Insulin resistance during growth hormone therapy in girls with Turner syndrome

Insulinooporność w czasie leczenia hormonem wzrostu u dziewcząt z zespołem Turnera

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ABSTRACT/STRESZCZENIE

Turner syndrome (TS) is a genetic disorder caused by aberrations of the X-chromosome in the form of monosomy, mosaicism, or other disorders, e.g. deletions, displacement, or rings. Insulin resistance is a state of reduced tissue sensitivity to insulin. The phenomenon involves reduced glucose uptake primarily by cells of fat and muscle tissues, despite normal or elevated concentrations of the hormone. Growth hormone has an antagonistic effect on insulin (pre-receptor mechanism) and impairs intracellular signal transmission (post-receptor mechanism). Since girls with Turner syndrome have short stature, exogenous growth hormone replacement therapy is applied in supra-physiological doses due to the reduced number of growth hormone receptors in patients with X chromosome monosomy. **Aim.** Assessment of insulin resistance estimated with the HOMA index prior to and after one-year and two-year treatment of girls with Turner syndrome with pharmacological doses of recombinant human growth hormone. **Patients and methods.** The study group consisted of 54 girls with TS verified by karyotyping. Before treatment with rHGH, after the one-year and two-year treatment, fasting blood glucose and insulin levels were re-assessed. The HOMA index was calculated based on the values obtained. **Results.** The glycaemia values did not differ significantly before and during the rHGH treatment. A significant decline in insulinaemia and the HOMA index was reported after 2 years. **Conclusions.** 1. Patients with Turner syndrome have higher pre-treatment levels of insulin resistance than healthy girls. (As result of comparison to the norm ranges in references) 2. Treatment with recombinant human growth hormone in patients with Turner syndrome does not exert an effect on the level of fasting glycaemia or glycaemia in OGTT. 3. Treatment with recombinant human growth hormone decreases insulin resistance in patients treated for 2 years. *Pediatr. Endocrinol.* 13/2014;3(48):17-24.

Zespół Turnera (TS) jest zaburzeniem genetycznym spowodowanym aberracjami chromosomu X w formie monosomii, mozaicyzmu lub innych zaburzeń takich jak delecja, przemieszczenie ramion lub chromosom X pierścieniowy. Oporność insulinowa jest stanem zmniejszonej wrażliwości tkanek na insulinę. To zjawisko wywołuje zmniejszenie zużycia glukozy w komórkach tłuszczowych i mięśniowych pomimo normalnego lub zwiększonego stężenia insuliny w surowicy krwi. Hormon wzrostu ma działanie antagonistyczne do insuliny (w mechanizmie przedreceptorowym)

oraz uszkadza transmisję sygnału insuliny wewnątrzkomórkowo (mechanizm postreceptorowy). U dziewczynek z zespołem Turnera ze względu na niskorosłość stosowana jest terapia hormonem wzrostu w dawkach ponadfizjologicznych, gdyż stwierdzono u nich zmniejszoną liczbę receptorów dla hormonu wzrostu. **Cel pracy.** Ocena insulinooporności mierzonej przy użyciu współczynnika HOMA u dziewczynek z TS przed leczeniem ludzkim rekombinowanym hormonem wzrostu, po roku i po dwóch latach leczenia. **Pacjenci i metody.** Grupa badana składała się z 54 dziewcząt z TS (stwierdzanym na podstawie kariotypu). Przed leczeniem, po roku i po dwóch latach leczenia oznaczano glukozę i insulinę na czczo i w teście OGTT. Na podstawie glikemii i insulinemii na czczo obliczano współczynnik HOMA. **Wyniki.** Wartości glikemii nie różniły się istotnie statystycznie przed i w trakcie leczenia rHGH. Obserwowano statystycznie istotny spadek insulinemii i współczynnika HOMA po 2 latach leczenia. **Wnioski.** 1. Pacjentki z TS przed leczeniem mają wyższą insulinooporność niż zdrowe dziewczynki. (Jest to wynik porównania do wartości referencyjnych z piśmiennictwa). 2. Leczenie rekombinowanym ludzkim hormonem wzrostu u pacjentek z TS nie wpływa na poziom glikemii na czczo ani na poziom glikemii w OGTT. 3. Leczenie rekombinowanym ludzkim hormonem wzrostu u pacjentek z TS obniża insulinooporność u pacjentek leczonych 2 lata. Endokrynol. Ped. 13/2014;3(48):17-24.

