively narrow range. Insulin and, to a lesser extent, insulin-like growth factors are responsible for a decrease in blood glucose, while glucagon, cortisol, epinephrine and growth hormone have the opposite role (1). Numerous disease states that involve impaired glucose metabolism have been discovered. A disease with the highest incidence is diabetes mellitus (2). Diabetes mellitus can be regarded as a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia, and often followed by life-threatening episodes that include ketoacidosis and hyperosmolar coma (1). Prolonged disease causes specific complications, many of which are related to pathological glycosylation (glycation) of various essential molecules and cellular structures. In general, structural modifications fall into two categories: (i) nonenzymatic glycation per se, which refers to the attachment of free carbohydrate to proteins in the Amadori adduct, and (ii) generation of advanced glycation endproducts (AGE), which refers to a heterogeneous group of carbohydrate-modified products derived from the Amadori adduct by oxidation, polymerization and other spontaneous reactions (3). Complications,
including cardiovascular disease, neuropathy, nephropathy and others, are frequently diagnosed in patients with diabetes mellitus (4–6). The opposite, hypoglycemia, is defined as a state in which blood glucose concentration is below the fasting range. A rapid decrease in glucose level produces weakness, shakiness, rapid pulse, headache etc. (1).

Insulin is a hormone that stimulates the uptake of glucose into cells, promotes intracellular transport and conversion into the other metabolic molecules. Proinsulin is produced from preproinsulin in pancreatic beta cells. Proinsulin is stored in secretory granules and is cleaved into insulin and C-peptide upon stimulation. A physiological role of insulin is well known, while C-peptide, being more stable than insulin in the circulation, can be regarded as a reliable monitor of insulin secretion (1).

Serial measurements of blood glucose levels before and after giving a specific amount of glucose orally provide a standard method to evaluate glucose metabolism. Oral glucose tolerance test (OGTT), recommended by the World Health Organization (WHO), is performed with 75 g of glucose for non-pregnant adults and the blood samples are collected at defined time intervals: 0 h (before glucose intake), 1 h and 2 h (after glucose consumption). Variations of the method include sampling every 30 min for 2 h, as well as after 3 h (1). The WHO criteria that differentiated healthy persons from diabetic were adopted in the National guide for clinical practice in Serbia (7). Metabolic response in healthy individuals exposed to OGTT should immediately for glucose and within a week for insulin and C-peptide. Meanwhile, sera were kept frozen at −20 °C, as C-peptide is unstable in serum at higher temperatures.

Glucose concentration was measured using RANDOX commercial reagent (GOD-PAP method); the reference levels according to the producer are 4.2–6.4 mmol/L. Insulin and C-peptide levels were determined by INEP commercial RIA kits; the reference values are established as 5–25 mU/L for insulin and 0.3–0.7 nmol/L for C-peptide. Data were statistically analyzed using the Student t-test for the significance of differences between the groups (patients vs. healthy persons).

Results

According to the results obtained for the concentration of blood glucose during OGTT and clinical history, the examined persons were divided into four groups. The first group (I, n = 21) was characterized as having the referent blood glucose concentration at 0 h (the lowest value was 4.0 and the highest 5.7 mmol/L), after 1 h the concentration increased (6.8–8.3 mmol/L) and returned to normal after 2 h (4.8–6.0 mmol/L). These individuals were assumed to be healthy in respect to glucose metabolism. Subjects collected as a second group (II, n = 25) exhibited delayed glucose clearance and most of them were monitored for 3 h. The fasting blood glucose concentration was referent (4.0–5.7 mmol/L), then increased after 1 h (7.4–10.3 mmol/L), decreased after 2 h (6.6–8.3 mmol/L), and returned to normal after 3 h (4.0–5.5 mmol/L). Patients in group three (III, n = 25) satisfied

