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Original paper

BIOCHEMICAL AND MOLECULAR BIOLOGICAL ASPECTS OF GLUCOSE INTOLERANCE IN ELDERLY PERSONS

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Summary: Changes in carbohydrate metabolism in elderly persons have drawn considerable attention but the findings from different studies are in contrast and are even controversial. The insulin receptors in erythrocytes were studied in elderly euglycaemic patients and in a middle-aged control group. The examined persons were also subjected to measurements of blood glucose, insulin and C-peptide concentrations, before and 3 hours after a dietetic meal. In the present study it was found that in spite of the maintained insulin level and C-peptide secretion, some structural and corresponding changes in membrane insulin receptors and the binding site caused differences in postreceptor responses in elderly persons. The examined groups, consisted of 29 males, average age of 66 years (65–70), with normal serum glucose level and 19 middle-aged males, average age of 42 years (32–48), with normal glycoregulation. In basal condition, elderly persons have both normal morning serum insulin (19.68 ± 4.00 mU/L) and C-peptide (2.04 ± 0.78 nmol/L) level. In elderly persons, the number of high affinity insulin receptors in erythrocytes membrane is 22.80 ± 6.18 but the formed insulin-high affinity receptors were not stable. Dissociation constant (Kd1) indicates its elevated dissociation (0.11 ± 0.04). At the same time the number of insulin low affinity binding sites is increased (13 273 ± 5 572) with a fast dissociation of the hormone (13.99 ± 3.37). Food intake raised the number of high affinity receptors compared to the basal value. Alteration in insulin binding affinity suggests the structural and corresponding changes in membrane receptors that may cause differences in postreceptors responses in elderly persons.

Key words: glucose intolerance, insulin, receptors, binding sites, elderly people

Introduction

Normal serum glucose value is a remarkable component in maintenance of homeostasis. Glucose is an essential substrate in vital organs metabolism such as the brain, the heart, the muscles, the liver, and the others. In certain circumstances, including the ageing, numerous very complex changes occur in carbohydrates, lipids and proteins metabolism. Insulin promotes glucose uptake in various tissues (1). All insulin biological effects are mediated by its previous binding to the specific tissue receptors. Thus, the changes in the characteristics of these receptors, as well as the changes in post receptors events may influence the metabolic processes (2). The age related changes in insulin binding to the specific receptors in various tissues occur (3), but the mechanisms of very complex peripheral insulin resistance pathophysiology and glucose intolerance in elderly persons are not well explained.

The aim of this study was to examine the quality of glucoregulation, erythrocytes insulin receptors, and insulin binding characteristics, plasma insulin level and the C-peptide serum concentration in elderly persons.

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Patients and Methods

Subjects. We studied a group of 29 euglycaemic male persons, average age of 66 years (65–70). All analyzed parameters were compared with those obtained in a control group, of 19 middle-aged males,

average age of 42 years (32–48) with normal glycoregulation.

Methods. The morning glucose values, plasma insulin and C-peptide serum levels were analyzed in blood samples obtained by venepunction before the breakfast (basal value) and three hours (3h) later i.e. after a dietetic meal, using the standardized biochemical test for glucose and radioimmunoassay (produced by INEP-Diagnostics, Yu) for determination of serum insulin and C-peptide serum concentrations. The values, assumed to be normal, were accepted in accordance with the methods used. The quality control was performed in all cases.

Insulin binding assay was performed in accordance with our own modification (4). Scatchard plots analysis data were used to determine the binding capacities and dissociation constants (5). The curvilinear Scatchard plots were resolved into two components; high affinity low capacity (N_1) and low affinity high capacity (N_2) component. The dissociation constants (Kd_1 and Kd_2) were also calculated (6). Binding capacities (mol/L) were converted to the receptors number per erythrocytes.

Oral glucose tolerance test (oGTT) was proved at the same manner in all analyzed persons, using 75.0 g of pure glucose.

Statistical analysis. The obtained data were evaluated by parametric statistics (7). The results were expressed by the mean value, resulting from two tests of the same sample with corresponding standard error ($\bar{x} \pm SE$). The significance of observed differences was tested by one-way analysis of variance (F_{emp}) in the same group (8) and by Student's t-test between the analyzed groups.

Results

The patients included in this study were not obese. Their body mass indexes (BMI) were normal, obtained by Mini nutrition assessment (9): 22 kg/m^2 ± 2.80 ($\bar{x} \pm SD$). Elderly persons (»physiological ageing«), i.e. group the examined, have normal morning blood glucose values (*Table I*).

