THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

METABOLIC ALTERATIONS IN CHILDHOOD OBESITY

Role of insulinresistance/hyperinsulinaemia and clinical impact of acanthosis nigricans

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1. Impact of childhood obesity.

In 1997 World Health Organization (WHO) declared obesity as a disease. Obesity is in economical developed and developing countries the most important nutritional disease, population studies show due to its pathological consequences strong correlation between overweight and lifetime of obese children. Importance of childhood obesity is due to its increasing prevalence, the associations with alterations of glucose and lipoprotein metabolism have been documented, and beside this transition of childhood obesity into obesity in adulthood is also often documented. Childhood obesity can be considered as risk factor for development of adulthood atherosclerotic diseases and type 2 diabetes mellitus (T2DM).

1.1. Prevalence of childhood obesity. Transition into adulthood obesity form

The prevalence of childhood and adolescent obesity has been increasing in the last decade all over the world. The ratio of overweight and obese children has been doubled during the past 15 years in the USA. Data of National Health and Nutrition Examination Survey (NHANES) in 1999-2002 show that 31% of children aged 6-19 were overweight. Ratio of obesity was 16.0% in this group. This tendency can be seen in Europe too: studies in 2006 figure the number of overweight children of 22 million and 5 million for obese children. Prevalence of overweight in boys was 31.5% and 32.9% in girls. Prevalence of obesity in boys was 6.8% and 9.0% in girls. Hungarian data show the increase of overweight and obesity while the comparison with the international data is not easy because the different age and gender related percentiles (90. and 97. vs. 85. and 95.) used to determine overweight and obesity in our country. Childhood obesity persist in most of the involved person, different studies show persistence 25% up to 80%. Infant obesity is not a strong predictor of adulthood obesity but childhood and adolescent obesity often turn into obesity in adulthood. Early and successfully treatment of childhood obesity can prevent atherosclerotic diseases and T2DM in adulthood.

1.2. Alterations of carbohydrate metabolism in childhood and adolescent obesity

Parallel to the increase of the prevalence of childhood obesity in the last decade, number of T2DM in childhood and adolescent also increasing. Importance of the fact was registrated first in the USA, especially in certain ethnic groups. Later studies from other part of the world, also from Europe demonstrated this tendency of increase of T2DM. In Hungary, the prevalence of T2DM in obese children was found to be 1-2%. Two other European study demonstrate the same prevalence, as long as prevalence of T2DM in American obese adolescents was found to be 4%. Alteration of glucose metabolism is characterized by insulin resistance, hyperinsulinaemia, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) and the pathological progress can ultimately result in type 2 diabetes mellitus.

During IGT insulin response of the pancreatic beta-cells mediated through glucose stimuli cannot compensate the insulin resistance; elevation of glucose level can be documented. In Hungary IGT was found in 15-18% of obese children. Other studies found prevalence of IGT in more than 20% of obese children and adolescents. In despite prevalence of IFG in childhood and adolescent obesity was not found more than 2.5%, so the frequency of IGT was substantially higher. Results confirm the diverse clinical and pathological mechanism of IFG and IGT. In the last years, cut-off point of IFG was changed; the increase of prevalence of IFG could be expected.

1.3. Alteration of lipoprotein metabolism in childhood and adolescent obesity

Obesity is a risk factor for atherosclerosis. Obesity, associated with alterations of lipoprotein metabolism, characterized by an abnormal „atherogenic" lipid profile consisting of elevated low density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and decreased high density lipoprotein cholesterol (HDL-C) and elevated small dens LDL molecules play an essential role in the atherogenic process. The term in the literature for dyslipidaemia, characterized by elevated TG and decreased HLD-L is atherogenic dyslipidaemia, it starts already in childhood obesity. The process of atherosclerosis starts also already in childhood and there is a correlation between lipid levels and autopsy findings of arterial lipid deposits. Demonstration of alterations of lipoprotein metabolism in childhood and adolescent obesity inform us of further atherosclerosis risk. In the process of atherosclerosis antagonism of oxidated LDL and HDL play an essential role. Oxidated LDL has an important role in the beginning steps of atherosclerosis, helps monocytes infiltration, smooth muscle cells migration, endothelial adhesion molecules production and the anticoagulation. HDL and binding enzymes like paraoxonase (PON1) – protect LDL from oxidation and stops the atherosclerosis procedure. Important antiatherogen function of the HDL is the reverse cholesterin transport, meaning peripheral LDL uptake and transport to the liver. These steps of the process of atherosclerosis are well known, investigations of the process in childhood obesity are rare.

1. 4. Metabolic syndrome in childhood and adolescent obesity

Obesity in childhood is associated with tripled increased risk of hypertension. Consequently, metabolic syndrome characterized by alterations of carbohydrate and lipoprotein metabolism and hypertension is also frequently found in children with obesity. Prevalence of metabolic syndrome in childhood and adolescent obesity was found 30-50% in recent studies. Insulin resistance and hyperinsulinaemia play an essential role in the development of abnormal glucose metabolism, dyslipidemia and hypertension, what is the characteristic cluster of

metabolic syndrome. Central adipose tissue plays an important role in the development of metabolic syndrome. Abdominal adipose tissue is not only passive lipid depot, but endocrine organ, producing adipocytokines (leptin adiponectint, resistint, tumor necrosis-factor-alfa and interleukin-6). In central obesity adiponectin levels decrease, production of the other adipocitokines increase, all this alterations correlated to the development of insulin resistance. Visceral adipose tissue is sensitive to the effect of free fatty acid (FFA) mobilization. Elevated FFA levels increase the hepatic gluconeogenesis, the insulin resistance of the muscles and increase the beta-cell insulin production, consent to development of metabolic syndrome.

According to these facts, by alterations of carbohydrate and lipoprotein metabolism in childhood obesity, insulin resistance and hyperinsulinaemia is to be investigated.

1. 5. Acanthosis nigricans in childhood and adolescent obesity

Childhood and adolescent obesity can be characterized beside metabolic alterations by physical examination markers such as acanthosis nigricans (AN), striae, acne and hirsutism. AN is a dermatosis with unknown aetiology and pathomechanism, it is characterized clinically by a dark, velvety, smooth thickening of the skin, and histologically by papillomatosis, hyperkeratosis and epidermal hyperpigmentation. AN is a physical marker for insulin resistance. It can be found not only by insulin resistance syndromes characterized by hyperinsulinaemia and insulin resistance but it associated with childhood obesity too. Obesity and AN at same time predict insulin resistance in obese children, due to the fact, that children with T2DM have often AN. This is why investigations of metabolic alterations in childhood simple obesity and obesity with AN can be important.

2. Aim of the thesis

We had three major aims of our studies followed with more correlated questions:

Investigation of carbohydrate metabolism in childhood and adolescent obesity

- Investigation of basal blood glucose, basal insulin level and homeostasis model assessment insulin resistance index (HOMA-IR), determination of prevalence of insulin resistance and hyperinsulinaemia, investigation of correlation between hyperinsulinaemia and puberty status of obese and overweight children and adolescent.
- Determination of alterations of glucoregulation in a numerous group of obese children: prevalence of IFG, IGT and T2DM.

- Correlation of alterations of glucoregulation and insulin resistance and hyperinsulinaemia, statistical analysis of correlations between 0'BG and 120'BG categories.
- Comparison of results obtained for IFG according to WHO and ADA criteria.