Introduction

Turner syndrome is a genetic disorder caused by aberrations of the X-chromosome in the form of monosomy, mosaicism, or other disorders, e.g. deletions, displacement, or rings. It is diagnosed in 1 out of 5000 girls. It is characterised by short stature, absence of oestrogen-dependent pubertal features, primary amenorrhea, sterility, and dysmorphism. Some cases may be accompanied by other defects of organs, primarily large blood vessels (coarctation of the aorta), heart (valvular disease), and kidneys [1].

Growth hormone (GH, somatotropin) is a polypeptide produced by eosinophilic cells of the anterior lobe of the pituitary gland. It is the major endocrine factor stimulating growth of the organism after the second year of life. GH increases protein synthesis, promotes lipolysis, and stimulates the growth of bone metaphysis. Growth hormone secretion is high in fetuses and neonates and decreases with age. In children, somatotropin secretion exhibits a circadian rhythm with the peak at night in stages 3 and 4 of non-rapid eye movement (NREM) sleep [2].

Insulin resistance is a state of reduced tissue sensitivity to insulin. The phenomenon involves reduced glucose uptake primarily by cells of fat and muscle tissues, despite normal or elevated concentrations of the hormone [3]. Insulin resistance develops in genetically predisposed individuals upon activation of relevant environmental stimuli. It can be either clinically silent or manifested by such disorders as impaired glucose tolerance, type 2 diabetes, hypercholesterolaemia, hypertriglyceridaemia, android obesity, hypertension, or hyperuricaemia. Three mechanisms of insulin resistance, i.e. pre-receptor, receptor, and post-receptor, have been

described. Two of the three mechanisms of the interactions between growth hormone and insulin are regarded as important. Growth hormone exhibits an antagonistic effect on insulin (pre-receptor mechanism) and impairs intracellular signal transmission (post-receptor mechanism) [4]. Since girls with Turner syndrome have short stature, exogenous growth hormone replacement therapy is applied in supra-physiological doses due to the reduced number of growth hormone receptors in patients with X chromosome monosomy [5,6].

Due to the defect in the pseudoautosomal region of the X-chromosome, haploidy of genes located on the short or long arm of the chromosome is observed, which is regarded a specific phenotype of Turner syndrome. Loss of one allele of the SHOX gene located on the X chromosome is the main factor responsible for short stature and skeletal deformities. The SHOX gene is expressed e.g. in chondrocytes and loss thereof leads to disturbances in bone development during growth resulting in the following defects: shield chest, elbow or knee valgity, auricular deformation, high arched palate, and shortened metacarpal IV [7].

Haploidy of the X-chromosome is also associated with genes involved in differentiation of gonads such as the ODG2 gene; absence of expression of the gene leads to gonadal dysgenesis. In a normally developed ovary, granulosa cells are responsible for production of approximately 95 % of blood oestradiol. In girls with Turner syndrome, the ovary has a form of bands of connective tissue that replace its normal structure; consequently, this leads to primary failure of this organ, reduced levels of serum oestradiol, and absence of puberty [7]. Oestrogen deficiency has a diabetogenic effect, which leads to impaired glucose tolerance in girls with TS. Giv-

en the primary ovarian failure, the hormonal therapy involves substitution with synthetic oestrogens (17-ethinyl oestradiol), which has an opposite effect on carbohydrate metabolism in the organism than that exerted by natural oestrogens, i.e. they reduce glucose tolerance and contribute to development of insulin resistance [2].

Early reports of impaired glucose tolerance (IGT) in TS [8,9] have been followed by studies finding several abnormalities of the glucose metabolism in both girls and women [10,11] with TS. Epidemiological studies have shown an increased risk of developing both type 1 diabetes (relative risk: 11.6) and type 2 diabetes (T2DM) (relative risk: 4.4) [11], in addition to increased mortality due to diabetes [12,13]. IGT is present in 25-78% of adult TS populations evaluated by oral glucose tolerance testing (OGTT) [10,11], and seems to be more prevalent in TS compared to both healthy controls and women with premature ovarian failure and thus reduced oestrogen exposure [10]. Other studies have suggested the presence of reduced insulin sensitivity [8,13] or impaired beta-cell function [10,11]. However, the exact mechanism behind the increased occurrence of type 2 diabetes is not clear.

