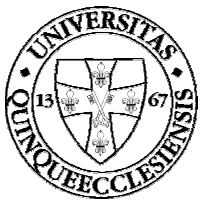
CHILDHOOD OBESITY: CAUSES AND CONSEQUENCES

PhD Thesis

Éva Erhardt MD



Department of Paediatrics, Medical Faculty, University of Pécs

2007

CHILDHOOD OBESITY: CAUSES AND CONSEQUENCES

PhD Thesis

Éva Erhardt MD

Department of Paediatrics, Medical Faculty, University of Pécs

Programme leader: Dénes Molnár MD, PhD, Dsc

2007

INTRODUCTION

An alarming rise in overweight and obesity was observed worldwide, particularly during the 1990's, in both the developed and developing countries. In Hungary, the prevalence of obesity increased from 12% to 16 % between 1980' and 1990's among schoolchildren. Obesity is a serious health risk; it is a major determinant of different diseases such as diabetes mellitus, hypertension, heart and kidney failure, atherosclerosis, cancer, infertility, birth complications and arthritis. On the other hand, obesity is largely preventable through changes in lifestyle.

Obesity is a heterogeneous group of conditions with multiple causes. Body weight is determined by an interaction between genetic, environmental and psychosocial factors.

Obesity is one of the features accompanying numerous genetic syndromes, like Prader-Willi (PWS), Cohen, Alstrom and Bardet-Biedl syndrome that have been genetically mapped. The first gene identification in obese human subjects was linked with the screening for genes identified previously in rodent models of monogenic obesity. However, the genetic susceptibility to obesity is in most cases polygenic, and is rarely the result of a Mendelian gene (monogenic obesity). Apart from rare obesity-associated syndromes, the genetic influences seem to operate through susceptibility genes. One of the two approaches to identify susceptibility genes is the candidate gene approach. It involves testing the association between obesity and a specific allele of a gene either in a family study or in a large cohort of unrelated controls and patients. Several candidate genes have been associated with human obesity and its metabolic complications, which include e.g. receptors that are important in thermogenesis (β3-adrenergic receptor /3-BAR / gene and the family of uncoupling proteins), as well as those involved in appetite regulation.

The effects of catecholamine are modulated through four subtypes of adrenoreceptors. The human 3-BAR is expressed predominantly in visceral fats, where it plays a role in determing the resting metabolic rate through its ability to stimulate lipolysis and thermogenesis. The involvement of 3-BAR in energy metabolism originates from observations indicating the prevalence of the genetic variant at the codon 64 of the 3-BAR gene leads to replacement of a tryptophan with an arginine (Trp64Arg). This polymorphism has been reported to be associated with abdominal obesity, a propensity for weight gain, high BMI, insulin resistance and an earlier onset of type 2 diabetes mellitus. Other studies, however, could not demonstrate these associations.

An obese child will remain obese in adulthood and become at risk for acquiring or increasing coronary heart disease in 30 % to 60 % of cases. Although genetics may have a part of it,

several environmental factors affect this phenomenon significantly. The first contact of the child with the environment is in the uterus. Over- or under-feeding in pregnancy has been associated with the development of obesity in later life. Beside of the prenatal period, the other critical period is the adolescence in the development and persistence of overweight in the paediatric age group. The period of 'adiposity rebound' may constitute a third. It is when the BMI begins to increase after reaching a nadir in early childhood. Small for gestational age (SGA) has been associated with increased risk of diseases such as diabetes or cardiovascular disease in adulthood. Infants with high birth weights appear to have an increased risk of subsequent overweight.

The presence of inherited and acquired thrombophilias has recently been linked to most cases of maternal venous thrombotic events as well as these adverse obstetric outcomes. One of the most common inherited thrombophilias is heterozygosity for the factor V Leiden mutation. Although there is no consensus on the association between the factor V Leiden mutation and early pregnancy loss, but the evidence suggests an association between the mutation and late (first-, second-, third-trimester) fetal loss, severe preeclampsia, abruption and severe intrauterine growth retardation (IUGR).

Over the past two decades, many papers have demonstrated in the literature that low birth-weight, thinness and short body length at birth are associated with increased risk of atherosclerosis, type 2 diabetes mellitus (T2DM), hypertension and metabolic syndrome. This observation, which originated at Hertfordshire in the United Kingdom, was confirmed in other countries such as Sweden and Netherlands. The association of low birth-weight with adult cardiovascular disease led to the 'fetal origins or thrifty phenotype hypothesis' formulated by Barker.

Obese children have a higher prevalence of T2DM and insulin resistance and the frequency of this complication appears to have risen in recent years paralleling the worldwide increase in obesity in this age group. On the basis of the available data, the prevalence of T2DM in Caucasian children and adolescents seems to be much lower then those reported in other races, but more representative, population-based surveys are needed. In April 2002, a questionnaire was distributed among European Childhood Obesity Group (ECOG) representatives from 16 European countries, which included several questions concerning the prevalence, risk factors and complications of childhood obesity, such as T2DM. From nine countries, altogether 184 European children were diagnosed with T2DM, 144 of them of Caucasian origin. The majority of them were overweight females and had positive family history for T2DM. Although general principles of treatment of T2DM in adolescents are

similar to the treatment of adults there is general agreement between the paediatricians dealing with this problem that they should not be a simple extrapolation. Treatment strategies should be based on symptoms at presentation. Asymptomatic children identified at routine testing should be counselled on the necessary lifestyle changes. Therapeutic strategies include lifestyle and behaviour modification, nutrition education, and psychological and family therapy interventions. Because obesity is the major problem in most adolescents with T2DM, dietetic advice is mandatory, although calorie intake should not be too restricted to ensure normal growth and pubertal development. Patients should be encouraged to increase their physical activity or at least to decrease inactivity.

Childhood onset of adult cardiovascular disease has become a significant public health problem that needs to be addressed globally and individually. Whether genetic, environmental, or fetal influences are the primary culprits in the epidemic of obesity-related adult cardiovascular diseases seen today remains unknown. In spite of this, the interventional focus should be placed on early life, and health care providers and public health professionals should pay attention to the elevated future coronary heart disease risk among children. Better understanding of the aetiology of these diseases hopefully will lead to more effective, targeted preventive measures and therapy.