Investigation of alterations of lipoprotein metabolism in childhood and adolescent obesity

- Investigation of alterations of lipoprotein metabolism, investigation of atherogen dyslipidaemia, pathological apolipoprotein and lipoprotein(a) values in childhood and adolescent obesity.
- Investigation of correlations of lipoprotein metabolism and insulin resistance, basal insulin levels and reactive hyperinsulinaemia.
- Investigation of atherogenic factors related to HDL function: investigation of activity of PON-1, LCAT and CETP and concentration of ICAM-1 and VCAM-1 in childhood obesity.
- Investigation of correlations between the results, obtained from atherogenis factors and insulin resistance and hyperinsulinaemia

Impact of AN in childhood and adolescent obesity

- Investigation of carbohydrate and lipoprotein metabolism in children with simple obesity and obesity with AN.
- Role of insulin resistance and basal and reactive hyperinsulinaemia in development of the alterations
- Comparison of carbohydrate (insulin resistance, basal and reactive hyperinsulinaemia values) and lipoprotein metabolism results obtained from children with OS and OAN.

3. Patients and methods

3.1. Patients

Cohorts of obese children and adolescent from outpatient department of Caucasian-European origin were examined for endocrine and metabolic abnormalities. Obesity was determined as a BMI percentiles of >97. BMI SD was ≥2 for age and gender of the children. Patients with obesity syndromes and other illnesses were excluded from this study.

3. 2. Methods of investigations

3.2.1. Physical examination

BMI were calculated using bodyweight and body height (the weight in kilograms divided by the square of the height in meters).

Puberty stadiums were defined and divided in groups by Tanner.

Grade of AN was divided in two groups, grade 1-3 by Burke et al. was defined as moderate and grade 4 by Burke et al. as severe AN.

3.2.2. Investigation of carbohydrate metabolism

3.2.2.1. Measurements of fasting glucose and insulin levels

Fasting blood glucose (FBG) was measured by the glucose-oxidase method on venous whole blood immediately deproteinized with perchloric acid. Fasting insulin was measured by commercial radioimmunassay (IRMA) (IMMUNOTECH).

3.2.2.2. Oral glucose tolerance test (OGTT)

According to WHO and ADA diagnostic and classification recommendations OGTT was performed, followed a diet consisting of at least 250 g of carbohydrates per day for 3 days before the study. After a 12-h overnight fast, all our children underwent an OGTT at 07.00 h. Baseline samples were obtained for measurement of fasting (venous) glucose and fasting insulin. Glucose was given orally (50 g/m2 body surface, up to a maximum of 75 g glucose). Blood samples were drawn after 30, 60, 90 and 120 min. At 0’ and 120’ glucose and insulin values were determined (0’BG, 120’BG, 0’INS, 120’INS) and summa glucose and insulin calculated (ΣVC, ΣINS).

3.2.2.3. Methods for investigation of the insulin resistance/insulin sensitivity

The HOMA for insulin resistance index (HOMA-IR) was estimated using the formula:

HOMA-IR = [0’INS) (mU/l) x (0’BG) (mmol/l)] : 22.5.

HOMA-BCF to evaluate the beta cell function of the pancreas, HOMA-BCF was estimated using the formula:

HOMA-BCF = (20 x Ins): (FBG – 3.4).

Quantitative insulin sensitivity check index – QUICK were calculated using the formula:

QUICKI = 1 / (log fasting glucose (mg/dl) + log fasting insulin (mU/ml)

Insulin sensitivity index - ISI by Matsuda were calculated using the formula:

ISI = 10000/ √((0' INS (mU/l) x 0' BG (mmol/l) x (average INS (mU/l) x average BG (mmol/l)

3.2.2.4. Evaluation of carbohydrate metabolism

Glucose concentrations were determined and categorized using WHO criteria. IFG was defined as a fasting plasma glucose of 6.1–6.9 mmol/l; impaired glucose tolerance, as a 2-h plasma glucose level of 7.8 mmol/l-11.1mmol/l and type 2 diabetes as a fasting glucose level ≥ of 7.0 mmol/l or a 2-h plasma glucose level ≥ of 11.1 mmol/l.

0' BG was determined and categorized using ADA criteria too. IFG was defined as a fasting plasma glucose of ≥ 5.6 mmol/l.

Elevated INS was described as a fasting insulin level > of 25 mU/l, and 120 min. insulin > of 45 mU/l. Increased HOMA-IR was defined as HOMA values > of 4. HOMA-BCF was defined as elevated as value was > 100.

3.2.3. Methods for investigation of lipoprotein metabolism

3.2.3.1. Investigation of lipoproteins, triglycerid, apolipoproteins and lipoprotein(a)

For investigation the lipoprotein metabolism T-C, TG, HDL-C was measured by colorimertic method. LDL cholesterol level was calculated by the Friedewald equation:

[LDL-C (mmol/l)] = [T-C (mmol/l] – [HDL-C (mmol/l)] – [TG (mmol/l)]/2.2)

ApoA-I, az apoB-100 és a Lp(a) were measured by immunnefelometric method.

Elevated values for lipoprotein metabolism were defined and categorized using the values for children and adolescents of National Cholesterol Education Program (NCEP) and IDF metabolic syndrome dyslipidaemia parameters: TC > 5.2 mmol/l, TG ≥1.7 mmol/l, HDL-C < 1.0 mmol/l, LDL-C > 3.4 mmol/l.

Abnormal values for apolipoproteins and lipoprotein(a) were: apo-AI<1.15 g/l; apoB100>1.0 g/l; Lp(a)>300 mg/l.

3.2.3.2. Methods for investigation of atherosclerosis factors

Measurement of the paraoxonase activity (PON1) was performed using enzyme reaction, with paraoxon (O,O diethyl-O-p-nitrophenylphosphatase, Sigma) substrate, 4-nitrophenol concentration was measured using spectrophotometer.

Measurements of the arylesterase activity (ARYL) *was performed using enzyme reaction*, with phenylacetat (Sigma) substrate, hydrolization was using spectrophotometer

Measurements of the LCAT and CETP activity was performed using ROAR Biomedical INC kit

Measurements of the concentration of the adhesion molecules (ICAM-1, VCAM-1) were performed using R&D kit, ELISA method**.**

Normal values are not defined, so we only investigated and compared the results of different groups.

3.2.3.3. Statistical analysis

For comparing normally distributed data from the groups of patients the Student t-test and for non-normally distributed data the Wilcoxon's rank-sum test was used; for investigating relationship among frequency of abnormal results in subgroup, the Fisher exact test was applied. Pearson correlation analysis was used to establish associations among the parameters investigated. Association between insulin resistance and metabolic parameters were estimated using multiple linear regression. Variables were transformed using the transformation yielding the greatest improvement, if necessary, in normality and linearity. Relationships between each possible pair of parameters were modelled, except for those insulin resistance parameters that are algorithmically convertible (HOMA-IR and QUICKI). The linearity assumption was graphically checked and adjustment for age, sex and BMI was applied in all models. Results were expressed as b coefficient and p-value.

4. Investigations and results

4.1. Alterations of glucoregulation in childhood and adolescent obesity. Association with insulin resistance and hyperinsulinemia.

4.1.1. Fasting blood glucose levels, insulin resistance and hyperinsulinaemia in obese children and adolescent, impact of puberty

Group of obese adolescent was investigated. FBG and basal insulin level was measured for determine the prevalence of IFG, insulin resistance and hyperinsulinaemia, and correlation between puberty status and hyperinsulinaemia was investigated.

Altogether 50 children, 28 girls and 22 boys of Caucasian-European origin, with obesity were involved into the study. The age of the children was 15.5±1.0 year, BMI was 31.0±3.5 kg/m^2. Adolescents were divided in groups by Tanner (T3, T4, T5). There were no significant differences in these parameters between the two subgroups.

