Age-Related Changes in Proinsulin Processing in Normoglycemic Individuals

Markéta VAŇKOVÁ[1], Daniela VEJRAŽKOVÁ[1], Petra LUKÁŠOVÁ[1], Josef VČELÁK[1], Denisa CHOCHOLOVÁ[2], Běla BENDLOVÁ[1]

[1]Institute of Endocrinology, Prague, Czech Republic, Faculty of Science, Charles University, Prague, Czech Republic

Received June 26, 2023
Accepted July 3, 2023

Summary

In order to understand the pathological changes associated with glucose homeostasis in old age, it is necessary to know the natural changes in the processing of proinsulin to mature insulin. While there is abundant information about insulin production and function in diabetics, the situation in healthy adults and the elderly has surprisingly rarely been investigated. The aim of the study was to determine how proinsulin secretion changes in individuals with normal glucose tolerance during the process of natural aging. A total of 761 individuals (539 women, 222 men) aged 18-90 years with normal fasting glycemia (less than 5.6 mmol/l) were divided into five groups according to age. Body composition and levels of fasting blood glucose, proinsulin, insulin, and C-peptide were determined, and the ratios of proinsulin to both insulin and C-peptide were calculated. The homeostasis model of β-cell function (HOMA F) and peripheral insulin resistance (HOMA R) were calculated. The effect of age was assessed using an ANOVA model consisting of the factors sex, age, and sex × age interaction. Statgraphics Centurion v. XVIII statistical software was used. Glycemia, insulin, C-peptide and HOMA R increased in both sexes up to 75 years. On the contrary, proinsulin levels as well as proinsulin/insulin and proinsulin/C-peptide ratios decreased with age up to 75 years. In normoglycemic and normotolerant people, both women and men, the aging process is associated with decreased insulin sensitivity compensated by potentiation of insulin production. In older age, there is also a gradual decrease in circulating proinsulin, which can be explained by its more efficient processing into active insulin by matured healthy beta cells.

Keywords

Proinsulin • Insulin • Age • Glucose tolerance • Type 2 diabetes mellitus

Corresponding author

Markéta Vaňková, Department of Molecular Endocrinology, Institute of Endocrinology, Narodni 8, 110 00 Prague 1, Czech Republic. E-mail: mvankova@endo.cz

Introduction

The insulin precursor protein proinsulin is initiated as preproinsulin, typically in the cytosol of pancreatic beta cells, although in early embryonic stages, still pre-pancreatic transcription of proinsulin mRNA has been proven, with a major impact on the development of the nervous system (reviewed by [1,2]). One beta cell of a healthy person can synthesize cca 6000 preproinsulin molecules per second [3]. Each molecule of preproinsulin is comprised of insulin A-chain, insulin B-chain, the area connecting the two chains named the connecting peptide or, for short, C-peptide, and a signal peptide. With the help of a signal peptide, preproinsulin is guided to the endoplasmic reticulum, where proinsulin folding takes place. Errors in this complex chaperone- and oxidoreductase-assisted process may contribute to deficient insulin production and type 2 diabetes mellitus (T2DM), as highlighted in discovery of several diabetogenic preproinsulin signal peptide mutations [4-8].

Proinsulin must be folded to become exportable from the endoplasmic reticulum and to form insulin. Human proinsulin is made up of 86 residues formed into the three chains (A-chain, B-chain, and C-peptide) that are stabilized by three disulfide bonds. Two of these disulfide bonds are between the A- and B-chains, and one is an intra-A-chain bond. The correct structure of

proinsulin is crucial for the correct folding of mature insulin. In humans, proinsulin is encoded by the *INS* gene and its misfolding underlies for example MIDY type of diabetes (Mutant *INS*-gene-induced Diabetes of Youth), which is characterized by insulin-deficient diabetes mellitus that commonly begins in the neonatal period. Although these patients do not have insulin autoantibodies, the disease has often been labeled as type 1 diabetes mellitus [9]. A correct genetic diagnosis in these non-autoimmune cases of diabetes can fundamentally determine treatment and thus clinical outcome. Folded proinsulin is transported to the Golgi apparatus where it is processed by a series of proteases. At least 99 % of proinsulin is converted to insulin *via* proteolytic cleavages by the proprotein convertases. The C-peptide is abstracted from the molecule and the A-chain and the B-chain remain connected by two disulfide bonds forming mature insulin. This hormone is stored in secretory granules, which can readily fuse with the plasma membrane upon nutrient stimulation and release their contents into the portal circulation. Within a few minutes following a meal, increase in glucose levels forces beta cells to secrete some of their insulin (the secreted content is, however, relatively small, usually not exceeding 5 %), with preferential secretion of newly synthesized insulin [10]. The hormone is then distributed to target cells, primarily including hepatocytes, skeletal muscle cells and adipocytes. Every beta cell can release over 3000 insulin molecules per second. Hyperglycemia thus concurrently with insulin secretion stimulates also *de novo* insulin synthesis to replenish insulin stores in beta cells and to maintain their maximal secretory capacity.

