INTERDISCIPLINARY APPROACH TO THE PREDIABETIC STATE: BONE – ENERGY HOMEOSTASIS AXIS, CLINICAL AND ETIOLOGICAL ASSOCIATIONS OF NON-ALCOHOLIC FATTY LIVER DISEASE AND INSULIN RESISTANCE

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Relevant publications

I. **Buday B**, Izsóné Katz M, Nagy E, Papp Zs, Korányi L: A metabolikus szindróma, 2-es típusú diabetes és a csontvesztés epidemiológiai összefüggései a Balaton felvidék felnőtt lakossága körében [Relationship of cardiovascular risk factors and bone status in a large adult population of the Balaton Region]. *Ca & Csont* 2007; 10(04): 132-137.

II. **Buday B,** Horváth T, Literáti Nagy B, Kulcsár E, Barta K, Salamon Cs, Péterfai É, Korányi L: A hagyományosan használt inzulinrezisztencia- és béta-sejt-funkciós indexek diagnosztikus értéke [The diagnostic value of traditional insulin sensitivity and beta cell function indices]. *Diabetologia Hungarica* 2007; 15: 93-105.

III. **Buday B,** Pach FP, Literati-Nagy B, Vitai M, Vécsei Z, Korányi L: Serum osteocalcin is associated with improved metabolic state via adiponectin in females versus testosterone in males. Gender specific nature of the bone-energy homeostasis axis. *Bone* 2013; 57(1): 98-104.

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IV. **Buday B**, Pach FP, Literáti-Nagy B, Vitai M, Kovács Gy, Vécsei Zs, Korányi L, Lengyel Cs: Sex influenced association of directly measured insulin sensitivity and serum transaminase levels: Why alanine aminotransferase only predicts cardiovascular risk in men?

Cardiovascular Diabetology 2015; 14: 55 (1-13).

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List of Other Publications

I. **Buday B**, Horváth T, Kulcsár E, Salamon Cs, Literáti-Nagy B, Barta K, Vitai M, Józsa R, Vecsei I, Bezzegh K, Kiss J, Péterfai E, Koltay L, Korányi L: The effect of prospektive insulin resistance on the relationship between glucose metabolism and bone status. *Hungarian Medical Journal* 2007;1(3). DOI: 10.1556/OH-HMJ.2007.28072

II. **Buday B**, Kulcsár E, Lieráti-Nagy B, Horváth T, Vitai M, Vecsei I, Bezzegh K, Kiss J, Péterfai É, Koltay L, Korányi L: Az osteocalcin helye a human cukor- és csontanyagcsere kapcsolatában. [The role of osteocalcin in the connection of bone and glucose metabolism in humans]. *Orvosi hetilap* 2008; 149(52):2453-61.

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VIII. **Buday B**, Pach FP, Literáti-Nagy B, Vecsei Zs, Korányi L: A csontátépülés és az energia háztartás nőkre jellemző kapcsolatai. *LAM-KID* 2011;1(2):30-35.

IX. Pauer J, Fék A, **Buday B**, Literáti-Nagy B, Pach P, Vitai M, Péterfai É, Korányi L: Anyagcsere-eltérések a 2-es típusú cukorbetegek egészséges, első fokú férfi rokonaiban. [Metabolic alterations in healthy men with first degree type 2 diabetic relatives]. *Orvosi Hetilap* 2013; 154 (5): 178-186

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XI. Fék A, **Buday B**, Kovács Gy, Vitai M, Vecsei Z, Pauer J, Literáti-Nagy B, Bezzegh K, Péterfai É, Korányi L: A genetikai diabeteskockázat hatása a csontanyagcsere-energiaháztartás kapcsolatokra. [The effect of genetic risk of diabetes on the correlations in bone and energy homeostasis]. *Orvosi Hetilap* 2015;156(25):1007-13.

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1. Introduction

Obesity and type 2 diabetes are one the greatest global challenge for all health care systems in the 21st century, moreover the incidence of osteoporosis shows an increasing tendency. The growing incidence of both obesity, type 2 diabetes (T2DM) and osteoporosis may be somewhat conflictive, as increased body mass index (BMI) seems to be protective against bone loss. Type 1 diabetic patients have decreased bone mineral density (BMD) and increased fracture risk while in T2DM this association is less strong; data exist about both increased or decreased BMD in T2DM patients.