Mean FBG was found normal in both groups, insulin concentration, HOMA-IR and HOMA-BFC was highly elevated in both of the groups. IFG was not found, FBG was normal of each

patient. Elevated insulin concentration was found in 40 children (80%). Elevated HOMA-IR was found also in 40 children (80%), HOMA-BCF in 45 children (90%). Significant difference was not found between the ratios of elevated INS, HOMA-IR and HOMA-BCF obtained from subgroups.

Investigated the results according to puberty status most elevated mean values of parameters were found in T4 subgroup, in T5 and T3 stadium average values were similar. Significant difference was found between HOMA-IR values ($p<0.05$), but prevalence of abnormal HOMA-IR was not different between puberty stadiums. Significant difference was not found between the results in different puberty stadium groups, the prevalence of abnormal HOMA-BCF values were not differ too.

Analyzing the results, significant positive difference was found between bodyweight (BW) and insulin concentration, BW and HOMA-IR, BMI and HOMA-IR, BW and HOMA-BCF, BMI and HOMA-BCF and FBG and insulin concentration indeed.

According to our results normal FBG, insulin resistance and hyperinsulinaemia is due to obesity in obese adolescents but transitory hyperinsulinaemia and insulin resistance due to puberty can play also a role of its development.

4.1.2. Alterations of glucoregulation in childhood and adolescent obesity. The role of insulin resistance and hyperinsulinemia.

Group of obese children and adolescent was investigated for determination of prevalence of alterations of carbohydrate metabolism. Correlation of alterations of glucoregulation, insulin resistance and hyperinsulinaemia was also investigated. Our further aims were to analyze correlations between 0' blood glucose (0'BG) and 120' blood glucose (120'BG) categories and to compare data obtained by using different IFG evaluation criteria, i.e. WHO criteria and ADA/IDF criteria.

Altogether 250 children, 132 girls and 118 boys of Caucasian-European origin, with obesity were involved into the study. The age of the children was 13.0±6.9 year; BMI was 34.0±5.4 kg/m^2. There were no significant differences in these parameters between the two subgroups.

A standard OGTT was performed. Serum glucose and insulin were determined at the 0^{th} and 120^{th} minutes of the test. HOMA-IR was calculated. Glucose concentrations were categorized using WHO and ADA/IDF criteria.

0' BG and 120'BG average values were normal, 0' and 120' INS average values were abnormal. HOMA-IR values of 12-18 year old children were significantly higher than those of younger children ($p<0.001$). Significant difference was not found between the other values

of girls and boys, however, the age related subgroups differed in each investigated parameters.

0’BG values corresponded to IFG in 3 patients (1.2%) according to WHO criteria, and in 11 patients (4.4%) according to ADA/IDF criteria. In 3 further cases (1.2%) fasting glucose level was suspect to T2DM. On the basis of the OGTT results IGT was detected in 34 patients (13.6%) and T2DM was found in 6 children (2.4%).

The 0’INS was increased 175 patients (70%), elevated 120’INS was found in 220 cases (88%). and elevated HOMA-IR index was detected 194 children (77.6%). Regarding the prevalence of abnormal values in each subgroups, it was observed that in children aged 12-18 elevated 0’INS and increased HOMA-IR values were more frequent than in subgroup of younger children ($p<0.05$ and $p<0.001$). Abnormal HOMA-IR was detected in all patients with IFG (evaluated either by WHO or by ADA/IDF criteria), in all patients with 0’BG suspect to T2DM, and in all cases with T2DM, but it was found only 27 cases from 34 patients with IGT (79%). Abnormal 0'INS was found in 2 cases from 3 patients with IFG according to WHO criteria and 10 cases from 11 patients with IFG according to IDF criteria, as well as in 26 cases of 34 children with IGT (76%), and in all children with T2DM.

The 0'BG was categorized according to WHO or ADA/IDF criteria. Using both criteria, significant associations were detected among numbers of children in 0'BG and 120'BG categories ($p<0.001$). Among 210 patients with normal 120'BG one had IFG according to WHO criteria, 7 patients using IDF criteria, and one further child was suspect to T2DM. Among 34 children with IGT the 0'BG corresponded to IG in patients using WHO criteria and in 4 patients according to ADA/IDF criteria. In patients with T2DM the 0'BG results raised the possibility of T2DM only in 2 patients; in 4 children the 0'BG levels were normal.

The fact that alterations of glucoregulation were substantially less than insulin resistance, basal and reactive hyperinsulinemia suggest that hyperinsulinemia can successfully compensate insulin resistance in majority of the obese children. However the ADA recommends the measurement of the fasting glucose as screening tool and OGTT is recommended only in the case of IFG. Our results suggest that an OGTT may be needed to identify those at risk of developing abnormal glucoregulation and T2DM.

4.2. Alterations of lipoprotein metabolism and atherogenetic factors in childhood and adolescent obesity

4.2.1. Atherogenic dyslipidaemia, insulin resistance and hyperinsulinaemia in obese children and adolescents

Alterations of lipoprotein metabolism were investigated in obese children. Correlation of alterations of lipoprotein metabolism, insulin resistance and hyperinsulinaemia were investigated too.

Altogether 51 children, 28 girls and 23 boys of Caucasian-European origin, with obesity were involved into the study. The age of the children was 15.5±1.1 year; BMI was 31.1±3.5 kg/m^2. T-C, TG and HDL-C, concentration of apoA-I, apoB-100 and Lp(a) were measured and LDL-C and HOMA-IR calculated.

Mean values of T-C, LDL-C, TG and HDL-C were normal. There was no significant difference between the two subgroups. Elevated T-C was found in 2 children, elevated LDL-C 1 case, increased TG and decreased HDL-C were found in 10 cases (20%) respectively. There was no significant difference between the prevalence of abnormal values of the two subgroups. Mean values of ApoA-I, apoB-100 and Lp(a) were normal. Elevated apoB-100 was found only in 1 case, decreased apoA-I concentration was detected in 7 children (14%). Elevated Lp(a) concentration was found in 15 children (30%). There was no significant difference between the prevalence of abnormal values of apoA-I és a Lp(a) of the two subgroups.

Investigated the parameters of HOMA-IR, FBG was normal, mean INS and HOMA-IR were abnormal (elevated); there was no significant differences between the two subgroups. FBG was normal of each patients but insulin concentration was elevated in each cases. HOMA-IR was elevated in 43 children (85%).

Significant positive correlation was found between T-C and LDL-C levels, significant negative between increased TG and decreased HDL-C values. Significant positive correlation was found between apoB-100 and BMI, FBG and HOMA-IR, T-C and LDL-C levels, just as between apoA-I and HDL-C concentration. Significant correlation of Lp(a) and other investigated parameters were not found. As regards to parameters of carbohydrate metabolism significant positive correlation was found between BMI and 0'INS, BMI and HOMA-IR as well as between 0'INS and FBG.

Atherogenic dyslipidemia, characterized by elevated TG and decreased HDL-C, is a frequent condition of childhood obesity. Basal hyperinsulinaemia and increased HOMA-IR were also

frequently found. Results of apolipoproteins correlated to alterations of lipid metabolism. Elevation of Lp(a) needs further investigations.

4.2.2. Investigations of atherogenetic factors in childhood and adolescent obesity

The process of atherosclerosis starts already in childhood. We investigated prevalence of dyslipidaemia in childhood obesity and the impact of atherogenic factors playing an essential role in the development of atherogenesis. We investigated the role of insulin resistance and hyperinsulinaemia in the development of alterations of lipoprotein metabolism. We investigated the atherogenic factors related to HDL function: activity of PON-1, LCAT and CETP and concentration of ICAM-1 and VCAM-1 in childhood obesity in children and adolescent with normal and decreased HDL-C concentration. We investigation the correlations between the results, obtained from atherogenic factors and insulin resistance and hyperinsulinaemia too.