Ageing as a risk factor for T2DM

Glucose intolerance and T2DM incidence typically increase with age. Among people aged 65 years or older, the prevalence of T2DM is as high as 29.2 % [11]. Diabetes develops as a consequence of functional decline in insulin secretion [12,13], which can be attributed to age-dependent increase of beta cell endoplasmic reticulum stress and subsequent morphologic alteration in its structure [14]. This adversely affects the function of the reticulum and thus the folding of proinsulin and subsequent secretion of functional insulin [15]. In addition to structural and functional changes in the endoplasmic reticulum, another factor contributes to the decline in beta cell function. With advancing age, all people develop some degree of insulin resistance [16], glucose levels gradually increase and a sustained increase in glycemia is a significant contributor to the proinsulin misfolding. At higher glucose levels, newly synthesized proinsulin forms protein complexes that interfere with proper proinsulin folding [17]. Decreased export of proinsulin limits insulin production, which leads to higher blood glucose. Thus, a vicious circle is spinning that promotes impaired glucose tolerance and T2DM.

Markers of deteriorating beta cell activity

The circulating proinsulin to C-peptide as well as proinsulin to insulin ratios serve as a markers of endoplasmic reticulum stress with the consequence of aberrant proinsulin processing [18,19]. Elevated blood proinsulin levels are considered a sign of deteriorating beta cell activity, when these cells produce large amounts of proinsulin, but due to age-dependent damage are unable to properly fold it into active insulin. Although more sophisticated indices based on dynamic models of glucose processing were calculated [20], their results correlated strongly with basal proinsulin to insulin ratio, confirming the usefulness and sufficient informative value of this index. Fasting proinsulin proved to be a very good indicator of glucose intolerance with increasing levels towards prediabetic and further towards diabetic states [21]. In healthy people, circulating proinsulin accounts for approximately 15 % of circulating insulin, but its concentrations can be as high as 50 % of circulating insulin in type 2 diabetics [22]. The common dysfunctional beta cell in T2DM is characterized by diminished glucose sensing, blunted first-phase insulin secretion, and increased proinsulin to insulin ratio [23]. Similarly, a large subset of individuals with longstanding type 1 diabetes mellitus (T1DM) revealed increased proinsulin-enriched but insulin-poor cells in their islets compared to nondiabetic controls. Persistent proinsulin secretion has also been observed in type 1 diabetics who no longer secrete any C-peptide *et al.* [24]. These observations suggest that, provided the etiology of the disease is well understood, agents targeting proinsulin processing may have therapeutic benefit in some types of diabetes.

Study aims

Evaluation of proinsulin secretion as a function of age was extensively studied in rodent models and in people with impaired glucose tolerance or T2DM [25,26]. In healthy normoglycemic adults it remains a fertile area

for research. Our aim was to study the effect of age on the concentration of basal proinsulin in the circulation in people who have been confirmed to have normal glucose concentration and tolerance even at an older age. We also intended to answer the question of whether there is any difference between men and women in this respect.

Methods

Subjects

A total of 761 individuals (539 women, 222 men) with normal fasting glycemia (less than 5.6 mmol/l) were examined between 1999 and 2023 at the Institute of Endocrinology in Prague as part of research projects for which the volunteers provided written informed consent. All the projects were approved by the constitutional ethics committee. Participants were divided into groups according to age: 1) 18-29.9 years 355 persons, 2) 30-44.9 years 234 persons, 3) 45-59.9 years 103 persons, 4) 60-74.9 years 58 persons, 5) 75-90 years 11 persons. Of this cohort, normal glucose tolerance in 120th min (lower than 7.8 mmol/l) has been verified in 699 (92 %) volunteers by an oral glucose tolerance test (OGTT) with 75g of glucose. In the remaining 62 (8 %) subjects, glucose tolerance was assessed from fasting glucose only either due to advanced age, so the burden of a glucose test would not be appropriate, or, in a few rare cases, due to difficulties with cannulation.

Anthropometric characterization

Body weight, height and waist- and hip-circumferences were measured in order to calculate the body mass index (BMI) and body adiposity index (BAI). BAI, a surrogate measure of body fat, was calculated as described elsewhere [27].

Biochemical characterization

For the evaluation of biochemical parameters such as glucose, proinsulin, insulin, and C-peptide, the peripheral blood was withdrawn on fasting in the morning. Blood samples were centrifuged and immediately processed or, in case of proinsulin, stored at -20 °C until analyzed. Blood glucose levels were measured by an enzymatic reference method with hexokinase, insulin, and C-peptide by ECLIA (Cobas 6000, Roche Diagnostics, Mannheim, Germany). Proinsulin levels were measured by ELISA (DRG Proinsulin ELISA, EIA-1560, Marburg, Germany) throughout the entire study. The ratios of insulin to C-peptide and proinsulin to both insulin and C-peptide were calculated. To assess insulin secretion and peripheral insulin sensitivity, the homeostasis model of β-cell function (HOMA F) and peripheral insulin resistance (HOMA R), resp., were calculated. The conversion factor between conventional units and Système International (SI) units for insulin was used: 1 mIU/l = 6.00 pmol/l [28].

Statistical analysis

For each metric variable, the parameters of the power transformation were found to make its distribution as close to a Gaussian distribution as possible. Due to the gender dependence of many of the variables of interest, data were assessed not only together but also separately for each gender and were evaluated using an ANOVA model followed by Bonferroni multiple comparisons. ANOVA assessed the effect of sex (A), age category (B), and the interaction between sex and age category (AxB). Bonferroni multiple comparisons assessed differences between combinations of all groups ($p<0.05$), with significance for those parameters for which 95 % confidence intervals did not overlap. Statgraphics Centurion v. XVIII statistical software from Statgraphics Technologies, Inc (The Plains, Virginia, USA) was used for the above analyses.