In order to investigate the relationship between insulin resistance (IR) / insulin sensitivity (IS) and other diseases / symptoms, like osteopenia and osteoporosis we need an easily accessible simple IR measuring method that is cheap, can be used in a large number of patients, reproducible and is validated via more sophisticated studies. Precise measurement of insulin resistance is also important for the prevention, diagnosis and the therapeutic follow up of type 2 diabetes. For measuring IS, today the "gold standard" is still the "hyperinsulinemic normoglycemic clamp" developed by *DeFronzo et al*. However, it is an expensive and time consuming method which cannot be used in large number of patients in clinical setting, so there have been a number of attempts to develop methods replacing the clamp, e.g. the HOMA indices (Homeostasis Model Assessment) which use data from fasting blood samples, or so are a number of indices derived from the OGTT (oral glucose tolerance test).

The first part of my work deals with the diagnostic evaluation of IS in terms of simple fasting and OGTT derived indices which still seem to be a hurdle in IS estimation since the most widely used HOMA indices in clinical practice do not correlate well with the gold standard clamp methods.

We aimed to gain further insights into the pathophysiology and diagnosis of IR and related complications by studying the association between transaminase levels and clamp measured insulin sensitivity, moreover we sought to explore a unique side of the gender specific aspect of insulin homeostasis / energy metabolism, with special regard on the non-alcoholic fatty liver disease which is one of the major link between insulin resistance and cardiovascular disease. By exploring the pathophysiology of the IR related steatohepatosis often associated with the 'unexplained' elevation of transaminase levels in overweight insulin resistant / type 2 diabetic patients, we might be able to improve the value of the HOMA

model with no extra costs, although possible gender related differences will have to be taken into account.

To explore the relationship between type 2 diabetes / IR and bone homeostasis first we analyzed data from a large epidemiologic study which included the screening results of more than 6000 people in Balaton Upper-Lands. Based on the results of this epidemiologic study, we conducted a cross over analysis on our existing clamp database where we measured markers of bone turnover, i.e. total non-carboxylated osteocalcin (OCN) levels and other metabolic-hormonal factors, like adipocytokines, lipids, lipoproteins, sex hormones. Previous human studies have shown that serum OCN concentration is negatively associated with the plasma glucose level and body fat mass and positively associated with insulin secretion, lower insulin resistance and higher serum adiponectin concentration. In most of this work, the homeostasis model assessment has mainly been used to assess β-cell function, insulin sensitivity and the involvement of OCN on glucose metabolism, although we and others have shown that fasting indices do not always correlate well with the real insulin resistance, therefore insulin sensitivity was measured by the gold standard clamp method. Recently, it has been demonstrated that osteoblasts are able to induce testosterone production by the testes, though they fail to influence oestrogen production by the ovaries. The role of testosterone in the bone–energy homeostasis is presumably gender-specific, as the effects of OCN were only demonstrated in Leydig cells and not in the ovarium; moreover, low testosterone levels are only associated with a metabolic syndrome in men.

2. Aims

Based on previous data and our preliminary assumptions discussed above our main goals were:

-To explore the diagnostic value of simple fasting and OGTT derived insulin sensitivity (and beta cell) indices compared to the gold standard clamp method.

-To try to justify possible new directions in the simple diagnosis of insulin resistance.

-To explore epidemiological characteristics of the relationship between bone loss and diabetes / insulin resistance.

-To explore molecular background of the bone – energy homeostasis axis, by analyzing the associations between clamp measured insulin sensitivity, OCN and other metabolic biomarkers

- Since former data suggested that basic sex differences exist in the pathogenesis and manifestations of insulin resistance and associated diseases, male and female populations were separately analyzed in most of our studies to address this issue.

3. Patients and methods – general considerations

3.1. Epidemiologic study

In our study we analyzed data from general screening tests amongst the adult population of Balaton Upper-lands between 2003 and 2006. All screenings were approved by National Public Health and Medical Officer's.

During screening tests anthropometric measurements (weight, height, abdominal circumference), sitting blood pressure (Omron 705CP digital tool), blood sugar determination from capillary blood (Personal Dcont and Optimum mechines, 77 Elektronika, Hungary), total cholesterol determination (Accutrend GCT 1537962 and Accutrend GC 1418246, Roche, Germany), and calcaneal ultrasound bone density measurement (GE-Lunar, Achilles Plus, USA) were carried out, which latter is an accepted method in the diagnostics and follow-up of osteoporosis / osteopenia.