Altogether 37 children, 17 girls and 20 boys of Caucasian-European origin, with obesity were involved into the study. The age of the children was 14.2±1.8 year; BMI was 35.9±6.1 kg/m^2. The children were divided in two subgroups, 19 children had OS and 18 OAN.

Investigated parameters of lipoprotein metabolism were similar to the investigated parameters before. HOMA-IR was calculated too. We investigated the atherogenic factors related to HDL function: activity of PON-1, LCAT and CETP and concentration of ICAM-1and VCAM-1 in childhood obesity in children and adolescent was measured.

As regard the lipoprotein metabolism elevated T-C and LDL-C ratio was found in 5/37, increased TG in 6/37, and decreased HDL-C in 9/37. Abnormal concentration of apoA-I was found in 13/37, apoB-100 in 5/34, prevalence of abnormal values of Lp(a) were 11/37. As regard the HOMA-IR parameters FBG was not elevated none of the investigated children, IFG was not found. ON other hand elevated INS were found in 27/37, elevated HOMA-IR in 31/37 of investigated children.

Significant positive correlation was found between T-C and LDL-C, T-C and apoB-100, LDL-C and apoB-100; significant negative correlation between HDL-C and TG. Between HDL-C and apoA-I; BMI and INS, BMI and HOMA-IR also significant positive correlation was found.

We investigated the atherogenic factors related to HDL function in childhood obesity in children and adolescent with normal and decreased HDL-C concentration. There was no significant difference of activity of PON-1, LCAT and CETP and concentration of ICAM-1 and VCAM-1. Significant elevated concentration of ICAM-1 was found in the subgroup of obese children with decreased HDL-C values ($p<0.05$) than in the subgroup of obese children

with normal HDL-C values. The tendency of the concentration of VCAM-1 was similar, but there was no significant difference between the two subgroups.

Investigated the parameters of atherogenesis significant negative correlation was found between PON1 activity and ICAM-1 concentration ($p<0.05$). Significant positive correlation was found between concentration of ICAM-1 and concentration of VCAM-1 ($p<0.001$), as well as between concentration of VCAM-1 and CETP activity ($p<0.05$)

These results prove that atherogenic dyslipidemia is a frequent condition of childhood obesity. In most of the cases elevated hyperinsulinaemia and HOMA-IR was documented. No significant correlation was found between factors related to HDL-C and insulin resistence/hyperinsulinaemia. Investigated factors suggest the complex role of dyslipidaemia and the decreased HDL-C is a marker of this.

4.3. Impact of AN in childhood and adolescent obesity

AN is a frequent condition in childhood and adolescent obesity. Recent studies suggest insulin resistance in presence of AN. AN seems to be the physical marker of insulin resistance and hyperinsulinaemia and risk factor of development of T2DM. That is why we investigated the carbohydrate and lipoprotein metabolism in children with SO and OAN.

4.3.1. Result the carbohydrate and lipoprotein metabolism in children with SO and OAN using the data of 4.2.2.

The investigated 37 obese children were divided in two subgroups, 19 children had SO 18 children had OAN. We compared the prevalence of abnormal values obtained from both of the subgroups.

As regard the abnormal parameters of carbohydrate metabolism prevalence of hyperinsulinaemia in the OAN subgroup was significant higher ($p<0.05$), than in OS (16/18 vs. 11/19), ratio of elevated HOA-IR values was not significant. The prevalence of decreased HDL-C and increased TG values were significant higher as regard the abnormal parameters of lipoprotein metabolism in children with OAN ($p<0.05$ and $p<0.01$), than in children with SO (7/18 vs. 2/19 and 6/18 vs. 0/19).

4.3.2. Alterations of carbohydrate and lipoprotein metabolism in childhood and adolescent simple obesity and obesity with AN.

A total of 113 obese children, 57 girls and 56 boys, of Caucasian European origin, were included into the study. The whole group of the obese children was divided into subgroups: 56 children had simple obesity (SO) and 57 children had obesity associated with acanthosis nigricans (OAN). The age of OS children was 13.2±2.2 year; BMI 31.9±4.9 kg/m^2, the age of

OAN children was 12.8±2.5 year; BMI 33.5±5.4 kg/m^2. There were no significant differences in these parameters between the two subgroups.

A standard OGTT, after a 12-h overnight fast, was performed with 1.75 g/kg, maximum of 75 g glucose load. Blood samples were obtained at 0, 30, 60, 90 and 120 minutes. Serum glucose and insulin were determined in all samples, and fasting blood glucose (0'BG), blood glucose at 120' min. (120'BG), and sum of blood glucose concentrations (ΣBG) as well as fasting insulin (0'INS), insulin at 120 min. (120'INS), and sum of insulin concentrations (ΣINS) were evaluated. Insulin sensitivity/resistance was evaluated using HOMA, HOMA-IR, QUICKI, Matsuda insulin sensitivity index (ISI) formulas.

From the fasting blood samples T-C, HDL-C and TG were also measured by colorimetric methods, and LDL-C was calculated by the Friedewald equation.

The mean values of 0'BG and 120'BG were in the normal ranges, the mean values of 0'INS, 120'INS were elevated. 120'INS and ΣINS values were significantly higher in OAN than in SO, $p<0.01$ in both comparisons. The prevalence of IFG in the whole group of investigated obese children was 3.5% according to WHO criteria and 5.2% according to ADA/IDF criteria respectively. The prevalence of IGT was 15.9%, and prevalence of T2DM was 1.1% in the whole group. Prevalence of abnormal values of 0'INS in 73% of cases, 120'INS 89% of cases was found. There was no significant differences between the prevalence of abnormal values from children with SO and OAN. Abnormal (elevated) HOMA-IR was more frequent in children with pronounced AN (grade 4 by Burke) than in moderate OAN (grade 1-3 by Burke). Significant lower ISI was found in OAN than in SO ($p<0.05$). There was significant positive correlation between HOMA-IR, QUICKI and ISI values.

TG value was significant higher in OAN, HDL-C significant lower ($p<0.05$ and $p<0.01$). T-C was elevated in 23%, LDL-C 21%, TG 25,6% of investigated group, decreased HDL-C was detected in 38% of the investigated children. Prevalence of elevated TG and decreased HDL-C ratio was more frequent in OAN ($p<0.05$ respectively).

Concerning to parameters of glucose metabolism, significant positive correlations were found between HOMA-IR and 120'INS ($p<0.05$), HOMA-IR and ΣINS ($p<0.05$), HOMA-IR and 120'BG ($p<0.0001$) as well as HOMA-IR and ΣBG ($p<0.0001$). As regards to the lipoprotein parameters, significant positive correlations were demonstrated between HOMA-IR and T-C ($p<0.05$), HOMA-IR and TG ($p<0.0001$), and a significant negative correlation was demonstrated between HOMA-IR and HDL-C ($p<0.05$). We investigated the correlation between insulin response and insulin resistance parameters (120'INS, ΣINS, HOMA-IR, 1/QUICKI, 1/ISI) and glucose response (120'VC, ΣVC) and parameters of atherogenic

dyslipidaemia (TG, HDL-C) using multiple linear regression analysis. Significant positive associations were found between both reactive glucose parameters and all reactive insulinemia parameters as well as insulin resistance parameters. From the lipoprotein parameters, TG showed significant positive association with the reactive insulinemia parameters as well as insulin resistance parameters, and negative associations were demonstrated between HDL-C and ΣINS as well as between HDL-C and 1/ISI. (p-values were: <0.05 - <0.0001).