Aim

The aim of the study was to assess insulin resistance estimated with the HOMA index prior to and after one-year and two-year treatment of girls with Turner syndrome with pharmacological doses of recombinant human growth hormone.

Patients and methods

The study group consisted of 54 girls with TS verified by karyotyping. 38 of the TS patients had a 45, X karyotype, 8 had 45, X/46, X, del(X), and 8 had a mosaic 45,X/46XY karyotype. All participants received growth hormone therapy. The examined group comprised 54 girls with Turner syndrome treated in the Department of Endocrinology and Diabetology, Medical University of Lublin. The girls were qualified for the programme of recombinant human growth hormone therapy by the Coordinating Team for Growth Hormone Therapy. During preliminary diagnosis, the karyotype was identified, thyroid hormones (girls with hypothyroidism received L-thyroxine substitution treatment) and fasting glucose and insulin were determined, and echocardiography was performed in each patient. The

patients were evaluated against the growth chart for Polish girls and their height was shown to be below percentile 3. The treatment included administration of preparations of recombinant human growth hormone in pharmacological doses of 0,7 – 2,0 IU/kg b.w./day. The preparations were administered subcutaneously in the evening. After a one-year- and two-year-long treatment, the levels of fasting glycaemia and insulinaemia were re-assessed. The values obtained were the basis for calculation of the HOMA index according to the formula:

$$\text{HOMA} = \text{insulin [IU/ml]} * \text{glucose [mg/dl]} / 22,5$$

An OGTT was performed, in which 75 g glucose was administered orally. Insulin and glucose were measured at baseline and at 60 and 120 min. Glucose was measured using ABBOTTchemical method, insulin – using direct chemiluminescence method ABBOTT, Hb A1C immunoturbidimetric method ABBOTT.

The exclusion criteria included known diabetes, BMI above 30, untreated hypo- or hyperthyroidism, present or past malignant disease, symptomatic heart disease, or daily use of prescribed medicine known to affect glucose metabolism.

The examinations were statistically analysed using Mann–Whitney–Wilcoxon test (Statistica 9.0).

All participants received oral and written information concerning the study prior to giving their written informed consent.

Results

Before therapy, the mean fasting glycaemia in the examined patients was $83,67 \pm 7,98$ mg/dl, which was in the normal range. None of the patients showed impaired glucose tolerance or diabetes. After a year of treatment, the mean fasting glycaemia was $86,02 \pm 8,27$ mg/dl. The difference between the levels of fasting glycaemia before and after the treatment was not statistically significant, likewise after 2 years of the HGH therapy (Tab. I, Fig. 1).

Similarly, the levels of glycaemia at 120 min in OGTT before the treatment ($121,48 \pm 30,04$ mg/dl), after a year ($105,94 \pm 17,16$ mg/dl), and two years ($110,14 \pm 22,17$ mg/dl) of the therapy (Tab. I, Fig. 1) did not differ significantly.

The mean level of fasting insulinaemia before the rHGH therapy was $15,79 \pm 8,44$ IU/ml and after a year-treatment $17,82 \pm 8,42$ IU/ml, which was statistically insignificant. After 2 years of the treatment, fasting insulinaemia levels decreased significantly and reached $10,83 \pm 6,17$ IU/ml. A similar

decrease in the serum insulin level was observed at 120 min of OGTT: the values before the treatment were $84,20 \pm 20,35$ IU/ml, after a year-long treatment $68,50 \pm 40,51$ IU/ml, and after 2 years $37,93 \pm 11,68$ IU/ml and the differences was statistically significant (Tab. I, Fig. 2).

The value of the HOMA index was $3,21 \pm 1,90$ at the beginning of the therapy and $3,82 \pm 1,84$ af-

ter the year-long treatment. After two years, a statistically significant decrease in HOMA values to $2,48 \pm 1,79$ was observed, which evidences a decline in insulin resistance (Tab. I, Fig. 3).