AIMS

1. Trp64Arg polymorphism of the β3-adrenergic receptor gene

1.1 To examine the frequency of Arg64 allele of the β_3-adrenergic receptor (3-BAR) gene, which is one of the known candidate genes, in healthy and obese Hungarian children.

1.2 To look for possible associations between this polymorphism and some clinical and metabolic characteristics of obese children.

2. Leiden mutation; size at birth and later risk factors

2.1 To test the prevalence of Leiden mutation in the mothers of premature infants and in the mothers of intrauterine-growth-retarded children.

2.2 To determine the association between size at birth and later risk factors (hypertension, hyperinsulinism, hyperglycaemia, dyslipidaemia) in prepubertal children.

3. Impaired glucose tolerance and type 2 diabetes mellitus

3.1 To examine the prevalence of impaired glucose tolerance (IGT) and T2DM in obese Hungarian children.

3.2 To assess the effects of a 6-months diet and life-style changes in the children with IGT and T2DM.

SUBJECTS

Written informed consents were obtained from the subjects and all parents of the children before enrolment in the different studies. All of the studies were approved by the ethic review committee of the University of Pécs.

1.1 In all, 295 obese children (male:168) were included in the study after the exclusion of any endocrinological disorder, nutritional-, growth- and renal problems or obesity syndromes. A total of 147 healthy, non-obese children (male: 68) recruited from elementary schools, served as controls. The average age of the children in the two groups was 12.6±3.2 and 12.4±1.7, respectively.

1.2 Obese children carrying the Arg64 allele (n=35, male: 23) were compared to randomly chosen, obese children without the Arg64 allele (n=35, male: 20).

2.1 White (Caucasian) mothers of premature (Group PM; n=50) and mothers of intrauterine growth retarded neonates (Group IUGR; n=56) were tested. The newborns were considered as premature when their gestational age was < 37 weeks. Intrauterine growth retarded children were born full term with birth weight, height and head circumference below the 10^{th} centile (proportional) or with birth weight below the 10^{th} centile, but with normal length and head circumference (disproportional).

2.2 229 children (134 boys, 95 girls) were examined at the age of 6-10. We compared children born full term with normal weight, height and head circumference (1st group), the children born full term with birth weight, height and head circumference less than 10th centile (2nd group), children born full term with birth weight less than 10th centile and with normal length and head circumference (3rd group) and children who were preterm at birth (4th group). The age of children at the time of investigation was comparable in the four groups.

3.1-2 Oral glucose tolerance test (OGTT) was performed in 289 obese (153 boys) (mean BMI±SD: 31.1±4.6 kg/m^2) adolescents (mean age±SD, 12.9±2.7 years). After 6 months, the OGTT was repeated in children with IGT and DM.

METHODS

Anthropometric measurements

Weight was obtained with subjects wearing light clothing to the nearest 0.1 kg on a standard beam scale. Height was measured to the nearest 0.1 cm by a Holtain stadiometer. In all examinations, children were considered as obese if their body weight exceeded the expected weight for height with more than 20%, and if body fat content with more than 25% in males and 30% in females. BMI was calculated according to the formula, real weight (kg)/height^2(m^2), while body fat (BF) was estimated from skinfold measurements, which were performed with a Holtain caliper, using Parizkova and Roth's formula. The body weight of control, healthy children was less than 120% of the expected weight for height.

Blood pressure measurements

Blood pressure (BP) was measured using a Mercury sphygmomanometer with proper cuff size in standard conditions. 3-5 occasional BP values were obtained and if the average of the blood pressure values was above the 95th centile for age and sex, 24 h ambulatory blood pressure monitoring (ABPM) was performed with a non-invasive recorder (Meditech, Hungary) using oscillometric method. Those children whose mean 24h arterial blood pressure value exceeded the 95th centile value for height and sex were considered hypertensive. (In the study of prepubertal children who were intrauterine growth retarded neonates, there was no possibility to perform the ABPM, so we considered children to be hypertensive when the lowest blood pressure value of the three measurements was above the 95th centile for age and sex.)

Laboratory tests

Determination of the Trp64Arg polymorphism in exon 1 of the 3-BAR gene

DNA was prepared from peripheral blood leukocytes by salting out procedure. Exon 1 was amplified with polymerase chain reaction (PCR) using the primers B*st*N UP: 5'-CGCCCAATACCGCCAACAG-3' and B*st*N DOWN: 5'-CCACCAGGAGTCCCATCACC-3' (product size 210 bp). Restriction enzyme digestion products were separated on a 3% agarose gel and visualized by staining with ethidium bromide.

Determination of factor V Leiden mutation

The factor V Leiden mutation was tested from dried blood-spot samples according to Zöller and Dahlbäck, by PCR method. The sequences of the PCR primers (Ransom Hill Bioscience, Ramona, CA) for the amplification were the following: forward primer: 5'GGGCTAATAGGACTACTTCTAATC3'; reverse primer: 5'TCTCTTGAAGGAAATGCCCCATTA3'. The DNA was digested by MnI restricted enzyme (Stratagene). The resulting PCR products were separated by electrophoresis on a 2.2 % agarose gel with ethidium-bromide staining.

Biochemical studies

Plasma glucose, serum insulin and lipid levels were determined from blood samples taken after an overnight fast in obese children. In obese children OGTT (1.75 g/kg ideal body weight, max. 75 g) was performed. Plasma glucose and serum insulin levels were determined at 0 and 120 min at the OGTT by the glucose oxidase method and by commercially available radioimmunoassay (RIA) kits, respectively. The criteria of impaired glucose tolerance and diabetes mellitus were based on the recommendation of American Diabetes Association. Insulin resistance was estimated by the Homeostasis Model Assessment (HOMA) using the formula: fasting serum insulin (μIU/ml) x fasting plasma glucose (mmol/l)/22.5. (In the study of prepubertal children who were intrauterine growth retarded neonates, the cut-off value for fasting insulin was 20 μIU/ml, while hyperglycaemia was considered when the fasting blood glucose level was more then 6.2 mmol/l.)

Serum cholesterol and triglyceride levels were determined by the enzymatic method with Boehringer kits; serum high-density lipoprotein (HDL) cholesterol was measured according to the method of Steele et al.. Serum cholesterol, triglyceride and HDL-cholesterol were considered high or low when they fell above or below the recommended values of the Hungarian Lipid Consensus Conference: serum cholesterol > 5.2, HDL-cholesterol < 0.9 mmol/l and serum triglyceride > 1.1 mmol/l (< 10 years)- > 1.5 mmol/l (> 10 years). If the value of any of these parameters was abnormal, the child was considered dyslipidaemic.