5. Discussion

5.1. Alterations of carbohydrate metabolism in childhood obesity.

For investigation of carbohydrate metabolism of obese children and adolescent, we used the determination of FBG, fasting insulin concentration and HOMA-IR. Using WHO and ADA/IDF criteria we determined IFG, using FBG and basal insulin concentration we conclude for the insulin resistance and for the hypersecretion of the pancreatic beta-cells.

In this study IFG was not found. In spite of normal FBG, insulin concentration and HOMA-IR of investigated obese children was elevated in 80% of cases, hypersecretion of the pancreatic beta-sells as regard of HOMA-BCF was detected in 90% of the cases. The fact that alterations of glucoregulation were substantially less than insulin resistance, basal and reactive hyperinsulinemia suggest that hyperinsulinemia can successfully compensate insulin resistance in majority of the obese children.

This methode give not information about prevalence of IGT. OGTT inform us about alterations of glucoregulation (IGT, IFG and T2DM). Using HOMA-IR together with OGTT, complex investigation of carbohydrate metabolism is possible.

In our study insulin resistance based on elevated HOMA-IR index was found in 78% of the investigated children. Basal hyperinsulinemia (0'INS) was detected in 70% of the patients, reactive hyperinsulinemia (120'INS) was found in 88 % of the cases.

In spite of the high frequency of insulin resistance, basal and reactive hyperinsulinemia, frequencies of abnormal glucoregulations in childhood obesity is substantially less. The ratio of IFG according to WHO criteria was only 1.2% and according to IDF was 4.4% but IGT was found in 13.6% of the investigated patients. These results correlate with previous Hungarian study and our earlier study too, on the other hand prevalence of IGT in childhood and adolescent obesity is more than 20% by some of the recent studies. The fact that alterations of glucoregulation were substantially less than insulin resistance, basal and reactive hyperinsulinemia suggest that hyperinsulinemia can successfully compensate insulin

resistance in majority of the obese children. Our results demonstrated T2DM in 2,4% of the investigated obese children. In previous Hungarian studies the prevalence of T2DM in childhood obesity was 1-2%. The same prevalence was found in two European study, however among American adolescents with obesity a prevalence of 4% was described.

We investigated the prevalence of insulin resistance, basal and reactive hyperinsulinemia in each category of glucoregulation alterations. In all patients with IFG, evaluated either by WHO or by ADA/IDF criteria, HOMA-IR values and reactive insulin levels were elevated, and the same results were found in patients with T2DM. On the other hand, in children with IGT elevated HOMA-IR index was found in 79%, while reactive insulin levels were elevated in all cases. This results show that reactive hyperinsulinaemia is a sensitive parameter of the alterations of glucoregulation and confirm the divers mechanism of IFG and IGT. While in IFG first phase of insulin response is decreased and the glycogenolysis in the liver is elevated, thus far in IGT insulin resistance is dominant, mainly in the skeletal muscles.

Association among the number of patients belonging to different 0'BG and 120'BG categories was also studied and a significant association was found. However, prevalence of IGT was substantially higher then prevalence of IFG: 11 times according to WHO criteria, 3 times according to ADA/IDF criteria. This means that in most IGT cases normal fasting glucose levels can be measured. This results show that the OGTT is a more sensitive and reliable method to define alterations of glucoregulation than the measurement of fasting glucose, even if the more severe IFG cut-off value is used. Therefore, this fact has to be taken in account in investigating alterations of glucoregulation in childhood obesity. The ADA recommends a selective screening of glucose metabolism in children with overweight and obesity (BMI >85th percentiles for gender and age) if the risk of T2DM increased (any two of the following risk factors: family history of T2DM, race/ethnicity, puberty, signs/symptoms of insulin resistance). The ADA recommends the measurement of the fasting glucose as screening tool, and OGTT in recommended only in the case of IFG. Our results suggest that an OGTT may be needed to identify those at risk of developing abnormal glucoregulation and T2DM. Previous investigations of IFG showed that the normal fasting glucose cut-off value (6.0 mmol/l) is not sensitive enough. ADA recommended in 2003. in his professional statement a cut-off value of 5.6 mmol/l. The IDF recently has used this cut-off value in establishing its new diagnostic criteria of metabolic syndrome. This is why we investigated prevalence of IFG using the cut-off value 5.6 mmol/l too. According to these evaluation method, prevalence of IFG was higher than it was by WHO criteria 4.4% of investigated children. However, even this prevalence was substantially less than the prevalence of IGT.

Since IFG is less frequent than IGT, there is a need for performing OGTT to demonstrate abnormalities of glucoregulation in children with obesity. In addition, for investigation of fasting glucose levels of overweight and obese children to detect IFG using IDF criteria, i.e. the cut-off value of 5.6 mmol/l is recommended.

5.2. Alterations of lipoprotein metabolism and atherogenetic factors in childhood and adolescent obesity

The role of the dyslipidaemia in the development of atherogenesis is well known, as well as the fact, that atherogenesis starts already in childhood. This is why we investigated alterations of lipoprotein metabolism in childhood and adolescent obesity.

Prevalence of abnormal values of alteration of lipoprotein metabolism varied in the presented studies. The variability is due to the differences of the investigated groups. On the other hand, our results prove that atherogenic dyslipidemia is a frequent condition of childhood obesity.

In a recent study we documented elevated ratio of atherogenic dyslipidaemia, 18% of investigated obese children and adolescent had increased TG and decreased HDL-C values. Several mechanisms how insulin resistance and hyperinsulinemia could cause an alteration in lipoprotein metabolism have been described. In our studies, insulin resistance based on elevated HOMA-IR was found in 78%-84% of the investigated children. Basal hyperinsulinemia (0' INS) was detected in 73%-86% of the patients, and reactive hyperinsulinemia (120'INS) was increased in 89%. In our first two studies significant positive correlation between TG and HDL-C to INS and HOMA-IR was not found. In a numerous third investigation significant positive correlation between HOMA-IR and parameters of lipoportein metabolism (T-C, TG) as well as significant negative correlation HOMA-IR and HDL-C was found. Significant positive correlation was found between elevated TG levels and all of the parameters of insulin response and insulin resistance and significant negative correlation between HDL-C and ΣINS and 1/ISI. In a recent study we found significant positive correlation between insulin values to TG levels, significant negative correlation between TG and HDL-C levels.

In the study showed in 4.2. concentration of apoA-I and apo100-B was measured. Mean values of concentration of apoB-100 was normal, elevated ratio correlated to the ratio of elevated LDL-C. Average value of apoA-I concentration was also normal, decreased ratio correlated to the ratio of decreased HDL-C values. We investigated the Lp(a) levels in obese children and adolescent, in 30% of the cases elevated Lp(a) was found. There was no significant correlation between investigated parameters and Lp(a) concentration. This correlated with the fact, that Lp(a) is a genetically determined lipoprotein fraction.

During the development of atherogenic dyslipidaemia, HDL functions are degradated. HDL-C play an essential role of reverse cholesterin transport, its important function, that enzymes related to HDL-C decrease the oxidation of LDL and elevation of oxidative LDL-C levels and inhibit the elevation of oxidative LDL-C mediated production of adhesion molecules.

This is why we investigated the atherogenic factors related to HDL-C function in childhood and adolescent obesity.

There was no significant correlation between the grade of insulin resistance/hyperinsulinaemia and HDL levels or activity of atherogenic factors related to HDL-C function (PON1, ARYL, CETP) or serum levels (ICAM-1, VCAM-1). There was no significant difference between these parameters as regard the subgroups of children with OS and OAN. It is explained that no differences between HOMA-IR and IRI was documented in both of the subgroups.

