The role of appetite regulation, adipose tissue cytokines and gene polymorphisms in obesity, insulin resistance and diabetes

PhD Theses

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1. Introduction

Although there can be big differences between the nutrient composition and energy content of meals, on the long run the balance of the energy homeostasis is closely regulated. The long term regulation of energy homeostasis is based upon peptides signalling the nutritional status of the body to the CNS. These peptides are the adiposity signals. The body needs to signal to the CNS not only the nutritional status but also the energy demand. The mediator of this is the ghrelin. The regulation of the energy intake may become disturbed, and this can result in obesity.

The adipose tissue plays an important role in energy homeostasis, and also produces many factors, which get into the systemic circulation and affects the whole body. The number of known cytokines grows every year. The role of some in the regulation of homeostasis is well established, for others we only have observations and guesses. Some of these factors are produced by the adipocytes themselves, other by the adipose tissue macrophages, whose number increases in obesity.

Genetic polymorphisms also play roles in obesity and insulin resistance. These polymorphisms may affect the differentiation of adipocytes or the type and amount of cytokines produced by the adipose tissue.

The decreased insulin secretion of the beta cells also has part in the manifestation of diabetes. Mitochondrial malfunction has an important role in this.

We planned to study the various mechanisms, the disturbances in appetite regulation, adipose tissue cytokine profile and insulin secretion leading to obesity, insulin resistance and diabetes in special patient groups.

Patients treated with atypical antipsychotics can develop marked hyperphagia and obesity, and also have a high incidence of diabetes.

The insulin resistance of pregnancy plays a role in the manifestation of gestational diabetes, especially in those who had insulin resistance or insulin secretion defect pre pregnancy.

In MIDD (Maternally Inherited Diabetes and Deafness) insulin secretion defect leads to diabetes.

Our studies were done in these patient groups.

2. Aims

The aim of these studies was to evaluate adipose tissue cytokines and appetite regulating signals in special obesity, insulin resistance and diabetes forms. It was also studied, whether gene polymorphisms having a role in obesity have different prevalence in these patients, and whether these polymorphisms affect the biomarkers of obesity and adipose tissue cytokine levels.

1. We measured fasting serum ghrelin levels in patients treated with atypical antipsychotics and patients with GDM.
2. We measured different adipose tissue cytokine fasting serum levels in these patient groups.
3. We compared the ghrelin and cytokine levels with those measured in control groups, to see the difference.
4. We studied the correlation of the ghrelin levels with cytokine levels, BMI and parameters of carbohydrate metabolism.
5. In the group treated with atypical antipsychotics we studied the prevalence of genetic polymorphisms which were already published as correlating with obesity and insulin resistance. We compared the results with the prevalence in normal population. We also studied the relationship with obesity and insulin resistance.
6. In two Hungarian families carrying the A3243G point mutation we studied the carbohydrate homeostasis with IVGTT, where we measured also C-peptide in those not treated with insulin.
7. In those carrying the mutation we measured the fasting C-peptide levels, islet cell autoantibody levels and HLA-DR and –DP status.

8. We compared the results with the clinical status of the mutation carriers.

3. Methods

3.1 Patients

The patient group treated with atypical antipsychotics consisted of 60 patients taking these medications for at least 1 year (clozapine, olanzapine, risperidon, quetiapine). We did not include patients with previously diagnosed glucose tolerance abnormality. We used two control groups, one age and gender matched healthy, non obese, non diabetic group, and an obese, not antipsychotic treated, age, gender, BMI and glucose tolerance matched control group.

To study women with GDM we included 30 insulin treated GDM patients between the 28th and 40th week of their pregnancy. We used healthy, age matched pregnant and non pregnant women as controls.

The MIDD group consisted of the members of two Hungarian families carrying the A3243G mitochondrial gene mutation. Healthy volunteers were used as controls, in them diabetes was excluded with OGTT, they did not have any trouble with hearing, and did not have suspicion for mitochondrial disease.

3.2 Serum tests

Serum ghrelin and resistin levels were measured with RIA, ELISA was used for TNF-α, leptin, resistin, sTNFRII and sFas serum level measure ment.

3.3 Genetic tests

We determined the polymorphisms with PCR RFLP. HLA haplotype was determined with sequence specific polymorphism (SSP) PCR.

3.4 Other methods

Islet cell antibodies (ICA) were determined by indirect immune fluorescence, on human frozen pancreas. The antibodies against glutamate decarboxilase 65 and the intracytoplasmic domene of IA-2 (GADA and anti-IA2) were determined by RIA.

For statistical analysis we used ANOVA with Bonferroni correction, linear correlation analysis (Spearman), forward stepwise linear regression and non-parametric Mann-Whitney test.

For the analysis and statistical presentation we used Prism 3 and SPSS 10 programs.

