Health status of young adults born with a low birth weight: investigation of the association between adrenal function, carbohydrate and bone homeostasis

Ph.D theses

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1. INTRODUCTION

The low birth weight is regarded as an independent risk factor for several disorders including coronary artery disease, type 2 diabetes, some malignancies, autoimmune disorders, osteoporosis and some psychiatric disorders.

Several reports indicated that hypothalamus – hypophysis – adrenal gland axis is stimulated in low birth weight neonates. This phenomenon persists during later life. Higher than normal basal cortisol levels were measured both in younger and older adults born with low birth weight (LBW). A previous observation of our team is that dehydro-epiandrosterone (DHEA) and dehydro-epiandrosterone-sulphate (DHEAS) levels are higher in adolescent LBW girls and young adult LBW women indicating the presence of functional adrenal hyperandrogenemia. Hypercortisolemia and hyperandrogenemia may increase the risk of cardiovascular disorders in later life.

The over-function of adrenal glands may have further effects; endogenous cortisol- and DHEA-levels may have an impact on bone density and homeostasis. The inverse relationship between cortisol levels and bone density is clearly demonstrated. Others observed an association between DHEAS levels and bone density in some populations. Low DHEAS production during menopause is an independent risk factor for fractures.

Hyperandrogenemia may have an impact on carbohydrate homeostasis; this may be of significance in

LBW groups. Prior data support that LBW girls present with an earlier occurrence of adrenarche. In this group adrenal hyperandrogenemia is present without clinical signs and symptoms. In addition higher fasting insulin levels and a more marked insulin response were detected during oral glucose tolerance test. The link between hyperandrogenemia and hyperinsulinemia is also supported by observations in patients with polycystic ovary syndrome.

During the period of my PhD work there was a trend to study the presence of genetic variants and its link to susceptibility to different conditions. The genetic variants associated with atherosclerosis and its complications were particularly investigated.
These were primarily focused to populations exhibiting an early development of atherosclerosis or any characteristics raising the notion that an inherited component is present. In the majority of chronic conditions including atherosclerosis the interaction between different genes and environmental conditions is probably of outmost importance. In several papers some protein elements with a suspected or demonstrated role in atherosclerosis such as those regulating lipid homeostasis, inflammation, endothelial function were tested. The selection of gene mutations to be investigated was primarily influenced by their prevalence in population, their functional effect and the number of patients enrolled into the study.

2. AIMS and GOALS

Based on these data and prior results of our research team it is clear that LBW young adult population forms a specific group that is characterized by systemic carbohydrate regulation disturbances, adrenal gland dysfunction and disturbed bone homeostasis. Therefore, the investigation of this population and the comparison with individuals born with normal birth weight may provide an opportunity to characterize and describe the association between these systems.

Enrolling LBW young adults I tested two major questions during my work:

1. Is there any link between parameters of bone homeostasis and altered adrenal function?
2. Does acute reactive hyperinsulinemia contribute to increased production of adrenal androgens?

I also performed an re-analysis of published reports in genetic polymorphisms. Re-calculating genotype distribution data collected from papers published in a leading journal (Atherosclerosis) I tested whether:

3. May the conclusions be altered if Hardy-Weinberg criteria are not considered routinely when genetic variants with Mendelian inheritance are investigated in epidemiological studies?

3. METHODS

In our study we asked those young adults to participate that fulfilled the following criteria (1) birth with a birth weight ≤2500 gram between 1977 and 1979 at the First Department of Obstetrics, Semmelweis University; (2) currently free of signs and symptoms of any severe disorder; (3) no drug therapy. Finally, this LBW group consisted of 33 LBW women and 37 LBW men. In addition we also enrolled 30 age-matched control individuals with normal birth weight.

Participants were sample at 7.30 am. after a 12 hour fasting state. Sera obtained for hormone measurements and bone homeostasis parameters were stored for -20 °C until measurements. After samplings individuals provided urinary sample for the determination of some ions and cross links (including deoxypiridinoline (DPD) levels). The next step was oral glucose tolerance test with the administration of 75 gram glucose. Simultaneously with sampling for glucose levels we also took blood for the measurement of serum DHEA, DHEAS, insulin and cortisol levels. DHEA/cortisol rate as an indirect indicator of ACTH-independent DHEA production was assessed before and after glucose administration and this ratio was used to estimate the acute effect of hyperinsulinemia on DHEA production.
We also performed osteodensitometry.

Laboratory tests were done with commercially available kits.

During our in silico analyzis we selected those papers published in the journal of Atheroscleosis between May, 1998 and May, 2003 that investigated the pathogenic role of genetic polymorphisms in any condition. Then we selected those papers from this pool that presented data on distribution of genetic polymorphisms with Mendelian inheritance. We also recorded whether authors reported any calculation of HWE.

Genotype distribution predicted according to Hardy-Weinberg rule was compared to reported frequencies with an appropriate algorithm.