We compared the result of HDL-C related factors obtained from obese children with normal and abnormal (decreased) HDL-C subgroups. No significant difference was not found between the two subgroups as regard the HDL-C related factors activity (PON1, ARYL, LCAT. Serum concentration of ICAM-1 was significant higher in children with decreased HDL-C levels, than in normal HDL-C level subgroup. The same tendency was according to the serum concentration of VCAM-1 but the difference was not significant. Significant positive correlation was found at the same time between ICAM-1 and VCAM-1 concentrations, showing the same effect of these enzymes. In other studies, investigated numerous groups of children, positive correlation was documented between insulin resistance and concentration of ICAM-1. We emphasize also that significant negative correlation was found between PON1 activity and the concentrations of ICAM-1. We found elevated activity of CETP in children with decreased HDL-C values, but the difference was not significant between the two subgroups. Obese children characterized by metabolic syndrome components increased concentration of CETP documented, which showed correlation to the decrease of HDL-C. Remarkable is the significant positive correlation between CETP activity and VCAM-1 concentration, which suggest the common influencing mechanism(s). However, the results suggest that atherogenic dyslipidemia has a complex influence to the parameters of HDL function in childhood obesity. Decrease of HDL-C has an important role of its development.

5.3. Alterations of carbohydrate and lipoprotein metabolism. The role of insulin resistance/hyperinsulinaemia.

Insulin resistance and hyperinsulinemia are frequent conditions in childhood obesity. Insulin resistance, which is a decreased response of insulin sensitivity tissues to insulin, is generally accepted as the "primum movens" in the development of metabolic alterations demonstrated in childhood obesity. In recent studies in children, insulin resistance and hyperinsulinemia were implicated in the association of obesity with abnormal glucose metabolism, dyslipidemia, hypertension, that is with the characteristic cluster of metabolic syndrome.

In our study for estimating insulin resistance the HOMA was performed and HOMA-IR was used as a marker for insulin resistance, besides the QUICKI and the Matsuda ISI were also calculated. However, the euglycemic-hyperinsulinemic clamp is the gold standard for investigating insulin resistance, several validation studies are now available demonstrating a good correlation of the HOMA-IR with euglycemic-hyperinsulinemic clamp technique. For this reason, the method is widely used to predict insulin resistance in childhood obesity.

In our study, strong significant associations were found among insulin resistance/sensitivity indices. To detect the hyperinsulinemia in children with obesity, not only the fasting insulin level but also the insulin concentrations during OGTT (120'INS and ΣINS) were investigated and in one of our studies ΣINS were also determined. These parameters detect hyperinsulinaemia. In our first study we calculated HOMA-BFC using FBG and basal insulin levels, to determine thy pancreas beta-cell function. HOMA-IR values were most our studies elevated. The ratio of basal hyperinsulinaemia was similar; the ratio of reactive hyperinsulinaemia was higher. The fact that alteration of glucoregulation was substantially less than insulin resistance, basal and reactive hyperinsulinemia, suggests that hyperinsulinemia can successfully compensate for insulin resistance in the majority of obese children. The patients with abnormal glucoregulation are in danger of developing T2DM, but using adequate interventions at this time, diabetes can be prevented.

Insulin resistance was associated with reactive increase of serum insulin and blood glucose during OGTT: significant positive correlations were found between HOMA-IR and 120'INS, ΣINS, 120'BG as well as ΣBG). In addition, significant associations were demonstrated between reactive insulinemia and reactive blood glucose parameters by multiple linear regression analysis. It is known that the relationship between insulin sensitivity and acute insulin release in response to glucose is regulated by a negative feedback, and this hyperbolic relationship has been described as glucose disposition index (DI). Its decrease, according to the "hyperbolic law of glucose tolerance", leads to IGT and, eventually, T2DM.

We have to go into details of transitory decreased insulin sensitivity and elevated hyperinsulinaemia in puberty. In our study investigated obese adolescent, HOMA-IR was significantly higher in children in puberty stadium T4 by Tanner. Ratio of prevalence of elevated HOMA-IR between the adolescent in different puberty stadium was not significant.
Our results are in accordance with another study, which documented in obese children more elevated insulin resistance in case of puberty stadium T4, which was not depending to grade of obesity. In the development of pubertal insulin resistance sex hormones and increased production of growth hormone play an essential role.
Association of obesity and dyslipidaemia in childhood is well documented; insulin resistance and hyperinsulinaemia play an important role in it. In our studies we documented the alterations of lipoprotein metabolism, characterized by so called atherogenic dyslipidaemia, elevated TG and decreased HDL-C levels. Hyperinsulinemia is known to enhance hepatic VLDL synthesis and thus directly contributes to the increased plasma TG and HDL-C levels; resistance to the action of insulin on lipoprotein lipase in peripheral tissues may also contribute to elevated TG and LDL-C levels, and insulin resistance may be responsible for the reduced level of HDL-C, by an increased rate of HDL-C degradation. In our study we found significant positive correlation between HOMA-IR values and T-C as well as TG levels. Significant negative correlation was found between HOMA-IR values and HDL-C concentrations. This, according to data from the literature, confirms the determining role of insulin resistance in the development of atherogenic dyslipidaemia, as much as the role of reactive hyperinsulinaemia. In our studies significant positive association was found between TG and 120'INS as well as ΣINS and significant negative association between HDL-C and ΣINS using multiple linear regression analysis.
Investigated the atherogenic factors related to HDL-C function in childhood obesity there was no significant correlation between insulin resistance, grade of hyperinsulinaemia and the HDL-C levels, or factor activities related to HDL-C function (PON1, ARYL, CETP) or serum levels (ICAM-1, VCAM-1). In the subgroup of obese children with abnormal HDL-C levels, factors related to HDL-C function were different from normal: ICAM-1 concentration was significant higher, tendency of elevation of VCAM-1 concentration and CETP activities was documented, significant negative association between ICAM-1 concentration and PON1 activity was found, significant negative association between VCAM-1 concentration and CETP activity was documented. PON1, LCAT and CETP activities as well as ICAM-1 and VCAM-1 concentrations did not differ in the groups with normal or decreased HDL-C level. There was no correlation of atherogenic factors activity/ serum levels to insulin resistance.

However, the results suggest that atherogenic dyslipidemia has a complex influence to the parameters of HDL function in childhood obesity. Decrease of HDL-C has an important role of its development.

5. 4. Impact of AN in childhood and adolescent obesity

Childhood obesity due to the insulin resistance and hyperinsulinaemia is a risk factor to developing T2DM. AN often associated with childhood obesity. Children with obesity associated with AN frequently have insulin resistance. This was found especially in certain ethnic groups like American Indian, Mexican, Afro-American and Japanese population, and the tendency was also demonstrated in the Caucasian population. AN is considered as an important risk factor for T2DM and a physical marker of insulin resistance in childhood obesity.

In our study we investigated insulin resistance in childhood obesity associated with OS and OAN, to detect the prevalence of hyperinsulinaemia basal insulin levels and insulin concentration during OGTT was measured. HOMA-IR was elevated 85% of the investigated children. 0'INS was elevated in 82 of 113 obese children (73 %). Increased 120'INS was found in 101 of 113 cases (89.3 %). As regard these results, insulin resistance is compensated by hyperinsulinaemia. IFG was detected in 5.2% of the investigated children using WHO criteria, IGT was documented using OGTT in 15,6% of the investigated obese children. Elevated T-C and LDL-C levels were found in more than 20% of the children. Ratio of the children with elevated TG levels were 25%, decreased HDL-C was detected in 40% of children.

Between HOMA-IR and T-C as well as TG levels significant positive correlation, between HOMA-IR and HDL-C significant negative correlation was found, this result suggest the association between insulin resistance, hyperinsulinaemia and alterations of lipoprotein metabolism.