4. Results

4.1 Studies of patients treated with atypical antipsychotics.

We found significantly higher fasting serum ghrelin levels in patients treated with atypical antipsychotics (X±SD: 1,318±0,6 ng/ml) than either normal (0,338±0,03 ng/ml, p< 0.0001) or obese controls (0,207±0,03 ng/ml, p< 0.0001).

There was no statistically significant difference in the TNF-α, resistin and leptin levels of the patients and the obese control group.

In patients treated with antipsychotics serum adiponectin level was 9,92±3,26 μg/ml. Adiponectin level was the lowest in the obese controls (6,45±2,97 μg/ml), and the highest in non obese controls (12,77±3,15 μg/ml). The difference was significant between any two groups (p<0,0001).

Serum sTNFR-2 levels were significantly higher in both the antipsychotic treated (8,63±2,55 ng/ml) and the obese control group (7,05±1,77 ng/ml) than in non obese controls (4,04±1,04 ng/ml). The difference between the antipsychotic treated group and the obese control group was also significant (p=0,0004). The TNF-α/sTNFR2 ratio was significantly higher in the non obese control group (1206±337, p<0,0001) than either in the antipsychotic treated (719±268), or the obese control group (880±290). In the antipsychotic treated group the TNFα/sTNFR2 ratio was significantly lower, than in the obese control group (p=0,0019).

The serum sFas level was significantly (p<0,0001) higher in both the antipsychotic treated (2,93±0,66 ng/ml) and the obese control group (2,52±0,55 ng/ml) than in the non obese control group (4,04±1,04 ng/ml). The difference between the antipsychotic treated group and the obese control group was also significant (p=0,0004).

Among the three groups the highest fasting C-peptide levels were found in the obese control group (4,07±2,33 ng/ml), which was significantly higher than that of the non obese control group (1,09±0,36 ng/ml, p<0,0001). In the antipsychotic treated group, with similar prevalence of glucose tolerance abnormalities we found significantly lower fasting C-peptide levels (3,04±2,15 ng/ml, p=0,0093), which were significantly higher than the levels found in the non obese controls (p<0,0001). Fasting proinsulin levels showed similar differences as the C-peptide levels. The fasting C-peptide/glucose ratio was significantly higher in the antipsychotic treated (0,62±0,43) and the obese control group (0,74±0,45) than in the non obese controls (0,25±0,09). There was no difference between the first two groups (p=0,0771).

We found a negative linear relationship between the fasting serum ghrelin levels and the BMI in the antipsychotic treated group (r=-0,37, p=0,0035). From the parameters of insulin resistance the HOMA-A and the proinsulin showed negative correlation with fasting ghrelin levels (r=-0,32, p=0,01 and r=-0,48, p<0,001).

The fasting serum ghrelin levels showed negative correlation with the fasting serum TNF-α and resistin levels in the antipsychotic treated group (r=-0,33, p=0,009 and r=-0,36, p=0,003). Serum adiponectin levels correlated negatively with BMI (r=-0,52, p<0,0001) and the indirect parameters of insulin resistance in the antipsychotic treated group. In this group the fasting serum TNF-α and resistin levels showed significant negative correlation with adiponectin levels (r=-0,34, p=0,007and r=-0,32, p=0,01). The TNF-α/sTNFR2 ratio had a significant positive correlation with HOMA-A (r=0,38, p=0,002) among antipsychotic treated patients. Serum FAS levels in the antipsychotic treated group showed significant positive correlation with BMI (r=0,43, p=0,0005) and negative significant correlation with ghrelin.

In carriers of TNF-α -308 A allele the indirect parameters of insulin resistance had significantly lower levels than in people with GG genotype (C-peptide AG 0,69±0,46 ng/ml, GG 1,1±0,72 ng/ml, p=0,03). In the carriers of the A allele the serum TNF-α and resistin levels were significantly lower (TNF-α AG 4,6±0,7 pg/ml, GG 6,0±1,8 pg/ml, p=0,019; resistin AG 7,3±2,7 ng/ml, GG 10,4±3,8 ng/ml, p=0,02), serum ghrelin levels were significantly higher than in those with only G alleles (AG 1793±582 pg/ml, GG 1221±522 pg/ml, p=0,01).

Carriers of the TLR4 Asp299Gly and Thre399Ile mutant allele had significantly lower BMI (25,1±4,0 kg/m2 vs. 30,1±7,4 kg/m2, p=0,01), and lower levels of indirect parameters of insulin resistance (insulin: 7,1±5,1 μU/ml vs. 12,0±7,8 μU/ml, p=0,02; proinsulin 9,4±1,9 pM vs. 15,3±7,5 pM, p=0,02; C-peptide 0,59±0,47 ng/ml vs. 1,12±0,71 ng/ml, p=0,009).