Measurements were not always done in a fasting state, although data were available about the time and the ingredients of the last meal and that the data whether subjects were taking antidiabetic drugs. We have analyzed the following parameters: gender, age, systolic and diastolic blood pressure, blood sugar, total-cholesterol, abdominal circumference (AC), BMI, T-score (the number of standard deviations above or below the mean calcaneus bone density of a healthy 30-year-old adult of the same sex and ethnicity). Based on available data and the results of the screening examinations we defined certain diseases / syndromes to be present in the population, like diabetes, hypertension, metabolic syndrome, osteopenia and osteoporosis.

3.2. Clamp studies

3.2.1. OGTT, IVGTT and clamp

All clamp studies were carried after receiving signed informed consents from the subjects between 2005 and 2010. Study was approved by the Hungarian Ethical Committee (A12988-2/2003-1018-EKU, ad.8-311/2009-1018EKU). Patients were categorized as normal glucose tolerant (NGT), having impaired fasting glucose (IFG), impaired glucose tolerant (IGT) or T2DM corresponding with the ADA criteria based on an OGTT test at screening within 3 weeks before the clamp study. Subjects with NGT, IGT/IFG or drug naïve T2DM were included in the study, patients who were taking any antidiabetic medications, suffering from endocrine disease or were hormone substituted, were excluded.

All subjects fasted on the day of the clamp examination. They first underwent an intravenous glucose tolerance testing (IVGTT) examination to assess insulin secretion (0.3 g/ bodyweight iv. glucose injection). Following the standard IVGTT procedure, a hyperinsulinaemic normoglycaemic clamp was carried out, as described by DeFronzo et al. For clamp index we used whole body glucose uptake (M1) and lean body (muscle) adjusted glucose uptake (M3). Glucose and insulin area under the curve (AUC) values were calculated using the trapezoidal rule, both from OGTT and IVGTT. Body composition was determined by dual-energy X-ray absorptiometry (DEXA). We determined the most frequently used fasting (HOMA, QUICKI, FFA-QUICKI models) and OGTT IS indices (MCR_{est}, $ISI_{Cederholm}$, OGIS, $ISI_{comp,}$ i.e. the 'Stumvoll indeces') to assess their diagnostic value compared to the gold standard clamp method.

3.2.2. Statistics

For the epidemiological study we used SPSS 10.0 statistical software. Data were represented as mean and standard deviation, or percentage of prevalence. For comparing disease prevalence within groups of different genders and ages we used the $\chi 2$ test and Fisher test. For the assessment of the association between two parameters we used bivariate correlations. For comparing numeric results we used 2 sided tests. A $p<0.05$ was considered as significant.

For the clamp studies all statistical analyses were performed with R Statistical Software. We used either mean and standard deviation, and median and mean absolute deviation (MAD)

for the expression of data points as not all data were normally distributed. The Wilcoxon rank sum test was used to assess group differences of biochemical and anthropometric parameters. In case of normally distributed parameters we used two sided t tests. Spearman correlations were used to assess the association between metabolic and other parameters. Partial correlation coefficients were used to assess the influence of possible confounding factors. A p value<0.05 was considered statistically significant. The Boruta algorithm (feature selection analysis) was used to find the most important attributes that were related to the M3 value in all of our clamp studies. Multiple regression analysis was used to find parameters that were independent predictors of the clamp M3 value.

4. Results

4.1. Diagnostic evaluation of simple insulin sensitivity indices

We performed OGTT examinations on 317 subjects during the screening period. (From the OGTT samples we determined glucose and insulin levels). We included 45 NGT, 67 IFG/IGT and 24 T2DM patients who all underwent an IVGTT followed by a hyperinsulinemic normoglycemic clamp on the first day of the study. We analyzed 1. Correlations between each analyzed fasting index and M1 in all subjects and different subset of subjects categorized by age, sex, glucose tolerance, beta cell function. 2. The cumulative value of all fasting indices (based on the strength of correlation coefficients and significance levels) in each assessed groups. Based on these analyses we found that most of the fasting indices showed moderate (r=0.4-0.6) correlations with M1 within all patients and in the homogenous subsets of patients. Correlations were missing using one sampling between fasting indices and M1 in the elder (age above median), normal glucose tolerant (NGT), and less severe (HbA1c under median) glucose intolerant groups which correlations improved if the mean of two samples were used (OGTT and IVGTT 0^{th} minute samples). In HOMA-2 (HOMA-S%, i.e. Oxford model) we found good correlations (r=0.6-0.8) only in the young NGT, diabetic and male groups. The sensitivity of fasting indices (average 50.4 ± 4.6%) is low, while their specificity (83.3 ± 1.6%) is high, i.e. there are a relatively large number of false negative but less of false positive cases. Cumulative evaluation of the fasting and OGTT derived indices based on group subanalyses show that OGTT indices have a greater value than fasting indices compared to clamp index, and have more homogenous results within the subgroups. $ISI_{cederholm}$, MCR_{est}, and $ISI_{est,}$ including bodyweight / BMI in their equations, derived from