During the 2 years of treatment with recombinant HGH, the levels of glycated haemoglobin did not differ significantly from the pre-treatment values and those reported after 1 year.

Table I. Biochemical investigations of glucose metabolism in girls with Turner syndrome

Tabela I. Badania biochemiczne glukozy u dziewcząt z zespołem Turnera

	Before treatment Przed leczeniem	After 1 year of treatment Po roku leczenia	p	After 2 years of treatment Po 2 latach leczenia	p
Fasting glucose [mg/dl] Glukoza na czczo	$83,87 \pm 7,98$	$86,02 \pm 8,27$	NS	$83,30 \pm 8,35$	NS
Glucose at 120 min. OGTT [mg/dl] Glukoza w 120 min. OGTT	$121,48 \pm 30,04$	$105,94 \pm 17,16$	NS	$110,14 \pm 22,17$	NS
Fasting insulin [IU/ml] Insulina na czczo	$15,79 \pm 8,44$	$17,82 \pm 8,42$	NS	$10,83 \pm 6,17$	0,007
Insulin at 120 min. OGTT [IU/ml] Insulina w 120 min. OGTT	$84,20 \pm 20,35$	$68,50 \pm 40,51$	NS	$37,93 \pm 11,68$	0,009
HOMA	$3,12 \pm 1,90$	$3,82 \pm 1,84$	NS	$2,48 \pm 1,79$	0,012
HbA1c [%]	$5,39 \pm 0,48$	$5,21 \pm 0,36$	NS	$5,39 \pm 0,44$	NS

Fig. 1. Glycaemia during treatment of girls with Turner syndrome [mg/dl]

Ryc. 1. Glikemia w trakcie leczenia dziewcząt z zespołem Turnera [mg/dl]

Fig. 2. Insulinaemia during treatment of girls with Turner syndrome [IU/ml]
Ryc. 2. Insulinemia w trakcie leczenia dziewcząt z zespołem Turnera [IU/ml]

Fig. 3. HOMA during treatment of girls with Turner syndrome
Ryc. 3. HOMA w trakcie leczenia dziewcząt z zespołem Turnera

Discussion

Healthy women with Turner syndrome are characterised by impaired glucose tolerance, insulin resistance, low physical capacity, and enlarged type IIa muscle fibers, indicating diminished oxygen and substrate supply for metabolic processes. These findings could be indicative of a prediabetic state [14].

Bakalov et al. found a 25% prevalence of T2DM among a large group of adults with Turner Syndrome tested by OGTT ($n = 224$) with a mean age of 35 years [23]. They went on to divide their study group by karyotype into patients with delXq with a T2DM rate of 9% (similar to the T2DM rate in the background population), 45, X with a rate of 18%, delXp with a rate of 23%, and impressively, a rate of 43% among subjects with isochromosome Xq. These data suggest that haploinsufficiency of genes on Xp increases the risk of T2DM to 18-23%, and additionally that haploinsufficiency of Xp combined with trisomy for Xq genes (karyotypes with isochromosome Xq) further increases the risk of T2DM [23]. Interestingly, a recent study in children with TS showed that previously GH-treated girls had less subcutaneous and visceral fat and less glucose intolerance than GH naïve girls, pointing towards either a long lasting protective effect of GH treatment on body composition and glucose homeostasis or that GH treatment actually corrected some underlying GH deficiency or a combination of both [16].

We recommend that all women with TS should be tested regularly for the presence of diabetes, and we suggest that the high rate of T2DM is due to faltering β -cell function as presented here has a genetic basis [17].