Table I Serum glucose, insulin and C-peptide morning levels in elderly and middle-aged persons

Parameters (reference values in brackets)	Elderly persons	Middle-aged control group
Glucose (3.8–6.1 mmol/L)	5.64 ± 0.30	4.56 ± 0.27
Insulin (5–30 mU/L)	15.04 ± 1.87*	19.68 ± 4.00*
C-peptide (2.1–4.0 nmol/L)	1.34 ± 0.43*	2.04 ± 0.78*
	*p<0.05	*p<0.05

The significant difference ($p<0.05$) in serum insulin and C-peptide levels between analyzed groups was proved. However, the curves, indicating the quality of glucoregulation, differed significantly (*Figure 1*).

In an attempt to elucidate the site of observed glucose intolerance during ageing, the binding of ^{125}I-insulin to isolated humans erythrocytes was analyzed in all persons included in the study. Scatchard analysis indicated that a decrease in receptors number (*Table II*), rather than receptors affinity seems to be the cause of the lowered specific insulin binding in elderly. This assumption is supported by the evidence indicating that the stability of formed insulin-receptor complex is higher ($p<0.10$) in the control group. However, the dissociation constant is not statistically significant between compared groups (*Table II*).

The results obtained by analysis of the low affinity binding sites demonstrate the heterogeneity. A higher number ($p<0.01$) of low affinity binding sites (13 273 ± 5 572, $\bar{x} \pm SE$) was observed in elderly. Insulin dissociates rapidly ($p<0.025$) from the complex, which were formed with low affinity binding sites (13.99 ± 3.37, $\bar{x} \pm SE$).

After a dietetic meal serum insulin level was higher (p (F)<0.01) in the group of elderly persons (46.56 ± 14.27 versus15.04 ± 1.87, $\bar{x} \pm SE$ before). Very similar finding for C-peptide serum concentration 3h after the meal was obtained (3.39 ± 0.86) when compared to the basal C-peptide (1.34 ± 0.43) level (p (F)<0.10).

However, in middle-aged group of persons both, serum insulin and C-peptide levels reached 55.24 ± 4.20 and 4.18 ± 0.52, respectively.

In order to elucidate the site of the observed insulin resistance occurring in the elderly, insulin erythrocytes receptors were analyzed 3 hours after the meal. The number of the high affinity receptors decreased (p (F)< 0.05) after the meal in euglycaemic elderly people (14.20 ± 2.80) in comparison with the

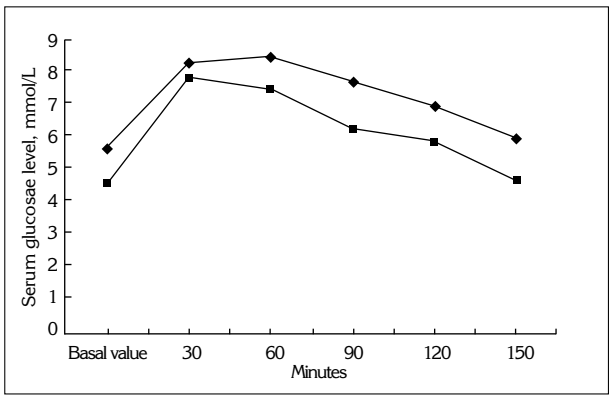

Figure 1. Glucose tolerance test (75 g) in elderly (–■–) and middle-aged (–◆–) persons

Table II Erythrocytes insulin receptors characteristics in elderly and middle-aged people

Groups	Specific binding of ^{125}I insulin/erythrocyte	Receptors	Number	Dissociation	constant
		High affinity (N_1)	Low affinity (N_2)	(Kd_1)	(Kd_2)
Elderly	2.66 ± 0.46	14.65 ± 1.70	13273 ± 5572	0.11 ± 0.04	13.99 ± 3.37
Middle-aged	3.55 ± 0.66	22.80 ± 6.18	5069 ± 1532	0.09 ± 0.02	5.25 ± 1.31
p	p<0.1	p<0.05	p<0.01	p<0.10	p<0.025

Table III Erythrocytes insulin receptors characteristics in analyzed groups 3h after the meal

Groups	Specific binding of ^{125}I insulin/erythrocyte	Receptors	Number	Dissociation	constant
		High affinity (N_1)	Low affinity (N_2)	(Kd_1)	(Kd_2)
Elderly	1.93 ± 0.18	14.20 ± 2.80	8074 ± 3087	0.38 ± 0.28	10.41 ± 4.08
Middle-aged	3.18 ± 0.46	26.82 ± 5.19	9108 ± 2425	0.11 ± 0.02	14.24 ± 3.30
p	p<0.01	p<0.05	p<0.10	p<0.05	p<0.10

same parameter before the meal (14.65 ± 1.70). The dissociation constant (0.38 ± 0.28) was significantly ($p<0.05$) higher if compared to that before the meal and the control (0.11 ± 0.02) group (*Table III*). Decreased insulin low affinity binding sites were found after the meal in elderly persons (8 074 ± 3 087; $\bar{x}$ ± SE) in physiological ageing process. This finding is statistically significantly lower ($p(F)<0.05$) in comparison with the same parameter in the elderly before the meal (13 273 ± 5 572) as well as compared to that observed in middle-aged control group (9 108 ± 2 425). Thus, it seems that the low affinity binding sites are »spare» binding sites, but without a stability of forming an insulin-binding site complex. The consequence is probably the postreceptor transmission dysfunction which can be the basis of the resistance to insulin action in elderly persons.