Results

Proinsulin processing was monitored in the wider metabolic landscape of related metabolites and derived indices of beta cell and insulin function. Table 1 shows monitored anthropometric and biochemical parameters in individual age categories evaluated together for both sexes and also separately for women and men. Differences in these parameters between individual age categories again processed both together and separately for women and men are demonstrated in Figures 1-3.

The effect of gender

Gender significantly affects body composition and fasting blood glucose levels.

The BMI is higher in men than in women up to the age category of 45-59.9 years. Over age 60, the BMI is comparable in men and women (Fig. 1a). Women have higher percentage of total body fat compared to men in all age groups (Fig. 1b).

Concerning glucose metabolism, men have

Table 1. Effects of age category and sex on anthropometric and biochemical parameters in normoglycemic men and women, as evaluated by the two-way ANOVA model.

Parameter	Sex	Age category					Factor	Factor	Interaction
		18-29.9 years	30-44.9 years	45-59.9 years	60-74.9 years	>75 years	Sex (A)	Age (B)	A×B
Number (women/men)		355 (258/97)	234 (163/71)	103 (67/36)	58 (44/14)	11 (7/4)			
BMI (kg/m^2)	All	22.8 (22.5, 23.1)	24.3 (23.9, 24.7)	25.9 (25.3, 26.5)	26.5 (25.6, 27.5)	24.6 (23, 26.4)			
	Women	22.3 (22.1, 22.6)	23.7 (23.4, 24.1)	24.7 (24.1, 25.4)	26.6 (25.6, 27.6)	25.1 (23.1, 27.4)	0.238	**p<0.001**	0.485
	Men	23.3 (22.8, 23.8)	24.9 (24.3, 25.6)	27.2 (26.1, 28.4)	26.4 (24.9, 28.3)	24.1 (21.8, 27.1)			
BAI (%)	All	24.3 (24.1, 24.6)	25.7 (25.4, 26.1)	27.5 (26.9, 28)	27.9 (27.1, 28.7)	27.9 (26.3, 29.6)			
	Women	26.9 (26.6, 27.2)	28.5 (28.1, 29)	29.8 (29.1, 30.5)	32 (31, 32.9)	32.4 (30.1, 34.9)	**p<0.001**	**p<0.001**	0.270
	Men	22 (21.7, 22.4)	23.3 (22.8, 23.8)	25.3 (24.6, 26.1)	24.5 (23.4, 25.7)	24.3 (22.2, 26.5)			
Glucose (mmol/l)	All	4.6 (4.57, 4.63)	4.8 (4.77, 4.84)	4.95 (4.9, 5)	5.04 (4.96, 5.11)	5.16 (5.02, 5.31)			
	Women	4.54 (4.51, 4.58)	4.75 (4.71, 4.79)	4.87 (4.81, 4.93)	5.01 (4.94, 5.08)	5.05 (4.87, 5.22)	**0.011**	**p<0.001**	0.915
	Men	4.66 (4.61, 4.71)	4.86 (4.8, 4.92)	5.03 (4.95, 5.11)	5.07 (4.94, 5.2)	5.28 (5.05, 5.5)			
Proinsulin (pmol/l)	All	3.51 (3.28, 3.76)	2.73 (2.49, 2.97)	2.78 (2.44, 3.14)	2.05 (1.65, 2.5)	1.97 (1.24, 2.94)			
	Women	3.67 (3.42, 3.93)	2.54 (2.29, 2.8)	2.49 (2.13, 2.9)	2.35 (1.92, 2.85)	3.15 (1.95, 4.77)	0.173	**p<0.001**	0.137
	Men	3.36 (2.98, 3.78)	2.93 (2.53, 3.36)	3.08 (2.51, 3.73)	1.77 (1.18, 2.53)	1.12 (0.402, 2.34)			
Insulin (pmol/l)	All	33.2 (31.9, 34.6)	38.1 (36.2, 40.1)	39.9 (37.1, 43)	45 (40.3, 50.2)	33.6 (27.1, 41.7)			
	Women	36 (34.5, 37.7)	37.5 (35.5, 39.6)	35 (32.2, 38.2)	50.7 (45.5, 56.7)	36.2 (28, 47.3)	0.501	**p<0.001**	**0.004**
	Men	30.7 (28.6, 32.9)	38.7 (35.5, 42.3)	45.7 (40.6, 51.5)	40 (33.2, 48.4)	31.1 (22.3, 44)			
C-peptide (pmol/l)	All	535 (522, 549)	561 (544, 579)	656 (625, 690)	669 (622, 722)	570 (498, 658)			
	Women	561 (546, 576)	564 (545, 584)	575 (545, 607)	723 (671, 781)	622 (526, 748)	0.509	**p<0.001**	**p<0.001**
	Men	511 (490, 534)	559 (530, 589)	757 (696, 828)	622 (551, 707)	524 (428, 656)			
HOMA R	All	1.12 (1.08, 1.17)	1.35 (1.28, 1.42)	1.44 (1.34, 1.56)	1.64 (1.46, 1.85)	1.28 (1.02, 1.61)			
	Women	1.21 (1.15, 1.26)	1.3 (1.23, 1.38)	1.25 (1.14, 1.37)	1.86 (1.66, 2.1)	1.35 (1.03, 1.8)	0.712	**p<0.001**	**0.006**
	Men	1.05 (0.973, 1.12)	1.39 (1.27, 1.53)	1.67 (1.47, 1.9)	1.45 (1.19, 1.78)	1.21 (0.85, 1.76)			
HOMA F	All	107 (103, 112)	98.9 (93.8, 104)	95.3 (88.3, 103)	108 (96.8, 121)	68.6 (55, 85.6)			
	Women	126 (120, 132)	107 (101, 113)	90 (82.3, 98.4)	116 (104, 130)	79 (60.5, 104)	**0.047**	**0.024**	**0.020**
	Men	91.9 (85.4, 99)	91.6 (83.9, 100)	101 (89.3, 114)	101 (83.1, 123)	59.6 (41.9, 84.8)			
(Proinsulin/Insulin)*1000	All	102 (94, 110)	70.4 (63.5, 77.8)	63.5 (54.7, 73.5)	43.4 (33.8, 54.8)	54.2 (33.1, 84.1)			
	Women	99.4 (91.6, 108)	65.3 (58.2, 72.9)	65.7 (55, 78)	44.9 (35.2, 56.3)	81.8 (47.7, 132)	0.326	**p<0.001**	0.597
	Men	104 (90.9, 118)	75.8 (63.9, 89.4)	61.4 (48, 774)	41.9 (26.7, 62.6)	34.3 (13, 72.9)			
(Proinsulin/C-peptide)*1000	All	6.27 (5.85,6.7)	4.54 (4.15, 4.96)	3.91 (3.41, 4.46)	2.91 (2.32, 3.59)	3.31 (2.1, 4.95)			
	Women	6.24 (5.81, 6.69)	4.34 (3.93, 4.79)	4.09 (3.49, 4.76)	3.16 (2.55, 3.86)	5.15 (3.18, 7.87)	0.139	**p<0.001**	0.502
	Men	6.29 (5.6, 7.05)	4.75 (4.09, 5.48)	3.73 (2.98, 4.61)	2.68 (1.77, 3.86)	1.99 (0.76, 4.05)			