Statistical analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 7.5, 8.0 and 10.0. Data are presented as means±SD. Statistical significance of the differences between groups was evaluated using the Fisher's exact or Chi-square or Student's t-test or ANOVA, when appropriate.

RESULTS

1. Trp64Arg polymorphism of the 3-BAR gene

(8, 11, 13, 14; abstr: 2,6)*

1.1 The frequency of Trp64Arg polymorphism in normal and obese Hungarian children was similar. The mutation occurred in 14 healthy (male: 7) and 35 obese children (male: 23), of whom 2 were Arg64Arg homozygote and 33 were Trp64Arg heterozygote.

1.2 The obese children with Arg64 allele were compared to a group of obese children without it. The latter group was formed by a computer-generated randomisation. The anthropometric data of obese children with and without polymorphism are shown in Table 1.

Table 1. Anthropometric data of obese children with and without polymorphism (mean±SD)

	Trp64Trp (n=35) (male: 20)	**Trp64Arg/Arg64Arg (n=33+2) (male: 23)**
Age (years)	12.3 ± 2.9	12.6 ± 2.9
Body height (cm)	155.7 ± 15.9	161.4 ± 15.4
Body weight (kg)	75.6 ± 17.7	81.2 ± 23.2 **
BMI (kg/m^2)	30.9 ± 3.9	35.0 ± 10.9 ***
BF (%)	36.5 ± 2.3	38.8 ± 3.9 ***
Systolic blood pressure (mmHg)	114.5 ± 8.3	125.2 ± 10.1 *
Diastolic blood pressure (mmHg)	72.5 ± 9.0	73.2 ± 8.4

**** p < 0.05, ** p < 0.01, * p < 0.001 BMI: body mass index; BF: body fat*

The weight of obese children with Arg64 allele was significantly higher ($p<0.01$) than those without the polymorphism. Similar tendency ($p<0.05$) was observed in the BMI and BF values.

*Numbers in parenthesis are serial numbers of the papers and abstracts which were written in the issue of the thesis.

The serum insulin levels and HOMA were significantly higher in children carriers of Arg64 allele as compared to those not having this. Since the two groups were significantly different in respect of BF and BW, and these factors are closely related to insulin levels and HOMA index, therefore these latter two parameters were corrected for BW and BF. The corrected values remained significantly different between the two groups. Serum triglyceride, total cholesterol and HDL-cholesterol levels were not different between the two groups. Systolic BP of subjects with Trp64Arg and/or Arg64Arg genotype was also significantly higher ($p<0.001$) than that of those with the Trp64Trp genotype (Table 1.).

2. Leiden mutation; size at birth and later risk factors
(3,4,5)

2.1 The prevalence of the Leiden mutation in an apparently healthy Hungarian Caucasian population sample of our region was 6.33 %, which was comparable with other European prevalence rates. In the group with IUGR the prevalence of heterozygosity was not significantly different from that of the healthy Hungarian population, while in the preterm the prevalence was 18 %. As compared to the 6.3% prevalence rate of the healthy Hungarian population, this 18 % value of the mothers of premature neonates proved to be significantly higher ($p<0.01$). The difference between Groups PM and IUGR (18% versus 7.2%) was also significant statistically (Table 2.).

Table 2. Prevalence of factor V Leiden mutation in the two groups

	Normal (% of total)	**Heterozygotes (% of total)**	**Homozygotes (% of total)**
Group PM (n=50) (mothers of prematures)	**41 (82)**	**9 (18)**	**-**
Group IUGR (n=56) (mothers of intrauterine growth retarded neonates)	**52 (92.8)**	**3 (5.4)**	**1 (1.8)**

Figures in parenthesis indicate percent of total.

2.2 According to the results cardiovascular risk factors cannot be found among children at the age of 6-10 who were born with low birth weight. The anthropometric data of children are shown in Table 3.

Table 3. Anthropometric data of children at the time of examination (prepubertal stage) (mean±SD)

	Full term neonates			**Premature**
	normal (n=24)	**proportional IUGR (n=90)**	**disproportional IUGR (n=25)**	**(n=90)**
	1. group	**2. group**	**3. group**	**4. group**
Age (years)	**8.8±1.5**	**8.1±1.5**	**8.7±1.7**	**9.2±1.6**
BW (kg)	**32.2±7.4**	**24.9±7.2* ****	**28.0±8.6**	**30.1±9.2**
BH (cm)	**134.4±10.1**	**122.6±9.3 ****	**128.2±9.9**	**130.3±8.3**
BF (%)	**22.9±6.8**	**20.5±5.9**	**20.2±4.4 ****	**20.9±6.4**
LBM (kg)	**24.4±4.2**	**19.6±4.6* ****	**22.2±6.7 ****	**23.4±6.2**

**p<0.01, ** p<0.001*

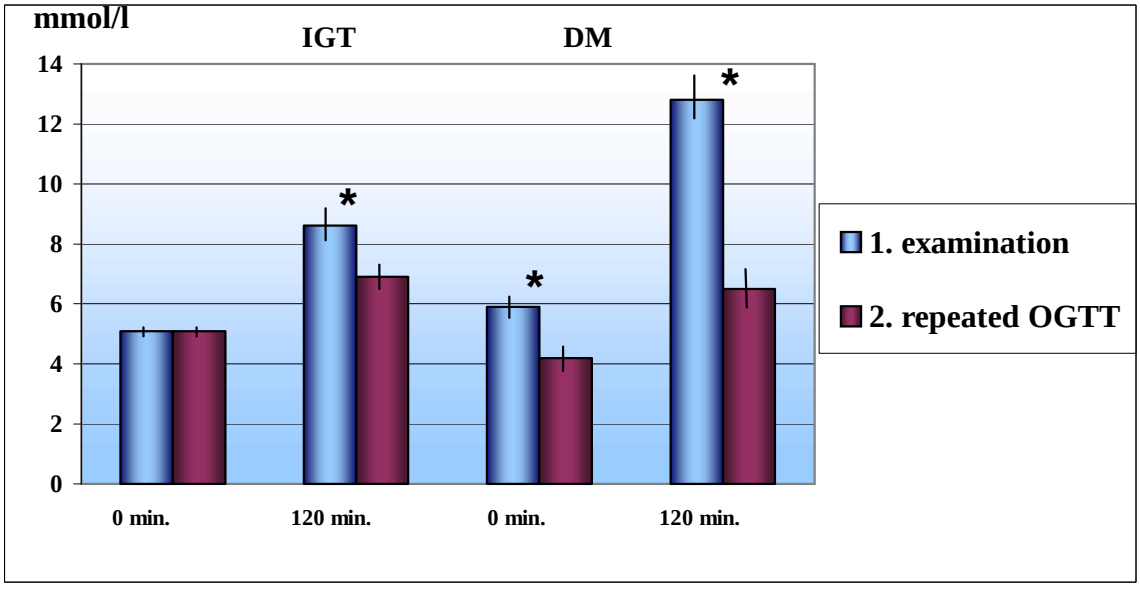
BW: body weight, BH: body height, BF: body fat; LBM: lean body mass