Discussion

Although it is accepted that glucose intolerance is a common feature in advanced years, the effect of ageing on carbohydrate metabolism has not been completely clarified. Ageing is often accompanied by hyperglycaemia in non-diabetics (10) and may induce further deterioration of glucose homeostasis. The possible explanation for this, in a mild degree disturbed carbohydrate metabolism in elderly persons, could be some modification of insulin action at the target tissue level, including the receptor and post-receptor events, by changing the endocrine status, which occurred in ageing processes (11). However, the mechanisms by which some hormones modulate these processes are not yet understood, and the mechanism of insulin resistance as the consequence of ageing is still unknown. Thus, the goal of this study is to examine the possibility if the glucoregulation in elderly persons is due to either the modification of receptors number and affinity or to changes in endogenous insulin secretion, indicated by C-peptide. All the above-mentioned data suggested the existence of some changes in insulin receptor characteristics. The number of high affinity receptors was not paralleled by an enhanced effect of hormone because the dissociation constant of high affinity receptors was higher in elderly persons than in middle-aged controls. Additionally, alteration in insulin binding affinity suggests some structural and corresponding changes in membranes receptors, that may cause some further differences in biological expected, post-receptor response, in elderly persons.

The data of this study support the hypothesis that ageing induced insulin resistance and mostly post-insulin binding defects, which could be at least partially located at the insulin receptor kinase, which is able to phosphorylate various proteins and other substrates (12), as well as at the level of synthesis and activity of enzymes involved in glucose metabolism, but not in the synthesis of insulin receptors.

This study provides the one more evidence on the hormone involvement in the ability of insulin receptor, which can regulate glucose level during the ageing. It seems, therefore, that ageing modulates the properties of insulin receptor complex and consequently the response to insulin action.

The obtained results suggest that the peripheral insulin resistance could be involved in very complex pathophysiology of glucose intolerance usually occurring in the elderly during normal ageing process.

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BIOHEMIJSKI I MOLEKULARNO BIOLOŠKI ASPEKTI INTOLERANCIJE GLUKOZE KOD STARIJIH OSOBA

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Kratak sadržaj: Promene metabolizma ugljenih hidrata starijih osoba zaslužuju pažnju, uprkos različitim stavovima. Kod starijih normoglikemičnih osoba, analizirani su receptori za insulin na eritrocitima i nalaz je upoređen sa istim kod srednjedobnih osoba. Takođe je svim osobama, obuhvaćenih studijom, određena koncentracija glikemije našte, insulina seruma i C-peptida, pre jela i 3 časa posle obroka. Iako je lučenje insulina i C-peptida očuvano, kod starijih osoba postoje izvesne promene konformacije receptora i vezujućih mesta za insulin, što uzrokuje razlike u postreceptorskom odgovoru na ovaj hormon. Studijom je obuhvaćeno 29 normoglikemičnih osoba muškog pola prosečne starosti 66 godina (65–70) i 19 osoba prosečne starosti 42 godine (32–48) normalne glikoregulacije. U bazalnim uslovima starije osobe imaju normalne koncentracije insulina (19,68 ± 4,00 mU/L i C-peptida (2,04 ± 0,78 nmol/L). Broj receptora visokog afiniteta na eritrocitima prema insulinu, kod starijih osoba je 22,80 ± 6,188, ali formirani kompleks nije stabilan, što potvrđuje niska konstanta disocijacije (Kd_1: 0,11 ± 0,04). Istovremeno, broj vezujućih mesta niskog afiniteta je povećan (13 273 ± 5 572), sa brzim disociranjem hormona iz kompleksa (13,99 ± 3,37). Unos hrane povećava broj visokoafinitetnih receptora u poređenju sa bazalnim vrednostima. Poremećaj afiniteta vezivanja insulina sugeriše strukturne i promene konformacije receptora što moze uzrokovati razlike u postreceptorskom odgovoru starijih osoba na delovanje insulina.

Ključne reči: intolerancija glikoze, insulin, receptori, vezujuća mesta, starije osobe

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