Data are presented as transformed means with 95% confidence intervals. "p" is the statistical significance level for the sex factor (A), the age category factor (B), and their interaction (AxB).

higher fasting blood glucose levels than women up to the age group of 45-59.9 years. In older people, the blood glucose levels are already similar in both sexes (Fig. 2a). As for proinsulin, insulin, C-peptide and the derived proinsulin/insulin and proinsulin/C peptide ratios, gender factor does not show a significant effect, as well as in the insulin resistance assessed by HOMA R. According to HOMA F index, women of reproductive age have slightly higher beta cell function than men, the difference is significant in age categories up to 44.9 years. Above the age of 45, HOMA F values for men and women are already comparable (Fig. 3b).

The effect of age

Age influences all of the monitored parameters, as can be seen in Table 1. The values of anthropometric measures (BMI, BAI) increase with age. In women, the percentage of body fat increases continuously across age categories, while in men it reaches its maximum in the age category 45-59.9 years and does not increase further (Fig. 1b). Regardless of gender, glycemia, insulin, C-peptide and insulin resistance index HOMA R increase up to 75 years (Figs 2,3a). On the contrary, proinsulin levels as well as values of proinsulin/insulin and proinsulin/C-peptide ratios decrease, see Figure 2 b) and Figure 3. In the group of normoglycemic people over 75 years of age, which, however, consists of only 11 people, the concentrations of insulin and C peptide decrease, as well as HOMA R and HOMA F indices. However, the decrease in the oldest age category is not evident in glycemia, proinsulin, proinsulin/insulin and proinsulin/C peptide ratios (Figs 2, 3).

Interaction of gender and age

During the aging process, men and women show significant differences in insulin and C-peptide levels, as well as in derived homeostatic models HOMA R and HOMA F values (Figs 2, 3). In women, insulin, C-peptide and HOMA R index of insulin resistance are almost equal in the age period between 18 and 59.9 years and then, in the age category 60-74.9 years, their levels rise significantly. In men, there is a gradual increase in insulin, C-peptide and HOMA R with the maximum levels in the age category of 45-59.9 years, i.e. much earlier than it occurs in women. In the oldest age category over 75 years, both in men and women, insulin and

C-peptide levels are falling. There is also a noticeable decrease in beta cell function index HOMA F in this oldest age category in both sexes, although it should be taken into account that it is represented by only 11 persons, 7 women and 4 men. For women, in contrast to men, this decline is preceded by a temporary rise of beta cell function in the age category 60-74.9 years (Fig. 3b). As already mentioned, a remarkable difference between the sexes in the highest age category is observed in the concentration of proinsulin, which increases in women and decreases in men (Fig. 2b). This phenomenon is also reflected in the values of the proinsulin/insulin and proinsulin/C-peptide indices (Fig. 3c,d), however, a larger cohort of normoglycemic seniors is needed to confirm this observation.