4. RESULTS

4.1 ADRENAL GLAND, BIRTH WEIGHT AND BONE MARKERS

We noticed an inverse relationship between osteocalcin and birth weight in women ($y = 23.6 - 0.0051x$, $r = -0.54$, $p < 0.01$). A direct association was demonstrated between DHEAS and osteocalcin levels ($y = 7.33 + 0.95x$, $r = 0.34$, $p < 0.05$), and DHEAS and urinary DPD excretion ($y = 2.42 + 0.059x$, $r = 0.38$, $p < 0.02$).
During step-wise regression analysis the association between DHEAS and osteocalcin levels remained significant. Birth weight and free estradiol index related inversely to osteocalcin.
The link between DHEAS and urinary DPD excretion remained significant in the step-wise regression model, while other factors had no independent effect on DPD excretion. Cortisol and BMD values did not associate with any of bone parameters.

In men linear regression analysis revealed an inverse correlation between birth weight and osteocalcin levels ($y = 31.9 - 0.0055x$, $r = -0.45$, $p < 0.01$), and birth weight and urinary DPD excretion ($y = 33.9 - 0.0054x$, $p < 0.01$). Step-wise regression analysis also demonstrated an inverse relationship between DHEAS and osteocalcin , and birth weight and osteocalcin, while no significant link between DHEAS and urinary DPD excretion was noted. The participants' age and SHBG levels had independent effect on DPD excretion. Cortisol and BMD values had no effect on bone parameters in men either.

Then the participants were sorted to quartiles according to their DHEAS levels. The antropometric parameters, BMD levels, hormone levels and bone parameters were compared between quartiles. BMD values, PTH, and 25OHD levels did not differ between quartiles. No difference in estradiol and testosterone levels was observed either. Androstenedione, osteocalcin and DPD levels were higher in quartile 3 & 4 than in quartile 1. Quartiles, however, did not associate with birth weight or any other laboratory parameter. The only exception was osteocalcin exhibiting lower levels in quartile 4 than in quartile 1.

4.2 HYPERINSULINEMIA AND HYPERANDROGENEMIA

In men DHEA levels decreased by 30th min of OGTT, irrespectively of birth weight. Then we measured lower levels than the baseline in each time point. The lowest DHEA levels was measured in LBW and control men in 120th and 90th min., respectively. Although the decrease of DHEA-levels was higher in LBW than in control men in each time point, the difference was not significant in any time point. DHEAS levels were stable during OGTT in men.

In LBW women DHEA levels decreased by 30th min of OGTT; then DHEA levels decreased further and we measured lower than basal levels in each time point. In control women DHEA levels were significantly lower than the basal level just in the 120th min of OGTT. In LBW women DHEAS levels were lower than the basal

after the 30th min of OGTT, albeit the maximum decrease in DHEAS-level was just 9%. In control women DHEAS was stable during OGTT.

Basal blood glucose, insulin and cortisol levels and the maximum increase in glucose and insulin during OGTT did not depend on birth weight and gender. During OGTT the decrease in cortisol levels did not depend on LBW; in men, they slightly exceeded those measured in women. Basal DHEAS/cortisol levels were comparable in men and women and in LBW and normal birth weight participants. The ratio of smallest DHEA/smallest cortisol levels was significantly higher during than before OGTT both n LBW and control subjects.

Serum cortisol and DHEA levels associated significantly both in women and men ($r = 0.42$, $p<0.01$, and $r=0.50$, $p<0.01$, respectively). No association between basal cortisol and DHEAS levels was observed. The change in blood glucose levels did not associated with DHEA change during OGTT.

The ratio of smallest DHEA/smallest cortisol levels associated with maximum insulin response and AUCins value during OGTT in women ($r=0.45$, $p<0.05$, and $r=0.48$, $p<0.05$, respectively). This association was not observed in men.

4.3 RE-ANALYSIS OF FULFILLMENT OF HARDY-WEINBERG EQUILIBRIUM IN THE JOURNAL OF ' ATHEROSCLEROSIS'

We selected and reanalyzed 503 genotype distribution published in 134 papers. According to our calculation 45 genotypes from 36 papers did not fulfill HWE criteria. However, authors indicated the deviation from HWE criteria just in 8 genotypes (6 papers); and was not mentioned in 30 papers. In 19 papers out of the 30 papers, however, the authors declared that they had performed HW calculation.

5. THESES

1. Bone homeostasis in young adults is associated with birth weight. In addition, bone homeostasis is largely influenced by dehydroepiandrosterone-sulphate (DHEAS) levels. The impact of DHEAS on bone homeostasis is affected by the gender: bone turnover is increased in fertile women, while it is decreased in men by DHEAS.

2. Reactive hyperinsulinemia occurring during glucose tolerance test may activate androgen pathways in adrenal cortex and, hence, dehydroepiandrosterone production. Acute hyperinsulinemia may moderate the diurnal decrease of dehydroepiandrosterone.

3. Before using any conclusion of studies investigating genetic polymorphisms with Mendelian inheritance, interested readers should check whether genotype distribution fulfills Hardy-Weinberg equilibrium.

PAPERS PUBLISHED IN THIS FIELD

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