With the PPARγ Pro12Ala polymorphism the carriers of the Ala allele had significantly higher BMI (35,71 vs. 27,67 kg/m^2, p=0,0074), so they had higher HOMA A (7,635 vs. 4,46, p=0,04), TNF-α (7,48 vs. 5,39 pg/ml, p=0,0006) and leptin levels (49,33 vs. 28,07 mg/ml, p=0,007). Ghrelin and adiponectin levels were significantly lower in carriers of the rare allele (ghrelin: 909 vs. 1418 pg/ml, p=0,0064; adiponectin 7,5 vs. 10,5 μg/ml, p=0,004).

4.2 Studies of women with GDM

In women with GDM the fasting serum ghrelin levels were significantly lower, than in non diabetic pregnant women in the 3rd trimester (226±21 pg/ml vs. 252±36 pg/ml). Serum ghrelin levels in GDM and also in the 3rd trimester of normal pregnancy were significantly lower than in healthy non pregnant women (309±21 pg/ml) and in the 1st trimester of normal pregnancy (314±41 pg/ml). In normal pregnancy the

fasting serum ghrelin levels were significantly higher than in any other studied group (377±38 pg/ml, all p<0,0001).

In GDM the fasting serum TNF-α, leptin and resistin levels were significantly higher than in the 3rd trimester of normal pregnancy. Serum TNF-α, leptin and resistin levels in GDM and in the 3rd trimester of normal pregnancy were significantly higher than in healthy nonpregnant women.

In GDM we found significantly lower fasting serum adiponectin levels than in the 3rd trimester of normal pregnancy (7,52±1,85 μg/ml vs. 8,06±2,44 μg/ml, p<0,01). Serum adiponectin levels in GDM and in the 3rd trimester of normal pregnancy were significantly lower than in healthy nonpregnant women (12,5±3,6 μg/ml, p<0,001) and also than that of the total healthy pregnant group (9,79±3,14 μg/ml, p<0,001).

In GDM we found significantly higher fasting serum sTNFR2 levels (9,07±7,39 ng/ml), than in the 3rd trimester of normal pregnancy (5,75±2,1 ng/ml). The sTNFR2 in both GDM and in normal pregnancy were significantly higher, than in healthy nonpregnant women (3,3±0,81 ng/ml).

In GDM (with euglycaemia) we found significantly higher fasting serum C-peptide levels (6,82± 2,51 ng/ml), than in the 3rd trimester of normal pregnancy (3,36±1,21 ng/ml). We found similar significant differences in the C peptide/glucose ratio between the studied groups (GDM 1,53±0,64; P3 0,7±0,3; whole pregnant group 0,44±0,25; healthy nonpregnants 0,24±0,08, all p<0,01).

Fasting serum ghrelin levels showed in all pregnant groups significant (p< 0,05) negative correlation with BMI (GDM r=-0,66, p=0,0001; P3 r=-0,59, p= 0,01), resistin, TNF-α (GDM r=-0,53, p=0,002; P3 r=-0,63, p=0,01), sTNFR-2 (GDM r=-0,51, p=0,003; P3 r=-0,53, p=0,03), leptin (GDM r=-0,41,

p=0,02; P3 r=-0,58, p=0,05) and C-peptide (GDM r=-0,52, p=0,002; P3 r=-0,53, p=0,04), and in GDM with the insulin dose needed to maintain normal sugar levels (r=-0,39, p=0,03). The correlation with fasting serum adiponectin levels was significant and positive in all pregnant groups (GDM r=0,53, p=0,02; P3 r=0,57, p=0,02).

Fasting serum resistin levels did not show significant correlation with BMI, but showed a significant ($p<0,05$) positive correlation with serum TNF-α, serum sTNFR2 and serum C peptide levels and with the insulin dose needed to maintain normal glucose levels in GDM. Same positive correlations were observed (apart from the insulin dose) in normal pregnancy. We found no such correlations in nonpregnant healthy women.

In GDM the fasting serum adiponectin levels showed significant negative correlation with TNF-α (r=-0,65, p< 0,0001), leptin (r=-0,75, p= 0,0004), fasting C-peptide levels (r=-0,83, p< 0.0001), BMI (r=-0,67, p< 0,0001), and fasting C-peptide/glucose ratio (r=-0,46, p=0,0109). In normal pregnancy we found similar significant negative correlation (TNF-α: r=-0,56, p= 0,0002; leptin: r=-0,45, p=0,003; fasting C-peptide: r=-0,70, p<0,0001; BMI: r=-0,51, p= 0,0007; C-peptide/glucose ratio: r=-0,43, p=0,0046). In healthy nonpregnant women the adiponectin showed significant negative correlation only with leptin (r=-0,44, p=0,0134), fasting C-peptide (r=-0,46, p= 0,01), and BMI (r=-0,57, p=0,0008). Adiponectin levels were the strongest predictors of fasting C-peptide levels in all groups.