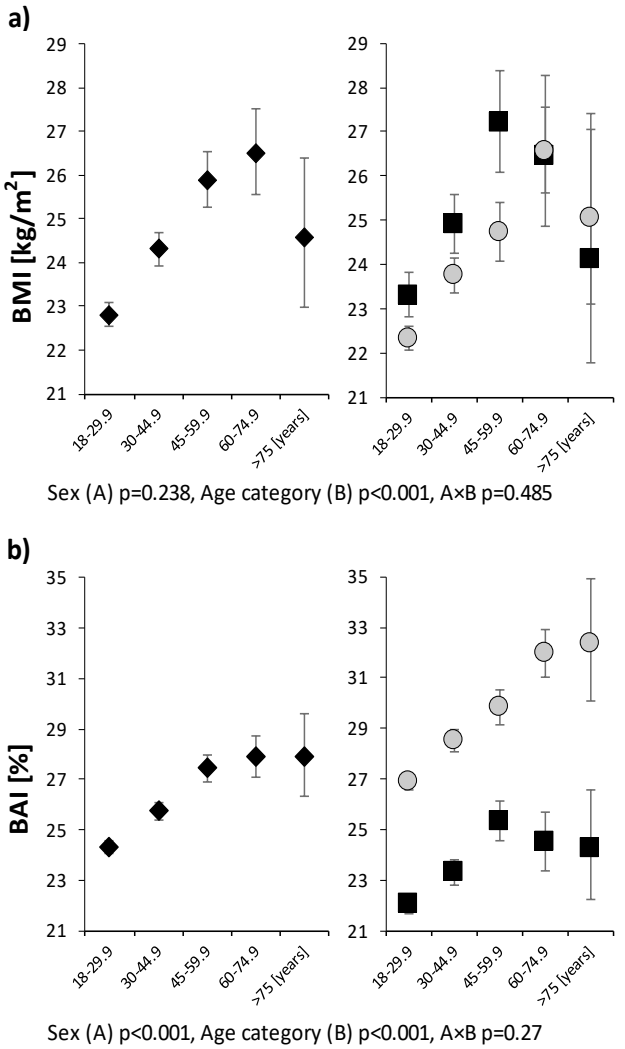

Fig. 1. Dependence of anthropometric parameters on age categories processed both together and separately for women and for men: **a**) body mass index (BMI), **b**) body adiposity index (BAI). ◆ both sexes, ■ men, ○ women

Discussion

In normoglycemic adults, we have confirmed that fasting glycemia levels increase slightly with increasing age, which is documented by scientific findings [16] and it is in line with the daily routine practice of physicians. In parallel, the concentration of proinsulin in the circulation generally slightly decreases, with the differences being particularly pronounced when comparing very young participants under 30 years of age with those over 60 years of age. As for active fasting insulin, its serum concentration tends to increase with age in our participants, a gradual increase can be observed up to 75 years of age, with slight decreasee thereafter. This observation of a combination of an increase in circulating insulin with a decrease in circulating proinsulin can be explained by more efficient processing and utilization of already formed proinsulin into functional insulin by older and functionally mature healthy beta cells, so that a smaller amount of released proinsulin can be detected in the circulation. This interpretation is based on the findings of several studies summarized in the review article evaluating the effect of aging on the function of both rodent and human pancreatic beta cells, although these excellent studies were mostly performed on tissue cultures. They reveal that beta cells from healthy old mice and humans secrete more insulin under fasting conditions as well as in response to glucose stimulus in comparison with young beta cells [29]. In this context, beta cell senescence emerges as a functionally beneficial epigenetically programmed process of age-related maturation process improving the function of differentiated beta cells. Our observation is also in accordance with cross-sectional analysis of beta cell function in healthy humans showing increased insulin secretion in old subjects, under both basal and stimulated conditions [30]. Also, our data harmonize well with a longitudinal study of 4000 adults showing that people who do not develop impaired glucose tolerance compensate for the gradual rise of insulin resistance by increasing insulin secretion in response to glucose challenge. This is exactly what we have observed in our participants, although exclusively under fasting conditions: up to 75 years of age, an increase of fasting insulin is linked with an increase of HOMA R index assessing insulin resistance. However, HOMA F index evaluating beta cell function did not rise systematically with age in our cohort. As for C-peptide and its changes in the elderly, it behaves quite identically to insulin,