In our study 120' INS and ΣINS values were significantly higher in obesity with AN than in simple obesity. HOMA-IR did not differ in the two groups but ISI was lower in OAN compared to SO. The frequencies of abnormal basal and reactive hyperinsulinemia, IFG and IGT as well as increased HOMA-IR values did not significantly differ in the two subgroups, although ratios of abnormal results were higher in cases with AN. However, it was ascertained that all children with severe AN (grade 4) had an increased HOMA-IR. These results prove an important role of the reactive hyperinsulinemia in development of AN, and they suggest that in the cases with sever AN the probability of insulin resistance is high.

As regards to lipoprotein metabolism, significant differences were found in TG and HDL values between the subgroups of SO and OAN. In addition, the frequencies of increased TG and decreased HDL-C were higher in the subgroup of OAN compared to SO, according to the study presented in 4.2.1

These results suggest that atherogenic dyslipidemia can be more pronounced and alterations of lipoprotein and carbohydrate metabolism are frequently found in childhood obesity if it is associated with AN. Severe AN in childhood obesity can be considered as a physical marker of insulin resistance.

6. Conclusion

- Insulin resistance, based on elevated HOMA-IR, basal and reactive hyperinsulinemia were found in vast majority of the investigated children.
- The fact that alteration of glucoregulation was substantially less than insulin resistance, basal and reactive hyperinsulinemia, suggests that hyperinsulinemia can successfully compensate for insulin resistance in the majority of obese children. In obese adolescent insulin resistance and hyperinsulinemia essentially due to the obesity itself, however puberty can also contribute to their development.
- In our studies the frequencies of IFG, IGT and T2DM were in accordance with other European studies performed in childhood obesity.
- Insulin resistance and hyperinsulinaemia has important role in the development of alterations of carbohydrate metabolism in childhood obesity
- Since IFG was less frequent than IGT, there is a need for performing OGTT to demonstrate abnormality of glucoregulation in obese children, also when using ADA criteria to define IFG.
- Obese children investigated in our studies were characterised a rather high frequency of atherogenic dyslipidemia, i.e. elevated TG and decreased HDL-C levels beside of elevated basal and reactive insulin levels and elevated HOMA-IR, suggesting insulin resistance.
- Ratio of pathological apoB-100 and apoA-I values correlate to lipoprotein alterations in childhood obesity. Elevated ratio of Lp(a) levels in obese children need further investigation.

- Paraoxonase, LCAT and CETP activities as well as ICAM1 and VCAM1 concentrations did not differ in the groups with normal or decreased HDL-C level. There was no correlation of atherogenic factors activity/ serum levels to insulin resistance.
- However, the results suggest that atherogenic dyslipidemia has a complex influence to the parameters of HDL function in childhood obesity. Decrease of HDL-C has an important role of its development.
- In our study 120' INS and ΣINS values were significantly higher in obesity with AN than in simple obesity. HOMA-IR did not differ in the two groups but ISI was lower in OAN compared to SO. The frequencies of abnormal basal and reactive hyperinsulinemia, IFG and IGT as well as increased HOMA-IR values did not significantly differ in the two subgroups, although ratios of abnormal results were higher in cases with AN. However, it was ascertained that all children with severe AN (grade 4) had an increased HOMA-IR.
- These results prove an important role of the reactive hyperinsulinemia in development of AN, and they suggest that in the cases with sever AN the probability of insulin resistance is high.
- As regards to lipoprotein metabolism, significant differences were found in TG and HDL values between the subgroups of SO and OAN. In addition, the frequencies of increased TG and decreased HDL-C were higher in the subgroup of OAN compared to SO.
- These results suggest that atherogenic dyslipidemia can be more pronounced in childhood obesity if it is associated with AN.
- Severe AN in childhood obesity can be considered as a physical marker of insulin resistance.

Own publications thesis based on (in Hungarian)

1. Juhász É, **Felszeghy E**, Ilyés I. A szénhidrát anyagcsere vizsgálata serdülőkorú elhízott gyerekekben. Diabetologica Hungarica 2004; 12: 281-286.

2. **Felszeghy E**, Fejes M, Ilyés I. Inzulinrezisztencia és atherogén dyslipidaemia gyermekkori elhízásban. Metabolizmus 2005; 3: 28-31.

3. **Felszeghy E,** Juhász É, Káposzta R, Ilyés I. A glükoreguláció zavarai gyermekkori elhízásban, összefüggés az inzulinrezisztenciával és hyperinzulinaemiaval. Metabolizmus 2008; 6: 234-238.

4. **E Felszeghy**, E Juhasz, R Kaposzta, I Ilyes. Alterations of glucoregulation in childhood obesity – association with insulin resistance and hyperinsulinemia J Pediatr Endocrinol Metab. 2008; 21: 847-853. **IF:0,938**

5. Ilyés I, **Felszeghy E**, Váradi Z, Seres I, Paragh Gy. Hyperinsulinaemia /inzulinrezisztencia, atherogen dyslipidaemia és az atherogenesis egyéb faktorai gyermekkori egyszerű és acanthosis nigricans-szal társult elhízásban. Metabolizmus. 2009; 7: 106-110.

6. **E Felszeghy**, R Kaposzta, E Juhasz, L Kardos, I Ilyes: Alterations of carbohydrate and lipoprotein metabolism in childhood obesity. Impact of insulin resistance and acanthosis nigricans. J Pediatr Endocrinol Metab. 2009; 12: 1117-1126. **IF:0,938**

Own publication related to the thesis (in Hungarian)

1. Ilyés I**, Felszeghy E**, Oláh A. Az acanthosis nigricans és az inzulinrezisztencia összefüggése gyermekkori elhízásban. Diabetológia Hungarica 2002; 10: 197-200.

2. Kosaras É, **Felszeghy E**, Ilyés I. A szénhidrát- és lipoprotein-anyagcsere eltérései gyermekkori elhízásban. Gyermekgyógyászat 2003; 54: 20-26.

3. Molnár A, **Felszeghy E**, Oláh A, Ilyés I. A szénhidrát- és lipoprotein-anyagcsere eltérései gyermekkori elhízásban acanthosis nigricans (AN) társulása esetén. Gyermekgyógyászat 2003; 54: 501-508.

4. Józsa L, **Felszeghy E**, Gönczi F, Ilyés I. Életmódtábor a gyermekkori elhízás komplex kezelésére. Egészségtudomány 2004; 48: 309-318.

5. Ilyés I, **Felszeghy E.** 2-es típusú diabetes mellitus gyermek és serdülőkorban. Gyermekorvos Továbbképzés 2004; 12: 34-39.