Adiponectin levels showed significant positive correlation in all pregnant groups with the body mass of the neonates (GDM neonates 3151±672 g, r=0,4345, p=0,0164; normal pregnancy neonates: 3562±359 g, r=0,6124, p=0,0041), their head circumference (r=0,47, p=0,008) and length (r=0,59,

p=0,0006). The body mass of babies born from a GDM pregnancy was significantly lower (p=0,001), even though there was no difference in the gestational week at birth (GDM: 38,22±0,51 weeks, NDM: 38.92±0,32 weeks).

4.3 Studies of families with the A3243G mitochondrial gene mutation (MIDD)

The A3243 mitochondrial mutation was found in every studied family member in the peripheral leukocytes, in the first family in 5-15%, in the second 10-30%. The persons carrying the mutation were significantly lower and had lower BMI than the mean of 500 healthy Hungarian blood donors. The fasting serum C-peptide levels did not differ in the MIDD and the control group. We still found measurable fasting C-peptide levels in the 2 patients with > 10 years of diabetes. The fasting C-peptide levels did not show correlation with the duration of diabetes. We performed IVGTT in the 4 MIDD patients not receiving insulin and in the controls. In the mutation carriers the first phase of C-peptide reaction (the first phase of insulin secretion) was missing. In MIDD patients in the first family we found ICA positivity in one family member (30 JDFU), in the other family both studied members had ICA positivity (20 JDFU and. 10 JDFU). We did not find antibodies against GAD or IA-2in any patient. In the MIDD families we did not find HLA DR or DQ alleles strongly associated with Type 1 diabetes.

5. Conclusions

1. We were among the first to observe high fasting serum ghrelin levels in patients taking atypical antipsychotics for a prolonged time, and realized the potential role in the pathomechanism of obesity and metabolic abnormalities caused by atypical antipsychotics.
2. We found significant negative correlation between serum ghrelin levels and BMI in antipsychotic treated patients, similar to that in normal obesity, but antipsychotic treated people had much higher ghrelin levels with a given BMI. This might suggest a partial impairment of the regulation of ghrelin secretion.
3. In atypical antipsychotic induced obesity we found similar changes in cytokine levels a sin normal obesity. Leptin and resistin levels were significantly higher compared to non obese people, and indirect parameters of insulin resistance were also increased. This shows, that the resulting adipose tissue does not differ from the adipose tissue of normal obesity in terms of metabolic changes.
4. Adiponectin levels were significantly lower in the antipsychotic treated group than in normal controls, but significantly higher than in BMI matched obese controls. This might mean that the impaired suppression of adiponectin secretion might play a role in the dysregulation of appetite control.
5. In patients treated with antipsychotics we found elevated levels of the potential antiapoptotic marker sTNFR2 and sFas compared to both control groups. This may suggest decreased adipose tissue apoptosis.
6. The TNF-α promoter -308 and TLR4 Asp299Gly and Thr399Ile polymorphisms affect cytokine levels and insulin sensitivity in patients treated with antipsychotics. Carrying -308 A, TLR4 299Gly or 399Ile alleles is associated with

lower TNF-α, resistin, leptin, higher adiponectin, sTNFRII, sFas levels and lower parameters of insulin resistance.

7. In the case of PPARγ Pro12Ala polymorphism carrying the rare Ala allele was associated with higher BMI. This might be enhanced by eating patterns of the patients, through environment-gene interactions. Higher BMI is associated with increased leptin, resistin and insulin resistance.
8. During normal pregnancy we found an elevation of fasting serum ghrelin levels in the 2nd trimester and a decrease in the 3rd trimester. In GDM the fasting serum ghrelin level was significantly lower in the 3rd trimester than in normal pregnancy. The changes in ghrelin levels during pregnancy might trigger and terminate the physiological increase in body mass.
9. In normal pregnancy serum resistin levels rise throughout the pregnancy. In GDM we found significantly higher levels, than in the 3rd trimester of normal pregnancy. The resistin level did not show significant correlation with BMI, although it still showed significant positive correlation with insulin resistance, and was an independent predictor of insulin resistance.
10. In GDM we found significantly lower adiponectin levels than in the 3rd trimester of normal pregnancy. The adiponectin level correlated negatively with insulin resistance and was the strongest independent predictor of insulin resistance. The adiponectin levels showed significant positive correlation with the size of the offspring.
11. In the two Hungarian families with A3243G mitochondrial DNA mutation we detected the loss of the first phase of insulin secretion, even without diabetes. We did not find HLA haplotype strongly correlated with type 1 diabetes or GADA or IA2A positivity. The ICA positivity in some family members suggest, that the mitochondrial

dysfunction might trigger some other autoantibody, which does not lead to the total destruction of beta cells, because we could still detect C-peptide levels after long diabetes duration.

6. Publications

Publications related to the dissertation

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