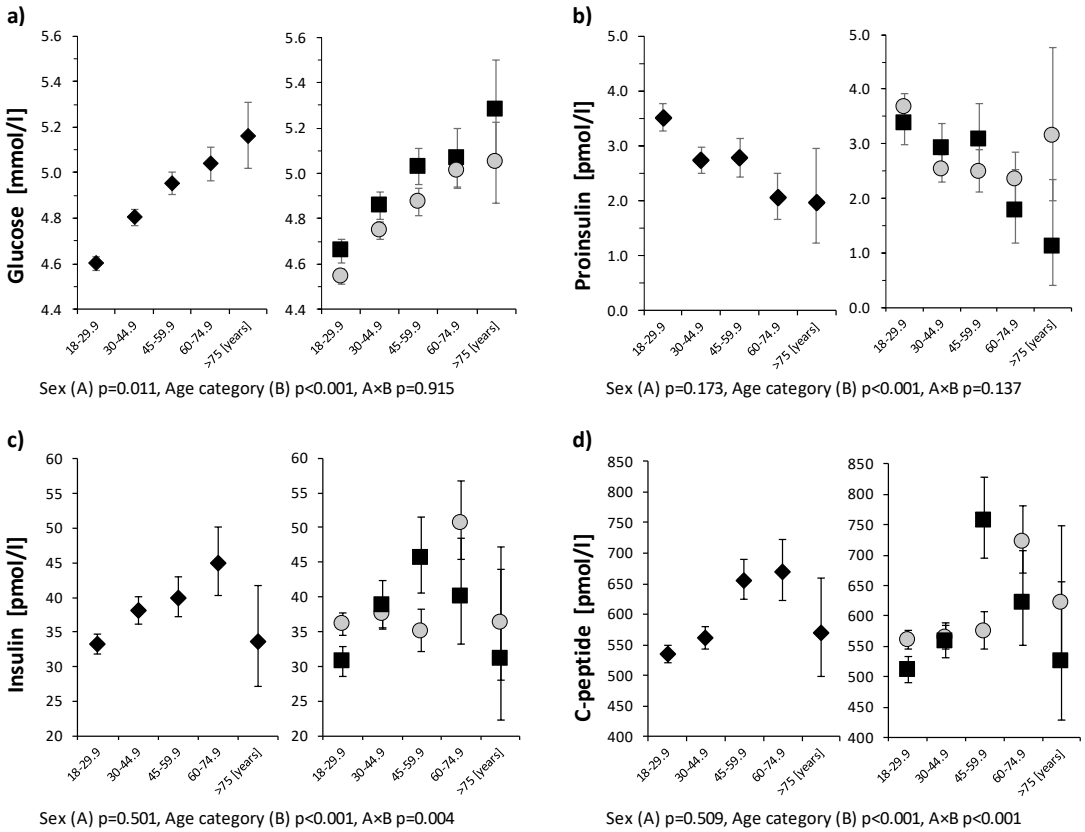

Fig. 2. Dependence of metabolic parameters on age categories processed both together and separately for women and for men: **a**) Glucose, **b**) Proinsulin, **c**) Insulin, **d**) C-peptide. ◆ both sexes, ■ men, ○ women

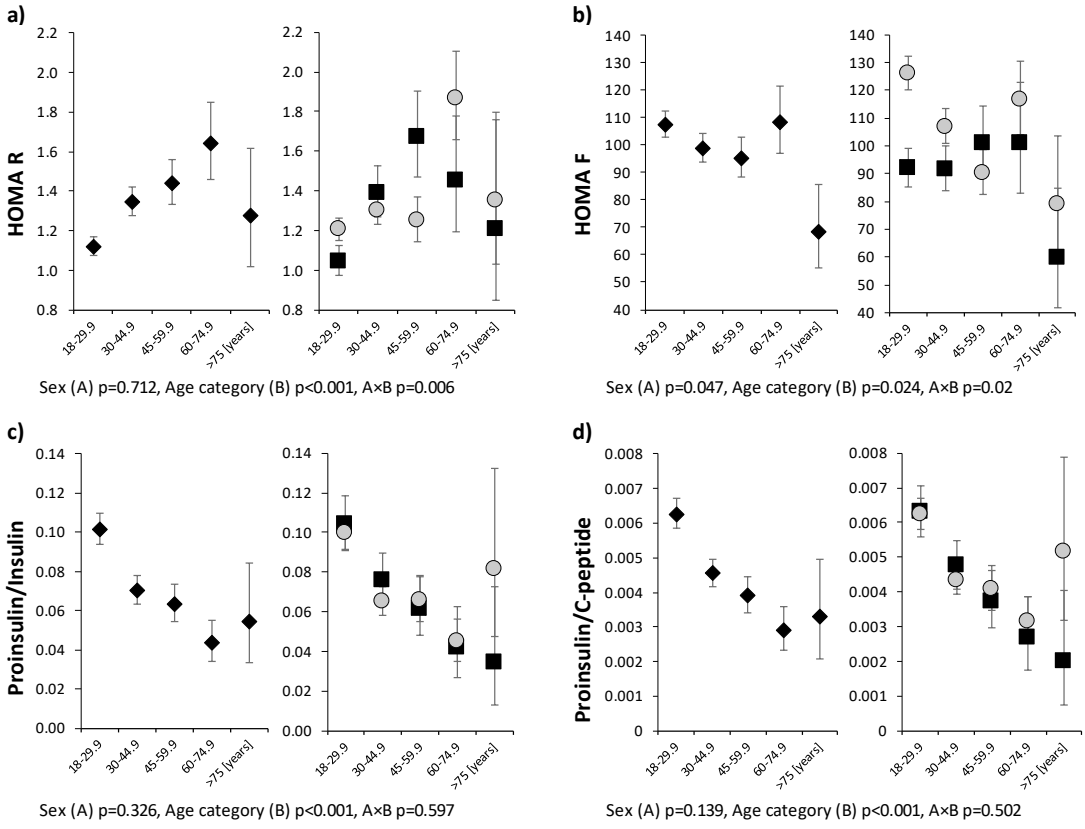

Fig. 3. Dependence of metabolic parameters on age categories processed both together and separately for women and for men: **a**) insulin resistance index HOMA R, **b**) beta cell function index HOMA F, **c**) Proinsulin/Insulin ratio, **d**) Proinsulin/C-peptide ratio. ◆ both sexes, ■ men, ○ women

which is very clearly visible in Figure 2 c) and d). As a result, both the proinsulin to insulin and proinsulin to C-peptide indices almost identically gradually decline with increasing age, with the exception of the oldest group over 75 years, where stagnation is evident. Thus, based on our data, it can be concluded that the indicative value of both the evaluated indices are very comparable and interchangeable.

Conversely, in young people, high levels of proinsulin may reflect the imperfect efficiency of conversion of proinsulin to active insulin by healthy but functionally immature beta cells, so more of it is released into the circulation. But also another involved circumstance comes into consideration. The proinsulin binding receptors are present in the nervous system and it has been proven that proinsulin promotes neural proliferation and differentiation [1], supports the development and maturation of the nervous system and has a neuroprotective effect, both prenatally and postnatally [2]. Therefore, if we consider that brain maturation continues until the age of 35 [31], higher levels of circulating proinsulin in young people may indicate its persistent supportive effect during the nervous system development and maturation. In the following decades, proinsulin exerts its neuroprotective function, which, however, weakens with increasing age, opening the way to the manifestation of neurodegenerative diseases.