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (mU/L)</th>
<th>C-peptide (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>1h</td>
</tr>
<tr>
<td>I</td>
<td>15 ± 4.9</td>
<td>116 ± 52.8</td>
</tr>
<tr>
<td>II</td>
<td>17 ± 4.7</td>
<td>209 ± 63.2</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>p = 0.0004</td>
</tr>
<tr>
<td>III</td>
<td>18 ± 8.1</td>
<td>131 ± 75.1</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IV</td>
<td>11 ± 2.5</td>
<td>63 ± 31.1</td>
</tr>
<tr>
<td></td>
<td>p = 0.009</td>
<td>p = 0.0004</td>
</tr>
</tbody>
</table>
the OGTT criteria that classified them as diabetics, they also had other signs of diabetes mellitus confirmed biochemically and/or clinically. Their fasting blood glucose was normal to elevated (5.4–13.9 mmol/L), increased after 1 h (8.8–22.4 mmol/L), then moderately dropped or continued to increase after 2 h (7.5–24.0 mmol/L). Finally, subjects with low blood glucose levels were established as a fourth group (IV, n = 16). At 0 h their concentration of glucose was low to normal (3.1–5.1 mmol/L), it remained almost unchanged after 1 h (4.0–5.7 mmol/L) and most often decreased to very low after 2 h (2.2–4.2 mmol/L).

The concentrations of insulin and C-peptide were measured in each sample and the results (mean ± standard deviation) are presented in Table I. Although there were significant interindividual variations, which can be seen from the SD values, specific patterns of insulin and C-peptide alterations during OGTT emerged. Figures 1a and 1b present typical insulin and C-peptide responses in four groups of people during OGTT.

Healthy individuals exhibited increase in insulin and C-peptide levels 1 h after the stimulation and they decreased after 2 h. Persons with delayed glucose clearance had slightly higher C-peptide levels at 0 h and significantly higher insulin and C-peptide concentrations compared to healthy subjects after 1 h and 2 h. Concentrations of insulin and C-peptide remained very high after 2 h and dropped after 3 h (data not shown). Diabetic patients had higher than normal basal levels of C-peptide. After 1 h insulin and C-peptide increased, on average, as in healthy people but further continued to increase significantly. After 2 h the concentration of insulin and C-peptide in groups II and III were within the same range. Subjects with low blood glucose levels demonstrated lower insulin concentrations at all time intervals, but the concentration of C-peptide leveled with that of the healthy ones.

Discussion

The results for insulin concentration during OGTT in healthy persons are in accordance with the data reported in Tietz Textbook of Clinical Chemistry, 0 h: 6–21 mU/L, 1 h: 20–120 mU/L and 2 h: 18–56 mU/L (8) and Human Clinical Endocrinology, 0 h: 20 ± 2 mU/L, 1 h: 75 ± 10 mU/L and 2 h: 45 ± 6 mU/L (9). In obese persons both basal and stimulated levels may be high, 0 h: 80 ± 5 mU/L, 1 h: 130 ± 7 mU/L and 2 h: 80 ± 5 mU/L (9). Impaired glucose tolerance was diagnosed in individuals who had fasting blood glucose levels less than those required for a diagnosis of diabetes mellitus, but had a glucose response during OGTT between normal and diabetic (1). Insulin and C-peptide response, on the other hand, did not follow that pattern. The initial response after 1 h was, on average, more intense than in diabetics, suggesting greater pancreatic sensitivity to glucose stimulation and hormonal secretion which, unfortunately, was less successful in decreasing the blood glucose. The state of relative insulin resistance at cellular level is most often responsible for this insensitivity (10). Mathematical analysis of the relationship between insulin sensitivity and secretion has revealed a hyperbolic function, such that the product of two variables is constant (11). Measurements of insulin sensitivity provide clinicians and clinical researchers with valuable tool to evaluate the efficiency of both current and potentially useful therapeutic preparations. Although several methods had been developed and validated to evaluate insulin sensitivity, none of these methods can be universally used in all patients (12). Evenmore, a method suitable for clinical or basic research may not necessarily be a practical method for use in regular practice or for epidemiological studies. Insulin activity may be also influ-
enced by the presence of anti-insulin or anti-insulin receptor antibodies (13, 14). Two hours after the glucose intake, insulin/C-peptide status in both groups of patients (II and III), on average, became the same. These results should be, however, interpreted with caution due to very high standard deviation values in group III. All examined diabetic persons had normal to high insulin levels upon stimulation, which classified them as insulin-independent cases of diabetes mellitus (1). Subtyping of these patients would, possibly, narrow insulin and C-peptide ranges for each subgroup.