Bakalov et al. compared the levels of glucose and insulin after oral glucose tolerance testing in girls with Turner syndrome and in girls with the 46,XX karyotype. Their investigations demonstrated that after 2 hours of glucose ingestion the level of the carbohydrate in girls with Turner syndrome was slightly higher than in girls with the normal karyotype, whereas the insulin level exhibited a reverse relationship. However, the authors emphasised that higher glucose levels were found in older girls, which is related to lower tissue sensitivity to insulin, whereas the reduced insulin secretion in younger girls was compensated by higher insulin sensitivity. The authors explain the lower insulin levels with impaired insulin secretion after glucose load without a significant decline in insulin sensitivity. Transient

dysfunction of pancreas β cells is regarded as the cause of the phenomenon, and an increased blood glucose concentration is a consequence of this state [11]. Nevertheless, the level of this carbohydrate returns to the initial state after treatment and is within the normal range.[11] Our studies indicate that the treatment with recombinant human growth hormone applied in girls with Turner syndrome does not have a significant effect on carbohydrate metabolism in their organisms, as the levels of fasting glucose and at 120 min of the OGTT did not differ significantly before and after long-term rHGH substitution. In turn, insulin resistance changed significantly, hence the pancreas of the HGH-treated girls secreted less insulin; the level of insulinaemia declined markedly especially after glucose load. Importantly, fasting insulin values $> 15 \mu\text{IU/ml}$ are regarded as abnormal levels. In the examined girls, the fasting insulin levels both before and during the treatment oscillated around the value.

The decline in insulin resistance is reflected by the decreased HOMA index after the 2-year-long HGH therapy. This is not associated with changes occurring during puberty, since the girls did not mature spontaneously but received hormone replacement therapy. In adults, the value of $\text{HOMA-IR} > 2.5$ indicates insulin resistance. In the paediatric population, these values are higher, particularly during the adolescence period. According to Kurtoglu et al. [18], the index is 2,67 in boys and 2,22 in girls before puberty and 5,22 in boys and 3,82 in girls during the puberty period. Our patients should be compared with a population of pre-pubertal girls. Our comparison shows that, before and after the 1-year-long rHGH therapy, insulin resistance is higher than in healthy girls and this ratio is compensated after two years of the treatment.

In his investigation, Jeffcoat emphasises emergence of many suggestions that insulin resistance itself may result from growth hormone deficiency, particularly when combined with abdominal obesity. The author claims that it is difficult to draw consistent conclusions from the investigations hitherto conducted on the impact of the rGH therapy on tissue insulin sensitivity due to the heterogeneity of examined groups and duration of observations, although an increase in the level of both insulin and glucose in blood serum is postulated in all papers. In some investigations, an increase in postprandial glucose and glycated haemoglobin was observed, and considerable insulin resistance or diabetes was diagnosed in some cases. However, the author

stresses that the increases in the insulin or glucose levels observed during rGH therapy were too low to be clinically relevant. [19] The insulin resistance diagnosed in the girls examined in the present study was likely to be associated with absence of cell sensitivity to GH.

The quantity and distribution of fat tissue in the organism has an impact on carbohydrate metabolism. In particular, storage of visceral adipose tissue is related to an increased risk of insulin resistance. Given the predisposition for excessive accumulation of the tissue in girls with Turner syndrome, the patients are at greater risk of carbohydrate metabolism disorders, decreased insulin sensitivity, and consequently development of type 2 diabetes. In her doctoral dissertation, Hanna Magnuszewska describes, based on many authors' studies, a positive long-term effect of recombinant human growth hor-

mone therapy on lipid metabolism. After the therapy, reduction in abdominal fat tissue was observed in the patients, which contributed to an increase in tissue insulin sensitivity [20].

Results

1. Patients with Turner syndrome have higher pre-treatment levels of insulin resistance than healthy girls. (As result of comparison to the norm ranges in references)

2. Treatment with recombinant human growth hormone in patients with Turner syndrome does not affect the level of fasting glycaemia or glycaemia in OGTT.