The novelty of our study lies in a detailed comparison of intersex differences, even distributed into age categories. To the best of our knowledge, there is only one study that has looked at differences between women and men in terms of proinsulin concentrations and its changes with age [32]. In this work, a group of 224 individuals (87 men and 137 women) was formed into age quartiles and proinsulin has been shown to increase with age with subsequent drop in people over 67 years. However, the study differed in design significantly, as people with impaired glucose tolerance were included. For our purpose, we consider it necessary to carefully select an exclusively normoglycemic cohort, since we are trying to understand the involvement of proinsulin in the physiology of a healthy, albeit aging, organism. Therefore, we have verified glucose tolerance in the vast majority of participants with a glucose tolerance test. In 8 % of subjects for whom the test was not feasible (the reasons have been previously explained, see methods section), fasting blood glucose was assessed and only participants with a value <5.6 mmol/l were included. However, according to our previous analysis based on OGTT results of 1098 people, impaired glucose tolerance detected exclusively on the basis of the 120th min with concurrently normal fasting blood glucose was detected in only 4 % of individuals. Thus, our approach does not introduce substantial bias into the results.

We consider the lower proportion of men compared to women to be a disadvantage of the study. The reason lies in the lower willingness of men to participate in the research project, while women readily welcomed the opportunity to check whether their glucose regulation was in good condition. Another weakness of the study can be seen in the lower representation of the oldest age category. This is mainly caused by the fact that perfectly healthy glucose tolerance is relatively rare in older people over 70 years of age.

In conclusion, our data demonstrate that increasing age is accompanied by a slight decrease in insulin sensitivity, which, however, is compensated by a potentiation of insulin production in healthy normoglycemic and normotolerant people. The aging process of normoglycemic subjects is further associated with a slow persistent decline in circulating proinsulin levels, both in women and men, which can be explained by its more efficient processing into active insulin by matured healthy beta cells. Gradual age-dependent decrease of the proinsulin/insulin and proinsulin/ C-peptide indices is consistent with this assumption. In the following years, we plan to verify described age-dependent dynamics of proinsulin and associated glycoregulatory hormones on data collected during a 20-year-long longitudinal study, when changes in the described parameters were monitored during the aging process of the involved volunteers.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Supported by Ministry of Health of the Czech Republic, grants NU20-01-00308, NV18-01-00399 and RVO 00023761.

References

1. de la Rosa EJ, de Pablo F. Proinsulin: from hormonal precursor to neuroprotective factor. Front Mol Neurosci 2011;4:20. https://doi.org/10.3389/fnmol.2011.00020
2. de Pablo F, Hernandez-Sanchez C, de la Rosa EJ. The Prohormone Proinsulin as a Neuroprotective Factor: Past History and Future Prospects. Front Mol Neurosci 2018;11:426. https://doi.org/10.3389/fnmol.2018.00426
3. Schuit FC, Kiekens R, Pipeleers DG. Measuring the balance between insulin synthesis and insulin release. Biochem Biophys Res Commun 1991;178:1182-1187. https://doi.org/10.1016/0006-291X(91)91017-7
4. Guo H, Xiong Y, Witkowski P, Cui J, Wang LJ, Sun J, et al. Inefficient translocation of preproinsulin contributes to pancreatic beta cell failure and late-onset diabetes. J Biol Chem 2014;289:16290-16302. https://doi.org/10.1074/jbc.M114.562355
5. Liu M, Hodish I, Haataja L, Lara-Lemus R, Rajpal G, Wright J, et al. Proinsulin misfolding and diabetes: mutant INS gene-induced diabetes of youth. Trends Endocrinol Metab 2010;21:652-659. https://doi.org/10.1016/j.tem.2010.07.001
6. Liu M, Lara-Lemus R, Shan SO, Wright J, Haataja L, Barbetti F, et al. Impaired cleavage of preproinsulin signal peptide linked to autosomal-dominant diabetes. Diabetes 2012;61:828-837. https://doi.org/10.2337/db11-0878
7. Liu M, Sun J, Cui J, Chen W, Guo H, Barbetti F, et al. INS-gene mutations: from genetics and beta cell biology to clinical disease. Mol Aspects Med 2015;42:3-18. https://doi.org/10.1016/j.mam.2014.12.001
8. Yan J, Jiang F, Zhang R, Xu T, Zhou Z, Ren W, et al. Whole-exome sequencing identifies a novel INS mutation causative of maturity-onset diabetes of the young 10. J Mol Cell Biol 2017;9:376-383. https://doi.org/10.1093/jmcb/mjx039
9. Stoy J, Steiner DF, Park SY, Ye H, Philipson LH, Bell GI. Clinical and molecular genetics of neonatal diabetes due to mutations in the insulin gene. Rev Endocr Metab Disord 2010;11:205-215. https://doi.org/10.1007/s11154-010-9151-3
10. Vasiljevic J, Torkko JM, Knoch KP, Solimena M. The making of insulin in health and disease. Diabetologia 2020;63:1981-1989. https://doi.org/10.1007/s00125-020-05192-7
11. American Diabetes Association. 2022, Accesed 22 June 2023. https://diabetes.org/about-us/statistics/about-diabetes
12. Muzumdar R, Ma X, Atzmon G, Vuguin P, Yang X, Barzilai N. Decrease in glucose-stimulated insulin secretion with aging is independent of insulin action. Diabetes 2004;53:441-446. https://doi.org/10.2337/diabetes.53.2.441
13. Szoke E, Shrayyef MZ, Messing S, Woerle HJ, van Haeften TW, Meyer C, et al. Effect of aging on glucose homeostasis: accelerated deterioration of beta-cell function in individuals with impaired glucose tolerance. Diabetes Care 2008;31:539-543. https://doi.org/10.2337/dc07-1443
14. Cechin SR, Lopez-Ocejo O, Karpinsky-Semper D, Buchwald P. Biphasic decline of beta-cell function with age in euglycemic nonobese diabetic mice parallels diabetes onset. IUBMB Life 2015;67:634-644. https://doi.org/10.1002/iub.1391
15. Yoshino H, Kawakami K, Yoshino G, Hirose T. Age-related changes of proinsulin processing in diabetic and non-diabetic Japanese individuals. Geriatr Gerontol Int 2018;18:1046-1050. https://doi.org/10.1111/ggi.13303
16. Ohn JH, Kwak SH, Cho YM, Lim S, Jang HC, Park KS, et al. 10-year trajectory of beta-cell function and insulin sensitivity in the development of type 2 diabetes: a community-based prospective cohort study. Lancet Diabetes Endocrinol 2016;4:27-34. https://doi.org/10.1016/S2213-8587(15)00336-8
17. Wang J, Chen Y, Yuan Q, Tang W, Zhang X, Osei K. Control of precursor maturation and disposal is an early regulative mechanism in the normal insulin production of pancreatic beta-cells. PLoS One 2011;6:e19446. https://doi.org/10.1371/journal.pone.0019446
18. Freese J, Al-Rawi R, Choat H, Martin A, Lunsford A, Tse H, et al. Proinsulin to C-peptide ratio in the first year after diagnosis of type 1 diabetes. J Clin Endocrinol Metab 2021;106:e4318-e4326. https://doi.org/10.1210/clinem/dgab463
19. Sims EK, Chaudhry Z, Watkins R, Syed F, Blum J, Ouyang F, et al. Elevations in the fasting serum proinsulin-to-c-peptide ratio precede the onset of Type 1 Diabetes. Diabetes Care 2016;39:1519-1526. https://doi.org/10.2337/dc15-2849