the OGTT, show the strongest correlations with M ($r > 0.6$ correlations in most groups). The average sensitivity and specificity of OGTT indices were slightly higher than that of the HOMA model (60.2 ± 8.0%, and 87.2 ± 1.8% respectively) but the diagnostic value based on the sensitivity values are still rather low.

4.2. Results of the clamp study on ALT – insulin sensitivity connections

We included 74 NGT, 74 glucose intolerant (IFG or IGT or T2DM) male, 47 NGT and 111 glucose intolerant female subjects respectively in the analysis. Mean HbA1c values were under 6.1 % in all groups, i.e. the population consisted of either normal glucose tolerant or mostly prediabetic (IGT/IFG or freshly diagnosed T2DM) subjects, both slightly overweight and obese individuals. Genders were analyzed separately as we expected basic sex difference in the role of alanine transaminase (ALT) in predicting camp measured insulin sensitivity.

After correcting with possible confounding factors, in males triglyceride, HDL-cholesterol, free fatty acid (FFA) and acute insulin response (AIR) showed significant correlations ($p<0.05$) with ALT (and AST). In females it was the clamp measured glucose uptake per se along with blood sugar values that stayed significantly associated after correction was done.

Multiple regression analysis was conducted in order to determine the ability of the 'important' attributes selected by feature selection analysis (Boruta Algorithm) to predict clamp measured M3 values, separately in the male and female populations. The results of the regression analysis show that $F = 29.95$ ($p < 2.2e-16$) for women, indicating that the 'important' variables (BMI, AC, FFA, Insulin and ALT) collectively have a significant effect on M3, ALT ($p=0.00991$) and BMI ($p=1.9e-05$) being significant independent predictors in women. In men the 'important' attributes (AC, Leptin, BMI, Insulin, TG, FFA, Glucose, and diastolic blood pressure) have a significant effect on M3 [$F = 14.71$ ($p < 2.36e-16$)], serum leptin ($p=0.00294$) and insulin ($p=0.00210$) levels being independent predictors of clamp M3 values. Linear regression model for fitted (i.e. determined by the equation including 'important' attributes determined by feature selection analysis) vs. measured M3 values gave an excellent estimation in women, less so in men.

4.3. Results of the epidemiological study about the association of metabolic syndrome, type 2 diabetes and bone loss in the adult population of Balaton Upper-lands

In our database all together data of 6287 screening results were available (mean age 56±13 years), 1561 men (mean age 56±13 years) and 4726 women (mean age 54±13 years). We have assessed the prevalence of diabetes, hypertension, hypercholesterolemia, metabolic syndrome, osteopenia and osteoporosis in this population. We have examined the associations between bone metabolic disorders and metabolic diseases, with special emphasis on the influence of gender and age. Categories do not mean definitive diagnoses, as one time capillary blood sugar and total-cholesterol determinations do not always enable us to adapt internationally accepted diagnostic guidelines, on the other hand we took these into consideration when defining diseases and syndromes.

When assessing the associations between T score and metabolic parameters we found that in women there is a significant positive correlation between BMI and T score except for the age group under 40 years; this correlation being the strongest within the population over 70 years ($r=+0.23$, $p<0.001$) and is missing in males. AC shows a weak positive association with T score in women under 40 ($r=+0.2537$, $p<0.01$) and in men between 40 and 50 years of age ($r=+0.234$, $p<0.05$). Glucose level shows significant positive correlation with T score only in men over 70 years ($r=+0,359$, $p<0,001$). No significant difference was noted in the prevalence of decreased bone density (osteoporosis or osteopenia determined by the calcaneus measurement) within the population with metabolic syndrome compared to those subjects without metabolic syndrome in either gender or age group. However we found that in women between 51 and 60 years osteopenia was more frequent within the diabetic then in the non-diabetic group (50 vs. 36.34%, $\chi 2$:5.237, $p<0.022$, OR: 1.711, 95% CI: 1.076–2.722), although the mean BMI in the diabetic group was significantly higher than in the normal glucose tolerant group (!) (29.42± 5.27 vs. 27.73±4.77, $p<0.008$). No such difference was noted in men in neither of the age groups.