BW: p<0.001 1. vs. 2. group; , p<0.01 2. vs. 4 group
BH: p< 0.001 1. vs. 2. 2. vs. 4 group
BF: p<0.001 1. vs. 3. group;
LBM: p< 0.001 1. vs. 2., 3. group, p<0.01 2. vs 4 group

Weight and height of the children in the 2nd group were significantly lower than in the 1st and 4th groups (2nd group vs. 4th group : p< 0.01; 2nd group vs. 1st group: p<0.001). The laboratory results were normal. Dyslipidaemia was found 21% in the 1st group, 17 % in the 2nd group, 16% in the 3rd group and 28% in premature. There was no significant difference among the four groups. The mean of the systolic and diastolic blood pressures were similar in the four groups. Hypertension was detected in 12.5 % of the 1st and 3rd groups, in 5.6 % of the 2nd group and in 8.9 % of the 4th group.

3. Impaired glucose tolerance and type 2 diabetes mellitus
(1,2,6,7,9,10,12; abstr: 1,3,4,5,7)

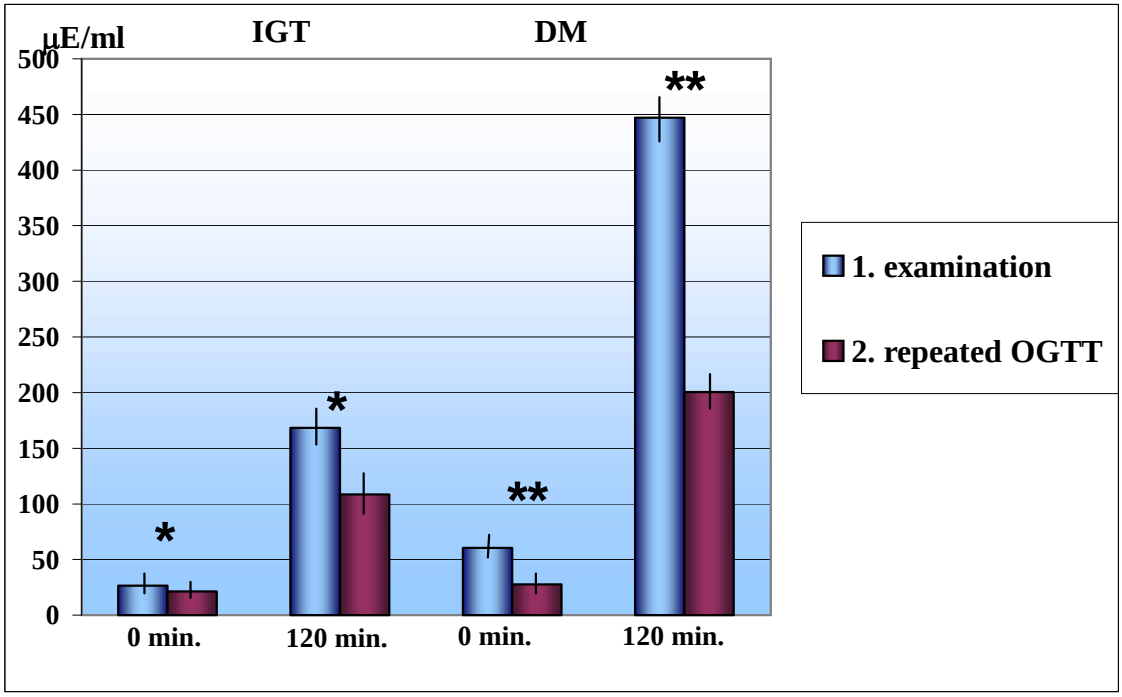
3.1 Because of the scarce European, especially Hungarian data of the prevalence of T2DM our aim was to evaluate the frequency of IGT and T2DM among clinically healthy, obese children. IGT was found in 50 children (17.3 %), while the prevalence of T2DM was 1.7 % (n=5), so altogether the disorders of carbohydrate metabolism could be detected in 19.0 % of the children (n=55).
The children with disturbed carbohydrate metabolism, a low calorie (1500 kcal/day), carbohydrate (200-250 g/day) diet, and regular exercise were recommended and they were called back for a repeated OGTT after six months.



** p < 0.001*

Figure 1. Changes of blood glucose (mean±SD) after a 6-months diet and exercise (n=36)

3.2 32 children with IGT and 4 children with T2DM took part in the repeated OGTT. Although the body weight was not changed significantly, BMI decreased significantly (30.4±4.9 vs 29.0±4.4 kg/m2; p<0.05). The changes of mean blood glucose and serum insulin levels are shown in Figure 1 and 2.. HOMA index also decreased significantly (6.7±3.7 vs 4.9±3.3).



** p< 0.001 ** p< 0.05*

Figure 2. Changes of serum insulin levels (mean±SD) after 6-months diet and exercise (n=36)

OBSERVATIONS AND PRACTICAL CONSEQUENCES OF THE STUDIES

1. Trp64Arg polymorphism of the 3-BAR gene

- The frequency of Trp64Arg polymorphism was similar in Hungary as compared to other European countries, and there was no difference between healthy and obese children, however the possession of Arg64 allele in obese children is associated with higher degree of obesity, insulin resistance and hypertension, but the number of cases need to be increased and further studies are needed to clarify these associations.
- To the best of my knowledge, this is the first study of children in Hungary investigating the frequency of a candidate gene of obesity and its effect on cardiovascular risk factors.
- Although, in the literature, there are controversial results of the role, not only, of Trp64Arg polymorphism of 3-BAR gene, but other candidate genes, in developing obesity, according to our findings and to know that obesity a multifactorial and heterogeneous disorder, therefore, preventive measures for obesity, to be fully effective in a population, must based on the modification of several potential risk factors simultaneously. Comprehensive, successful prevention programs are needed which should focus on promoting and supporting healthful lifestyles for all children at home, in school and in the society.
- Although the genetic analyses of the samples started at the Department of Clinical Genetics and Child Development of University of Pécs, these investigations made the possibility to create a scientific PCR laboratory at the Department of Paediatrics of University of Pécs.