Blood glucose concentration limits are hard to define for hypoglycemia, as glucose levels are often below fasting range and transient decline may occur (1). OGTT is not a test regularly recommended for diagnosis of hypoglycemia. Blood insulin concentration in persons with low blood glucose levels was found to be significantly lower than in healthy people, both after fasting and during OGTT. C-peptide levels, on the other hand, were within reference range at all sampling intervals. These data suggested that pancreatic stimulation was preserved, but secreted insulin was either cleared from the circulation faster than normal or it was present in a form that was not detected by the applied analytical method (immune complexes?).

Although OGTT and blood glucose determinations are routinely performed for diagnosis of diabetes mellitus and impaired glucose tolerance (15, 16), parallel measurement of insulin and C-peptide levels, in all cases of impaired glucose metabolism, certainly helps in diagnosis and management of these disorders. The results of this work may be useful in establishing reference ranges for insulin and C-peptide concentrations for defined time intervals during OGTT, in health and disease.

PROMENA KONCENTRACIJE INSULINA I C-PEPTIDA KOD ZDRAVIH LJUDI I OSOBA SA POREMEĆENIM METABOLIZMOM GLUKOZE TOKOM ORALNOG TESTA TOLERANCIJE GLUKOZE

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Kratak sadržaj: Metabolizam glukoze se uobičajeno procenjuje serijskim određivanjem koncentracije glukoze u krvi pre i posle oralnog unošenja određene količine glukoze. Iako je referentni opseg za koncentraciju glukoze utvrđen kod različitih stadijuma bolesti, u čijoj osnovi je poremećena homeostaza glukoze, referentne vrednosti za koncentraciju insulinina i C-peptida nisu jasno definisane. Cilj ovog rada je bio da ispita promenu koncentracije insulinina i C-peptida tokom OGTT. Zdrave osobe su ispoljile sledeći profil promene koncentracije insulinina i C-peptida: 15 ± 4,9 mU/L i 0,5 ± 0,19 nmol/L (0 h), 116 ± 52,8 mU/L i 2,3 ± 0,79 nmol/L (1 h) i 59 ± 26,7 mU/L i 2,0 ± 0,67 nmol/L (2 h). Osobe sa smanjenom tolerancijom glukoze su imale viši nivo koncentracije insulinina i C-peptida: 15 ± 4,9 mU/L i 0,5 ± 0,19 nmol/L (0 h), 209 ± 63,8 mU/L i 3,5 ± 1,00 nmol/L (1 h), 188 ± 48,8 mU/L i 3,6 ± 0,92 nmol/L (2 h). Dijabetičarima je izmeren viši bazalni nivo C-peptida, 0,8 ± 0,23 nmol/L, posle 1 h koncentracije insulinina i C-peptida su povećane slično kao kod zdravih ljudi, ali su nastavile i dalje da značajno rastu, 181 ± 137,6 mU/L i 3,7 ± 1,49 nmol/L. Kod osoba sa niskom koncentracijom glukoze u krvi utvrđena je niska koncentracija insulinina u svim testiranim vremenskim intervalima, 11 ± 2,5 mU/L (0 h), 63 ± 31,1 mU/L (1 h) i 44 ± 22,9 mU/L (2 h), dok je nivo C-peptida bio sličan kao kod zdravih. Rezultati ovog rada bi mogli biti korisni za određivanje referentnih opsega za koncentraciju insulinina i C-peptida, za utvrđene vremenske intervale tokom OGTT, u zdravom stanju i tokom bolesti.

Ključne reči: insulin, C-peptid, OGTT
References


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