If we analyzed normal weight and all NGT and diabetic populations separately we found that in the population >60 years the prevalence of decreased bone density was higher in the normal weight diabetic subjects then in the normal weight NGT group. The difference was border significant (63.63 vs. 26.2%, OR: 2.71, 95% CI: 0.969–7.6, $p=0.054$) in case of osteoporosis, only a tendency was noted for osteopenia (53.38 vs. 43.31%, $p=0.359$).

Meanwhile there was no difference between the frequency of osteopenia / osteoporosis in all non-diabetic versus all diabetic subjects.

4.4. Clamp study of bone – energy homeostasis connections

After obtaining signed informed consent, we included 135 women (aged 49 ± 9 years) and 155 men (aged 42 ± 13) in our study, as approved by the ethical committee. Subjects were classified based on results of a standard 75-g OGTT at screening. We included 47 normal glucose-tolerant (NGT) and 89 glucose-intolerant (GI) subjects in the female group; in the male group, there were 72 NGT and 83 GI (IFG, IGT and drug-naïve T2DM) subjects. All GI patients, which included impaired fasting glucose (IFG), impaired glucose-tolerant (IGT) and type 2 diabetes mellitus (2DM) patients, were drug-naive at the time of the study.

Although OCN mean values were slightly higher in the NGT than in the GI groups in both genders, no significant difference was observed in OCN levels between NGT and GI subjects. OCN mean values in our population were somewhat lower than described in healthy male and female population, although stayed in the normal ranges. Subjects with extreme values were excluded from the study. Significantly higher total testosterone values were found in NGT than in GI males, while no such difference was observed between the respective female groups.

In females, significant association was observed between OCN and adiponectin levels ($R=+0.254$, $p<0.001$) independent of age, HbA1c, BMI and BFP. Higher OCN values were associated with increasing age ($R=+0.231$, $p<0.01$). Significant ($p<0.05$) correlations between OCN and the indicators of improving metabolic state (lower OGTT glucose values, BMI, BFP, higher M1 andM3 levels) became obvious after the adjustment for age alone. Most of these associations ceased after further adjustment with HbA1c, BMI and BFP, except for fasting glucose. Further adjustment with adiponectin resulted in lost correlation between OCN and fasting glucose value, i.e. OCN effect on improving glycemic control might be partly mediated by adiponectin in females. Further adjustment was done with FSH levels in a subset of 68 women (where FSH levels were available) after which positive associations between improved metabolic state and OCN became ambiguous, similar to the influence of age: $R = -0.323$, $p = 0.0062$ with HbA1c, $R = -0.349$, $p = 0.0026$ with IVGTT glucose AUC, $R = -0.288$, $p = 0.0153$ with OGTT glucose 0^{th} min, $R = +0.260$, $p = 0.031$ with M3. A strong positive association ($R = +0.413$, $p = 0.00047$) was found between FSH levels and OCN as expected, independent age, BMI, body composition and HbA1c values.

In males, the OCN levels were significantly associated with improving metabolic state (decreasing OGTT insulin AUC [R=-0.179, p<0.05], leptin [R=-0.168, p<0.05], BMI [R=-0.199, p<0.05], BFP (body fat percent) [R=-0.172, p<0.05] and increasing insulin sensitivity, i.e., M1 and M3 values [R=+0.229 and +0.221 respectively]). These correlations disappeared after the adjustment for age, HBA1c, BMI and BFP. The significant positive correlation between OCN and testosterone levels (R=+0.243, p<0.01) was independent of age, HBA1c, BMI and BFP. After correction for testosterone alone, the significant positive association between OCN and M3, as well as the significant negative correlation with leptin and BMI was lost, i.e. metabolic associations of OCN were at least partly mediated by testosterone in males. Feature selection (Boruta algorithm) confirmed that age, IVGTT glucose 3^{th} , 5^{th} and 60^{th} minute values and adiponectin (mean Z: 9.76, 5.41, 4.09, 3.75 and 3.69, respectively) were the most important attributes in determining OCN levels in women. In men, M1, BMI, M3, leptin, BFP, OGTT 90 minute glucose and insulin values as well as testosterone (mean Z: 5.21, 4.95, 4.41, 4.04, 3.62, 3.57 and 3.54, respectively) but not adiponectin, were confirmed as the most important parameters independently associated with OCN amongst all metabolic factors examined.