2. Leiden mutation; size at birth and later risk factors

- Our results are in concert with the findings of some studies that the presence of Factor V, Leiden mutation may have a role in premature delivery.
- According to our results, cardiovascular risk factors cannot be proved among children at the age of 6-10 who were born as intrauterine growth retarded. Further studies are required to determine whether which stage of pregnancy might influence birth weight and later risk factors, and it can be important at what age of children should be examined for these risk factors.

3. Impaired glucose tolerance and type 2 diabetes mellitus

- According to our prevalence data of T2DM, it seems that not it is the major problem among obese youth, in spite of this, clinically healthy obese children have disturbances of carbohydrate metabolism, so screening for T2DM in children and adolescents, as suggested by the American Diabetes Association, is highly recommended.
- Impaired glucose tolerance and T2DM in asymptomatic obese children can be managed with dietary and lifestyle interventions, resulting improvement of the metabolic status of these children.
- According to this observation and assuming that T2DM is preventable, there are two components of the primary prevention. First of all, a populations strategy is needed, for altering the lifestyle and the environmental determinants of T2DM. Second, a high-risk strategy is needed for screening individuals at especially high risk for T2DM and bringing preventive care to them.

PUBLICATIONS IN THE ISSUE OF THE THESIS

Book chapter

1. E. Malecka-Tendera, E. Erhardt, D. Molnár: Type-2 diabetes in children and adolescents. Childhood obesity p. 167, Editor: giuseppe de Nicola, Napoli, 2004

Papers

2. Molnár D., Török K., Decsi T., Csábi Gy., Erhardt É.: Az elhízás következményei gyermekkorban. Táplálkozás-Allergia-Diéta 3/3-4: 9-15, 1998
3. Erhardt É., Molnár D., Storcz J., Márkus A., Török K.: Az intrauterin tápláltság szerepe a cardiovascularis kockázati tényezők alakulásában 6-10 éves gyermekekben. Orv Hetil 140(46): 2563-2567, 1999
4. E. Erhardt, J. Stankovics, D. Molnár, K. Adamovich, B. Melegh: High prevalence of factor V Leiden mutation in mothers of premature neonates. Biol Neonate 78(2): 145-146, 2000 **IF: 1.258**
5. Decsi T., Erhardt É., Márkus A., Molnár D.: Plasma lipids, phospholipid fatty acids and indices of glycaemia in ten-year-old children born as small for gestational age or preterm infants. Acta Paediatr 88:1-7, 1999 **IF: 1.130**
6. Decsi T., Csábi Gy., Török K., Erhardt E., Minda H., Burus I., Molnár S., Molnár D.: Polyunsaturated fatty acids in plasma lipids of obese children with and without metabolic cardiovascular syndrome. Lipids 35 (11): 1179-84, 2000 **IF: 1.769**
7. Erhardt É., Nyikos O., Csernus K., Molnár D.: Szénhidrátanyagcsere-zavarok előfordulása és változása diéta hatására kövér gyermekekben. Gyermekgyógyászat 54/4: 415-422, 2003
8. Erhardt É., Czakó M., Csernus K., Molnár D., Kosztolányi Gy.: A beta3-adrenoreceptor gén Trp64Arg polimorfizmus kapcsolata cardiovascularis kockázati tényezőkkel. Metabolizmus II/3: 147-150, 2004
9. E. Erhardt, D. Molnár: Is type 2 diabetes mellitus a significant problem in European adolescents ? Scand J Nutr 4/48: 155-160, 2004
10. E. Malecka-Tendera, E. Erhardt, D. Molnár: Type 2 diabetes mellitus in European children and adolescents. Acta Paediatrica 94: 543-546, 2005 **IF: 1.277**
11. E. Erhardt, M. Czakó, K. Csernus, D. Monár, Gy. Kosztolányi: The frequency of Trp64Arg polymorphism of the β3-adrenergic receptor gene in healthy and obese

Hungarian children and its association with cardivascular risk factors. Eur J Clin Nutr 59: 955-959, 2005 **IF: 2.163**

12. Erhardt É, Molnár D: 2-es típusú diabetes mellitus és elhízás gyermekkorban. Orvostovábbképző Szemle (Különszám) 12-15, 2005
13. Bokor Sz, Csernus K, Erhardt É, Burus I, Marosvölgyi T, Molnár D, Decsi T: A béta-3 adrenoreceptor gén Trp64Arg polimorfizmusának összefüggése a zsírsavellátottsággal elhízott gyermekekben. Gyermekgyógyászat 2: 125-129, 2006
14. Répásy J, Bokor Sz, Csernus K, Erhardt É, Molnár D: Béta-3 adrenoreceptor gén Trp64Arg polimorfizmusának szerepe elhízott gyermekek energiaegyensúlyában. Gyermekgyógyászat 4: 423-431, 2006

ABSTRACTS WHICH CAN BE CITED IN THE ISSUE OF THE THESIS

1. E. Erhardt, D. Molnár, Gy. Soltész: Impaired glucose tolerance and type 2 diabetes in obese Hungarian children. J Pediatr Endo and Metab 15 (Suppl 4): 1077, 2002 **IF: 1.146**
2. E. Erhardt, M. Czakó, D. Molnár: The Trp64Arg polymorphism of the beta3-adrenergic receptor gene in healthy and obese Hungarian children. Int J Obes Relat Metab Disord 27 (Suppl 1): S71, 2003 **IF: 2.794**
3. E. Erhardt, D.Molnár: A rare complication that can be ignored.Int J Obes Relat Metab Disord 27 (Suppl 2): S7, 2003 **IF: 2.794**
4. E. Malecka-Tendera, E. Erhardt, D. Molnár: Prevalence of type 2 diabetes mellitus in European children. Int J Obes Relat Metab Disord 27 (Suppl 2): S26, 2003 **IF: 2.794**
5. E. Malecka-Tendera, E. Erhardt, D. Molnár, ML Frelut: Type 2 diabetes – is it an important health problem in European obese children? Int J Obesity Relat Metab Disord 27 (S1): S85, 2003 **IF: 2.794**
6. E. Erhardt, K. Csernus, M. Czakó, D. Molnár: Frequencies of single-nucleotide polymorphisms of some candidate genes playing role in thermogenesis in Hungarian children. Int J Obesity Relat Metab Disord 28 (Suppl 1): S106, 2004 **IF: 3.459**
7. E. Erhardt, K. Csernus, Sz. Bokor, D. Molnár: Frequency and effect of Ala12 allele of PPARγ on cardiovascular risk factors in Hungarian children. Int J Obesity Relat Metab Disord 29 (Suppl 2): S146, 2005 **IF: 4.482**