Own publications not used in the thesis (in Hungarian)

1. Nagy B**, Felszeghy E.** Asthmás gyermekek Retaphyllin kezelése. Gyermekgyógyászat 1995; 46: 343-349.
2. **Felszeghy E**, Nagy B. Az idiopathiás pulmonalis haemosiderosis diagnosztikus és terápiás nehézségei négy eset kapcsán. Gyermekgyógyászat 1995; 46: 36-39.
3. **Felszeghy E,** Nábrádi Z, Nagy B, Nemes Z. Pulmonális lymphangiomyomatosis esete 11 éves lánygyermekben. Gyermekgyógyászat 1997; 48: 124-127.
4. Galuska L, **Felszeghy E**, Szakáll Sz, Garai I, Trón L. Spongioblastoma kimutatása különböző nukleáris medicinai módszerekkel. Pediáter 1998; 7: 7-9.
5. Kiss Cs, Szegedi I, Jakab Zs, Kiss M, **Felszeghy E**, Oláh É. Kolónia-stimuláló faktorok alkalmazása másodlagos granulocytopeniás gyermekekben. M Belorv Arch. 1998; 51, 427-432.
6. Nagy B, Varga A, **Felszeghy E.** A bronchoalveoláris mosás jelentősége alsó légúti fertőzések kórismézésében. Gyermekgyógyászat 1999; 50: 79-82.
7. Varga A, Nábrády Z, **Felszeghy E**, Nagy B. Requiem a bronchographiaért két eset kapcsán. Gyermekgyógyászat 1999; 50: 144-149.
8. **Felszeghy E**, Ilyés I, Fazekas K. Pseudohypoparathyroidismus gyermekkori esete. Gyermekgyógyászat 2000; 51: 168-171.
9. Nagy A, **Felszeghy E**. D-penicillaminnal és fototerápiával sikeresen kezelt Rh-isoimmunisatio. Gyermekgyógyászat 2000; 51: 81-83.
10. Ilyés I, Káposzta R, **Felszeghy E**, Nagy B, Nagy E. Autoimmun polyendocrinopátia gyermekkori esete. Gyermekgyógyászat 2000; 51: 375-381.
11. Ilyés I, **Felszeghy E.** Lehetőség a cardiovascularis betegségek megelőzésére? Az intrauterin retardáció késői anyagcsere és endokrin konzekvenciái. Magyar Alapellátási Archívum 2000; 3: 89-93.
12. Ilyés I, **Felszeghy E.** A növekedési hormon (GH) – inzulinszerű növekedési faktor (IGF-I) tengely működésének változása gyermekkori elhízásban. Diabetologica Hungarica 2001; 11: 7-12.
13. **Felszeghy E**, Mogyorósy G, Soós Gy, Bágyi P, Lukács G, Ilyés I. Primer hyperaldoszteronizmus gyermekkori esete. Gyermekgyógyászat 2003; 54: 509-515.
14. Orosz A, **Felszeghy E**, Ilyés I. Bioimpedancia és anyagcsere vizsgálatok gyermekkori elhízásban. Medicus Universalis 2004; 27: 69-72.

15. Juhász É, Majnik É, **Felszeghy E**, Ilyés I, Rácz K. PROP1 mutáció következtében létrejött multiplex hypophysishormon-hiány. Gyermekgyógyászat 2005; 56: 570-574.
16. Juhász É, **Felszeghy E**, Ilyés I. A hashitoxicosisról gyermek- és serdülőkorban. Gyermekgyógyászat 2006; 57: 478-481.
17. Szima S, **Felszeghy E**, Lenkey Á, Ilyés I. A juvenilis lymphocytas thyreoiditis (JTCL) felismerése és gondozása. Gyermekgyógyászat 2006; 57: 432-439.

Abstracts of posters

1. Ilyés I, **Felszeghy E**, Molnár A, Jancsó Z. Insulin resistance, alterations of carbohydrate and lipoprotein metabolism in children with obesity and acanthosis nigricans, 42nd Annual meeting of the European Society for Pediatric Endocrinology (ESPE), Ljubjana, Slovenia, 18-21. September. 2003, Horm Res. 2003; 60(S2): 94
2. Erhardt É, Csernus K, **Felszeghy E**, Ilyés I, Molnár D. Impaired glucose tolerance and type 2 diabetes in obese children. 12th European Congress on Obesity, Helsinki, Finland, 29 May-1 June. 2003, Int J Obes Relat Metab Disord. 2003; 27(S1): 24.
3. Csernus K, Erhardt É, **Felszeghy E**, Ilyés I, Molnár D. The prevalence of disorders of carbohydrate metabolism in clinically healthy obese children. 13th European Childhood Obesity Group Workshop, Tenuta Moreno, Mesagne (BR), Italy, 25-27. Sept. 2003, Int J Obes. 2003; 27(S1): 78.
4. **E Felszeghy**, J Jakkel, Z Jancso, I Ilyes. Insulin resistance, alteration of carbohydrate and lipoprotein metabolism in simple obesity and obesity with acanthosis nigricans in childhood, 44th Annual Meeting of the LWESPE (Lawson Wilkins Pediatric Endocrine Society)/ESPE, Lyon, Horm Res. 2005; 64(S1): 273.
5. **E Felszeghy**, J Jakkel, Z Jancso, I Ilyes. Insulin resistance, alteration of carbohydrate and lipoprotein metabolism in childhood simple obesity and obesity with acanthosis nigricans, 5th ECACN Meeting, 28-29 october 2005 in Budapest, J Am Coll Nutr. 2006; 25(N1): 78.
6. **E Felszeghy**, I Ilyes. Alteration of glucoregulation in childhood obesity, relation to insulin resistance and hyperinsulinemia, 45th Annual meeting of the ESPE, Rotterdam, Horm Res. 2006; 65(S4): 83.
7. **E Felszeghy**, Z. Varadi, I Ilyes, Gy Paragh. Insulin resistance, atherogenic dyslipidaemia, and other atherogenic factors in simple obesity and obesity with

acanthosis nigricans (AN) in childhood, 46th Annual Meeting of the ESPE, Horm Res. 2007; 68(S1): 134.

8. I Ilyes, **E Felszeghy**, Z Varadi, I Seres, Gy Paragh. Insulin resistance, atherogenic dyslipidemia, and other atherogenic factors in simple obesity and obesity with acanthosis nigricans (AN) in childhood, European Congress of Endocrinology, Berlin, 3-7 May, 2008, Endocrine Abstracts, 2008; 16: 497.
9. M F Hartmann, **E Felszeghy**, S Wudy. Diagnostic potential of GC/MS urine steroid profiling in the diagnosis of CAH due to 21-OH deficiency in neonates and infants, European Congress of Endocrinology, Berlin, 3-7 May, 2008, Endocrine Abstracts 2008; 16: 20.
10. F Péter, A Luczay, E Sólyom, B Gellén, T Niederland, **E Felszeghy**, L Ságodi, J Sólyom, Á Muzsnai. Growth hormone deficient children born small-for-gestational-age need higher than replacement dose of hGH for successful treatment, European Congress of Endocrinology, Berlin, 3-7 May, 2008, Endocrine Abstracts 2008; 16: 584.

International congress presentations

1. **E. Felszeghy**, A Molnar, A Olah, I Ilyes. Alteration of carbohydrate and lipoprotein metabolism in childhood obesity with acanthosis nigricans, MESPE 2003 Kőszeg
2. **E Felszeghy**, A Molnar, A Olah, I Ilyes. Alterations in carbohydrate and lipoprotein metabolism in childhood obesity associated with acanthosis nigricans. 6th International Congress on Prevention of Atherosclerosis in Childhood, 26-27.03.2004, Budapest
3. **E Felszeghy,** E Balogh, I Ilyes, A Ujfalusi, E Olah. X chromosome structural abnormalities in a family: the role of fluorescence in situ hybridization (FISH) in the definitive diagnosis, MESPE 2006 Pozsony
4. **E Felszeghy,** M F Hartmann, S Wudy. Diagnostic potential of GC-MS urinary steroid profiling in the diagnosis of CAH due to 21-OH deficiency in neonates and infants - Jubilee meeting of the Hungarian Section for Paediatric Endocrinology (HSPE) (1982-2007) as a Satellite Symposium of the 9th European Congress of Endocrinology Budapest, Hungary – 27. April 2007.
5. **Felszeghy E**, Paragh Gy, Ilyes I. Insulinresistance, atherogen dyslipidemia and other atherogenic factors in simple obesity and obesity with acanthosis nigricans in

childhood, 8th ECACN Meeting with the European Association of Paediatric Education (EAPE) about the Prevention, Pathophysiology and Treatment of Premature Chronic Illnesses in Budapest at May 14-15, 2009

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