20. Tura A, Pacini G, Kautzky-Willer A, Ludvik B, Prager R, Thomaseth K. Basal and dynamic proinsulin-insulin relationship to assess beta-cell function during OGTT in metabolic disorders. Am J Physiol Endocrinol Metab 2003;285:E155-162. https://doi.org/10.1152/ajpendo.00104.2002
21. Nakamura A, Miyoshi H, Ukawa S, Nakamura K, Nakagawa T, Terauchi Y, et al. Proinsulin is sensitive to reflect glucose intolerance. J Diabetes Investig 2020;11:75-79. https://doi.org/10.1111/jdi.13106
22. Ward WK, LaCava EC, Paquette TL, Beard JC, Wallum BJ, Porte D, Jr. Disproportionate elevation of immunoreactive proinsulin in type 2 (non-insulin-dependent) diabetes mellitus and in experimental insulin resistance. Diabetologia 1987;30:698-702. https://doi.org/10.1007/BF00296991
23. Halban PA, Polonsky KS, Bowden DW, Hawkins MA, Ling C, Mather KJ, et al. beta-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. J Clin Endocrinol Metab 2014;99:1983-1992. https://doi.org/10.1210/jc.2014-1425
24. Sims EK, Syed F, Nyalwidhe J, Bahnson HT, Haataja L, Speake C, et al. Abnormalities in proinsulin processing in islets from individuals with longstanding T1D. Transl Res 2019;213:90-99. https://doi.org/10.1016/j.trsl.2019.08.001
25. Alarcon C, Boland BB, Uchizono Y, Moore PC, Peterson B, Rajan S, et al. Pancreatic beta-cell adaptive plasticity in obesity increases insulin production but adversely affects secretory function. Diabetes 2016;65:438-450. https://doi.org/10.2337/db15-0792
26. Liu M, Weiss MA, Arunagiri A, Yong J, Rege N, Sun J, et al. Biosynthesis, structure, and folding of the insulin precursor protein. Diabetes Obes Metab 2018;20 Suppl 2:28-50. https://doi.org/10.1111/dom.13378
27. Bergman RN, Stefanovski D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, et al. A better index of body adiposity. Obesity (Silver Spring) 2011;19:1083-1089. https://doi.org/10.1038/oby.2011.38
28. Knopp JL, Holder-Pearson L, Chase JG. Insulin Units and Conversion Factors: A Story of Truth, Boots, and Faster Half-Truths. J Diabetes Sci Technol 2019;13:597-600. https://doi.org/10.1177/1932296818805074
29. Helman A, Avrahami D, Klochendler A, Glaser B, Kaestner KH, Ben-Porath I, et al. Effects of ageing and senescence on pancreatic beta-cell function. Diabetes Obes Metab 2016;18 Suppl 1:58-62. https://doi.org/10.1111/dom.12719
30. Gumbiner B, Polonsky KS, Beltz WF, Wallace P, Brechtel G, Fink RI. Effects of aging on insulin secretion. Diabetes 1989;38:1549-1556. https://doi.org/10.2337/diabetes.38.12.1549, https://doi.org/10.2337/diab.38.12.1549
31. McIlvain G, Schneider JM, Matyi MA, McGarry MD, Qi Z, Spielberg JM, et al. Mapping brain mechanical property maturation from childhood to adulthood. Neuroimage 2022;263:119590. https://doi.org/10.1016/j.neuroimage.2022.119590
32. Ateia S, Rusu E, Cristescu V, Enache G, Cheta DM, Radulian G. Proinsulin and age in general population. J Med Life 2013;6:424-429.