OTHER PUBLICATIONS AND ABSTRACTS

1. Erhardt É., Molnár D.: A bioelektromos impedancia analízis értékelése gyermekekben.Pediáter 4, 231-36, 1995
2. Molnár D, Jeges S, Erhardt É., Schutz Y.: Measured and predicted resting metabolic rate in obese and non-obese adolescents. J Pediatrics 127/4, 571-77, 1995 **IF: 2.859**
3. Erhardt É., Molnár D., Schutz Y.: No blunted postprandial thermogenesis in obese adolescents.Int J Obesity 19, 86, 1995 (abs) **IF: 1.832**
4. Molnár D., Erhardt É., Csábi Gy., Schutz Y.: Increased postabsorptive fat oxidation in obese adolescents. Int J Obesity l9, 42, 1995 (abs) **IF: 1.832**
5. Molnár D, Erhardt É, Schutz Y.: Postprandial thermogenesis in obese adolescents. Pediatr Res 38, 445, 1995 (abs) **IF: 2.857**
6. Erhardt É., Csábi Gy: Kövérség, életkor, nem és pubertás hatása a nyugalmi anyagcserére gyermekekben. Pediáter 5/2, 105-16, 1996
7. Erhardt É., Molnár D., Györkő Béláné, Angsterné Tarján Ágnes: Diétás felmérés kritikája.Gyermekgyógyászat 47/4, 308-11, 1996
8. Erhardt É., Harangi F.: Two cases of musculoskeletal syndrome associated with acne. Pediatric Dermatology 14/6, 456-459, 1997 **IF: 0.381**
9. Erhardt É., Hermann R., Soltész Gy., Kozári A.: Familiáris Addison-kór. Gyermekgyógyászat, 4: 447-50, 1997
10. Kozári A., Erhardt É., Pintér A., Szilágyi K., Magyarlaki T., Kálmán E., Soltész Gy.: Hashimoto betegség talaján kialakuló follicularis pajzsmirigy carcinoma.Gyermekgyógyászat, 4: 451-54, 1997
11. D. Molnár, T. Decsi, I. Burus, K. Török, É. Erhardt: Effect of weight reduction on plasma total antioxidative capacity in obese children Int J Obes Relat Met Disord 22/4, 23, 1998 (abs) **IF: 3.003**
12. E. Erhardt, R. Hermann, S. Davidovics, A. Kozári, E. Kálmán, G. Soltész: Graves disease associated with papillary thyroid carcinoma. Endocrine Regulations 32/4, 215, 1998 (abs)
13. R. Hermann, E. Erhardt, G. Soltész: Neurofibromatosis with Noonan's phenotype. Endocrine Regulations 32/4, 215, 1998 (abs)
14. É. Erhardt, J. Sólyom, J. Homoki, S. Juricskay, Gy. Soltész: Correlations of blood-spot 17-hydroxyprogesterone profiles and urinary steroid profiles in congenital adrenal hyperplasia. J Pediatr Endocrinol Metab 13: 205-210, 2000 **IF: 0.638**

15. Molnár D., Török K., Erhardt E., Jeges S.: Safety and efficacy of treatment with an ephedrine/caffeine mixture. The first double-blind placebo-controlled pilot study in adolescents. Int J Obes Relat Metab Disord 24(12): 1573-8, 2000 **IF: 2.982**
16. Erhardt É., Morava É., Czakó M., József I., Decsi T.: Izomhypotonia hátterében felfedezett Prader-Willi syndroma esete. Gyermekgyógyászat 51/4: 382-3844, 2000
17. Hermann R., Erhardt É., Kajtár P., Soltész Gy.: Beckwith-Wiedemann-syndroma bilaterális nephroblastomatosissal. Gyermekgyógyászat 51/4: 385-389, 2000
18. Hermann R., Erhardt É., Peter M., Sólyom J., Soltész Gy.: DAX-1 gén stopmutációja miatt kialakult X-kromoszómához kötött adrenalis hypoplasia congenital és hypogonadotrop hypogonadismus. Gyermekgyógyászat 51: 349-351, 2000
19. Sütő A., Erhardt É., Hermann R., Kozári A., Soltész Gy.: Véletlenül felfedezett tartós hypokalaemia esete (Gitelman-szindróma). Gyermekgyógyászat 51: 72-76, 2000
20. Kozári A., Erhardt É., Hock András, Soltész Gy.: Unilaterális, praepubertális gynecomastia. Gyermekgyógyászat 51/4: 373-374, 2000
21. T. Decsi, Gy. Csábi, K. Török, E. Erhardt, H. Minda, I. Burus, Sz. Molnár, D. Molnár: Indicators of enhanced delta-6 and diminished delta-5 desaturase activities in obese children with metabolic cardiovascular syndrome. (abs) J. Pediatr Gastroenterol Nutr 31 Suppl.2, 2000 **IF: 1.580**
22. H. Minda, T. Decsi, K. Török, E. Erhardt, I. Burus, Sz. Molnár, D. Molnár: Relationship between serum fatty acids and insulin sensitivity in obese children. (abs) Int J Obes Relat Metab Disord 25 (2): S90, 2001 **IF: 2.196**
23. T. Decsi, Gy. Csábi, K. Török, É. Erhardt, H. Minda, I. Burus, Sz. Molnár, D. Molnár: Omega-6 fatty acids in obese children with metabolic cardiovascular syndrome. (abs) Ped Res 49: 274, 2001 **IF: 3.289**
24. T. Decsi H. Minda, R. Hermann, A. Kozári, É. Erhardt, I Burus, Sz. Molnár, G. Soltész: Fatty acid composition of plasma lipid classes in diabetic children. (abs) Ped Res 50: 282, 2001 **IF: 3.289**
25. H. Minda, T. Decsi, É. Erhardt, I. Burus, D. Molnár: N-6 polyunsaturated fatty acids and insulin resistance in obese children. (abs) Ped Res 52: 781, 2002 **IF: 3.382**
26. T. Decsi, H. Minda, R. Hermann, A. Kozári, É. Erhardt, I. Burus, Sz. Molnár, Gy. Soltész: Polyunsaturated fatty acids in plasma and erythrocyte membrane lipids of diabetic children. Prostaglandins, Leukotriens and Essential Fatty Acids 67(4): 203-210, 2002 **IF: 0.958**