3. Treatment with recombinant human growth hormone decreases insulin resistance in patients treated for 2 years.

REFERENCES/PIŚMIENNICTWO

- [1] Kubicka K., Kawalec W.: *Pediatrica. Podręcznik dla studentów*. PZWL, Warszawa 2006, 531-537.
- [2] Traczyk W., Trzebski A.: *Fizjologia człowieka z elementami fizjologii stosowanej i klinicznej*. PZWL, Warszawa 2009, 346-352.
- [3] Witek J., Witek P., Pańkowska E.: Insulinooporność u dzieci. *Pediatric Endocrinology, Diabetes and Metabolism*, 2011, 206-212.
- [4] Grzesiuk W., Szydłarska D., Józwiak K.: Insulinooporność w endokrynopatiach. *Endokrynologia, Otyłość i Zaburzenia Przemiany Materii*, 2008, t. 5, nr 1, 38-44.
- [5] Binder G., Trebar B., Baur F., Schweizer R., Ranke M.B.: Homozygosity of the d3-growth hormone receptor polymorphism is associated with a high total effect of GH on growth and a low BMI in girls with Turner syndrome. *Clin Endocrinol (Oxf)*. 2008 Apr;68(4):567-72.
- [6] Baş F., Darendeliler F., Aycan Z. et al.: The exon 3-deleted/full-length growth hormone receptor polymorphism and response to growth hormone therapy in growth hormone deficiency and Turner syndrome: a multicenter study. *Horm. Res. Paediatr.*, 2012;77(2), 85-93.
- [7] Gawlik A., Antosz A., Wilk K., Małecka-Tendera E.: Opieka medyczna w zespole Turnera – z praktycznego punktu widzenia. *Endokrynologia Pediatria*, 2013; nr 3 (44), 55-57.
- [8] Forbes A.P., Engel E.: The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis, and their close relatives. *Metabolism*, 1963;12, 428-439.
- [9] Nielsen J., Johansen K., Yde H.: The frequency of diabetes mellitus in patients with Turner's syndrome and pure gonadal dysgenesis. Blood glucose, plasma insulin and growth hormone level during an oral glucose tolerance test. *Acta. Endocrinol. Copenh.*, 1969;62, 251-269.
- [10] Gravholt C.H., Naeraa R.W., Nyholm B. et al.: Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. The impact of sex hormone replacement. *Diabetes Care*, 1998;21, 1062-1070.
- [11] Bakalov V.K., Cooley M.M., Quon M.J. et al.: Impaired insulin secretion in the Turner metabolic syndrome. *J Clin Endocrinol Metab.* 2004;89:3516-3520.
- [12] Schoemaker MJ, Swerdlow AJ, Higgins CD, Wright AF, Jacobs PA. Mortality in women with turner syndrome in Great Britain: a national cohort study. *J. Clin. Endocrinol. Metab.*, 2008;93, 4735-4742.
- [13] Stochholm K., Juul S., Juel K. et al.: Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J. Clin. Endocrinol. Metab.*, 2006;91, 3897-3902.
- [14] Gravholt C.H., Nyholm B., Saltin B. et al.: Muscle fiber composition and capillary density in Turner syndrome: evidence of increased muscle fiber size related to insulin resistance. *Diabetes Care*, 2001 Sep;24(9), 1668-73.
- [15] Bakalov V.K., Cheng C., Zhou J., Bondy C.A.: X-chromosome gene dosage and the risk of diabetes in Turner syndrome. *J. Clin. Endocrinol. Metab.*, 2009;94, 3289-3296.
- [16] Wooten N., Bakalov V.K., Hill S., Bondy C.A.: Reduced abdominal adiposity and improved glucose tolerance in growth hormone-treated girls with Turner syndrome. *J. Clin. Endocrinol. Metab.*: 2008;93, 2109-2114.

- [17] Hjerrild B.E., Holst J.J., Juhl C.B. et al.: Delayed β -cell response and glucose intolerance in young women with Turner syndrome. *BMC Endocr. Disord.*, 2011;11, 6.
- [18] Kurtoglu S., Hatipoglu N., Mazicioglu M.: Insulin Resistance in Obese Children and Adolescents: HOMA-IR Cut-Off Levels in the Prepubertal and Pubertal Periods. *J. Cli. Res. Ped. Endo.*, 2010;2 [3], 100-106.
- [19] Jeffcoat W.: Growth hormone therapy and its relationship to insulin resistance, glucose intolerance and diabetes mellitus: a review of recent evidence. Department of Diabetes and Endocrinology, City Hospital, Nottingham, England – Monography.
- [20] Magnuszewska H.: Korzyści i ryzyko stosowania ludzkiego rekombinowanego hormonu wzrostu u pacjentek z rozpoznaniem zespołu Turnera. *Klinika Pediatrii, Diabetologii i Endokrynologii Gdańskiego Uniwersytetu Medycznego*, Gdańsk 2011, 9-16, praca doktorska.