27. T. Decsi, E. Erhardt, H. Minda, K. Török, I. Burus, D. Molnár: Childhood obesity itself is not related to altered fatty acid status. (abs) Ped Res 52: 781, 2002 **IF: 3.382**
28. K. Csernus, É. Lányi, E. Erhardt, D. Molnar: Markers of renal glomerular and tubular dysfunction in childhood obesity. (abs) Int J Obes Relat Metab Disord 27 (S1): S4, 2003 **IF: 2.794**
29. Kozári A., Erhardt É., Soltész Gy.: Prolactinomás eseteink. Gyermekgyógyászat 54/4: 458-460, 2003
30. Nagy E., Csernus K., Erhardt É., Molnár D.: Elhízáshoz társuló zsírmáj gyermekkorban. (abs) Obesitologia Hung. Suppl (3): 17, 2003
31. K. Csernus, E. Erhardt, E. Lányi, D. Molnár: Effect of childhood obesity on glomerular and tubular protein excretion. (abs) Int J Obesity Relat Metab Disord 28 (Suppl 1): S42, 2004 **IF: 3.459**
32. Nagy E., Erhardt É., Csernus K., Molnár D.: Az elhízás és az uncoupling protein-2 exon 8 ins/del polimorfizmusának szerepe a gyermekkori zsírmáj kialakulásában.(abs) Obesitologia Hungarica 4 (Suppl 2): 54. 2004
33. Csernus K., Lányi É., Erhardt É., Molnár D.: A gyermekkori elhízás és metabolikus szindróma hatása a glomeruláris és tubularis fehérjeürítésre.(abs) Gyermekgyógyászat 55(S2): 18, 2004
34. Lányi É., Csernus K., Erhardt É., Molnár D.: Keringő aktív ghrelin szintjének változása orális glükózterhelés során kövér gyermekekben. (abs) Gyermekgyógyászat 55(S2): 54, 2004
35. Csernus K, Erhardt É, Molnár D: Non-alcoholic fatty liver disease in childhood obesity and role of uncoupling protein-2. (abs) Int J Obes 28(Suppl 3): S110, 2004 **IF: 3.459**
36. K. Csernus, E. Lányi, E. Erhardt, D. Molnár: Effect of childhood obesity and obesity-related cardiovascular risk factors on glomerular and tubular protein excretion. Eur J Pediatr 164: 44-49, 2005 **IF: 1.382**
37. E. Erhardt, K. Csernus, D. Molnár: Examination of synergetic effects of some candidate genes playing role in thermogenesis. (abs) Obesity Reviews 6 (Suppl 1): S125, 2005
38. E. Lányi, K. Csernus, E. Erhardt, D. Molnár: Plasma levels of active form of ghrelin during an oral glucose tolerance test in obese children. (abs) Obesity Reviews 6 (Suppl 1): S123, 2005

39. Erhardt É, Kozári A, Lányi É, Hudák I, Gömöri É, Dóczi T, Soltész Gy: M. Cushing gyermekkori esete. Gyermekgyógyászat 4: 472-476, 2006
40. Decsi T, Szabo E, Burus I, Marosvolgyi T, Kozari A, Erhardt E, Soltesz G: Low contribution of n-3 polyunsaturated fatty acids to plasma and erythrocyte membrane lipids in diabetic young adults. Prostaglandins Leukot Essent Fatty Acids 76(3): 159-64, 2007 **IF: 2.261**
41. Lanyi E, Csernus K, Erhardt E, Toth K, Urban B, Lenard L, Molnar D: Plasma levels of acylated ghrelin during an oral glucose tolerance test in obese children. J Endocrinol Invest 30(2): 133-7, 2007 **IF: 1.469**
42. Bokor S, Csernus K, Erhardt E, Burus I, Molnar D, Decsi T: Association of n-6 long-chain polyunsaturated fatty acids to -866 G/A genotypes of the human uncoupling protein 2 gene in obese children. Acta Paediatr 96(9): 1350-4, 2007 **IF: 1.297**

LECTURES AND POSTERS IN THE ISSUE OF THE THESIS

1. A Magyar Elhízásellenes Alapítvány VII. Konferenciája
   1996. szeptember 18-22., Balatonlelle
   Erhardt É, Molnár D: *Intrauterin tápláltság szerepe a felnőttkori elhízásban és a kardiovaszkuláris betegségekben*
2. 7th International Workshop of European Childhood Obesity Group
   21-22nd November, 1997, Verona, Italy
   E. Erhardt, D. Molnár: *Size at birth and later risk factors*
3. A Magyar Elhízásellenes Alapítvány XII. Konferenciája
   1999. szeptember 19-20., Siófok
   Erhardt É, Molnár D., Stankovics J., Márkus A., Török K.: *Intrauterin növekedés, kardiovaszkuláris rizikófaktorok, Leiden mutáció*
4. 9th European Childhood Obesity Group Workshop
   8-10th October 1999, Malmö-Lund, Sweden
   E. Erhardt, D. Molnár, J. Stankovics, A. Márkus, K. Török: *Intrauterine growth retardation, cardiovascular risk factors, Leiden mutation*
5. Magyar Diabetes Társaság XV. Kongresszusa
   2000.04.13-16., Tihany
   Erhardt É., Molnár D., Stankovics J., Török K.: *Kardiovaszkuláris kockázati tényezők, intrauterin növekedés, Leiden mutáció*
6. A Gyermekendokrinológiai Szekció ENDOPED Tudományos Ülése
   2001. május 18-19, Hortobágy, Máta
   Erhardt É., Czakó M., Molnár D., Kosztolányi Gy. Soltész Gy.: *Beta3-adrenoreceptor gén polimorfizmus előfordulása kövér gyermekekben*
7. Magyar Gyermekdiabetológiai szekció Tudományos Ülése
   2001. 09.27.-30., Zalakaros
   Erhardt É., Molnár D., Soltész Gy.: *Csökkent glucose tolerancia előfordulása kövér gyermekekben*
8. 8th Middle European Workshop on Paediatric Endocrinology (MEWPE)
   16-18th November, 2001, Bled, Slovenia
   E. Erhardt, M. Czakó, D. Molnár, Gy. Kosztolányi: *The Trp64Arg polymorphism of the beta3 adrenergic receptor gene in normal and obese Hungarian children*
9. 12th European Childhood Obesity Group Workshop
   23-25 May, 2002, Prague, Czeh Republic
   E. Erhardt, M. Czakó, D. Molnár, Gy. Kosztolányi, G. Soltész: *The Trp64Arg polymorphism of the beta3-adrenergic receptor gene in normal and obese Hungarian children*
10. Magyar Diabetes Társaság XVI. Kongresszusa
    2002.05.30.-06.02., Debrecen
    Erhardt É., Csernus K., Molnár D., Soltész Gy.: *A 2-es típusú diabetes ritka, az IGT viszont gyakori tünetmentes, kövér gyermekekben*
11. 28th Annual Meeting of the International Society for Pediatric and Adolescent Diabetes (ISPAD)
    18-21 September 2002, Graz, Austria
    E. Erhardt, D. Molnár, Gy. Soltész: *Impaired glucose tolerance and type 2 diabetes in obese Hungarian children.*
12. 12th European Congress on Obesity
    29 May-1 June 2003, Helsinki, Finland
    E. Erhardt, M. Czakó, D. Molnár: *The Trp64Arg polymorphism of the beta3-adrenergic receptor gene in healthy and obese Hungarian children*

13. A Magyar Gyermekorvosok Társaságának 2003. évi Nagygyűlése
    2003. június, Szeged
    Erhardt É. Csernus K., Molnár D., Soltész Gy.: Szénhidrát-anyagcserezavarok kövér gyermekekben
14. 13th European Childhood Obesity Group Workshop
    25-27 September, 2003, Tenuta Moreno, Mesagne (BR), Italy
    E. Erhardt, D.Molnár: *A rare complication that can be ignored (felkért, plenáris előadás)*
15. Magyar Gyermekdiabetológiai szekció Tudományos Ülése
    2003. 10.17.-18., Szeged
    Erhardt E., Czakó M., Molnár D., Kosztolányi Gy., Soltész Gy.: *β_3-adrenoreceptor gén Trp64Arg polimorfizmus*
16. A Magyar Diabetes Társaság XVII. Kongresszusa
    2004. április 22-25., Tihany
    Erhardt É., Czakó M., Molnár D., Kosztolányi Gy., Soltész Gy.: *A beta-3-adrenoreceptor gén, Trp64Arg polimorfizmus előfordulása normál és kövér, magyar gyermekekben*.
17. 13th European Congress on Obesity
    26-29 May, 2004, Prague, Czeh Republic
    E. Erhardt E, K. Csernus, M. Czakó, D. Molnár: *Frequencies of single-nucleotude polymorphisms of some candidate genes playing role in thermogenesis in Hungarian children (poster).*
18. XIV. Symposium of Polish Pediatric Endocrinology
    15-17 October, 2004, Wisla, Poland
    E. Erhardt: Is type 2 diabetes mellitus a significant problem in European children? (felkért, plenáris előadás)
19. 14th European Congress on Obesity
    1-4 June, 2005, Athen, Greece
    E. Erhardt, K. Csernus, D. Molnár: *Examination of synergetic effects of some candidate genes playing role in thermogenesis (poster).*
20. ECOG International Workshop
    29 Sept-1 Oct, 2005, Vienna, Austria
    E. Erhardt, K. Csernus, Sz. Bokor, D. Molnár: Frequency and effect of Ala 12 allele of PPARγ on cardiovascular risk factors in Hungarian children (poster)
21. Obezitológiai szimpózium és továbbképző tanfolyam
    2005. október, Pécs
    Erhardt É: *A gyermekkori elhízás helyzete és kezelése (felkért előadás)*
22. Gyermekkori Diabetes és Obesitas Továbbképző Tanfolyam
    2006. március, Pécs
    Erhardt É: 2-es típusú diabetes mellitus (felkért előadás)
23. X. Családorvosi konferencia
    2007.10. 06., Budapest
    Erhardt Éva: Az elhízás genetikája (felkért előadás)

ACKNOWLEDGEMENTS

It was a great pleasure for me to carry out this work at the Department of Paediatrics, Medical Faculty, University of Pécs. A thesis is supposed to be a contribution by one person for a PhD; there are still a lot of people who have helped me out over the years. I have been fortunate enough to have the support of so many people and without it this would not have been possible.

Firstly, I would like to express my gratitude to Professor Molnár who invited me as a medical student to join his research group, and supported me throughout all my experimental and clinical studies. His open-minded personal and professional merits provided a continuouing inspiration not only for me, but for all people in the group. He was always sensitive for new theories thereby creating a helpful, warm atmosphere for young people.

I am extremely greatful to Professor Soltész who "introduced" me to Endocrinology and Diabetes. He greatly supported all my works and encourages me to perform these studies.

I cannot express my so many thanks to Professor Méhes, who unfortunately could not live this thesis. He was my first teacher in Paediatrics who always supported me, and he contributed to create a unique warm and friendly atmosphere at the Department.

Special thanks to Professor Kosztolányi who helped me a lot to start the genetic studies and continuously encourages me to perform this work.

This work would not have been possible without help, especially technical contribution in genetic studies, of Márta Czakó and Anna Erdélyi. Márta Czakó became one of my best friends during the years.

I am also grateful to Ágnes Angster for their support in clinical date and sample collection.

I would like to thank several colleagues and the staff of Endocrine Unit for their valuable contribution.

I would not have been able to carry out my PhD work without the love and continuous support of my husband and my little son. Most importantly of all, I would like to thank my parents and my sister for helping me during my life. It is through their encouragement and care that I have made it through all the steps to reach this point in life, and I could not have done it without them. My family has always taken care of me.