5. Discussion

5.1. Evaluation of simple indices / methods in the estimation of clamp measured insulin sensitivity

Quantitative determination of IS and/or beta cell function has a great significance both in clinical practice and in scientific studies. The correlation between routinely available fasting indices (i.e. HOMA model) and clamp techniques are quite diverse, some studies show strong others show moderate to week correlations with clamp data.

In our study, the correlations were only moderate (r= 0.4-0.6) between HOMA and M1 in most groups. We found weaker correlations in the NGT and IFG/IGT/DM groups with higher age (above median). Correlations between fasting and clamp indices were weaker in subjects with lower HbA1c and higher AIR (i.e. less severe metabolic state) than in subjects with higher HbA1c and lower AIR (i.e. more decreased beta cell function). According to our results the utility of HOMA-S% (Oxford Model) was far behind the expected, although today it is considered the most accepted, best fasting index. Correlations between QUICKI and M1 was not improved by including FFA in the equation according to results which is in contrast

with previous studies. We found that the three OGTT indices including bodyweight or BMI in their equations, MCR_{est}, ISI_{est} and $ISI_{cederholm}$ showed the best correlation with clamp IS. Based on the correlations measured within the different groups, the cumulative scores approached 90%, although their sensitivity was only slightly higher than that of the fasting indices (67% vs. 50%).

In summary, including BMI in the IS index improves its value compared to equations based only on glucose and insulin values in this mostly overweight population with varying glucose tolerance. Besides, OGTT derived IS indices show significantly better results than the HOMA derived models and a fairly homogenous picture within most of the subgroups analyzed in our study and as such can be recommended to measure IS in most patients where they are feasible and clamp results are not available.

A gender (and racial) difference in the utility of insulin-based fasting and OGTT-based models has recently been described, as both gender and race, had a significant effect on explaining the predictability of clamp-measured glucose disposal rates. Although we did not find significant difference between genders in the value of either fasting or OGTT indices (all of the subjects were Caucasian in our study), we did find some basic gender difference when we analyzed the association between liver enzymes and insulin sensitivity.

One of the most important findings of our study in this healthy/prediabetic population is that after the adjustment for confounding factors such as age, BMI, abdominal circumference, body fat percent, HbA1c, alcohol consumption and FSH, all three liver enzymes (ALT, aspartate transaminase (AST) and gamma-glutamyl transferase (GGT)) stayed significantly associated with clamp-measured insulin sensitivity (i.e. muscle glucose uptake) in women but disappeared in men. This difference was only applicable for the gold standard clamp measured peripheral insulin sensitivity, i.e. the association with the estimated OGTT derived Hepatic Insulin Resistance Index (although stronger in females than in males) disappeared in both genders after the correction was done. Moreover, the multiple regression model has found that ALT was a significant independent predictor of clamp insulin sensitivity besides BMI in females. In men, this was fasting insulin and leptin but none of the liver enzymes. Our results support the hypothesis that a very well definable difference exists in the progression /association of NAFLD with metabolic parameters in the adult population. In women, it is the insulin resistance per se which might indicate liver fat accumulation, and vice versa, elevated ALT levels may indicate decreased insulin sensitivity before hyperinsulinemia

develops. In men, ALT (also AST and GGT) elevations coexist with other metabolic changes followed/caused by insulin resistance. Therefore in men liver enzyme elevation per se is not an indicator of decreased insulin sensitivity but a general metabolic deterioration along with insulin resistance with no independent associations with the clamp M3 value.

Hence, according to our results slightly elevated ALT may strongly indicate the presence of insulin resistance in females even without hyperinsulinemia, especially in overweight women. A future direction could be to compile transaminase levels with the HOMA index, which may increase the diagnostic value of the HOMA model, especially in females, as our unpublished data have also confirmed this notion.

As a conclusion we found that: 1. Conventional fasting IS indices only correlate moderately with the clamp results, 2. OGTT derived IS indices correlate well with the clamp data so whenever it is possible they should be used instead of the less precise fasting indices, 3. Liver transaminase levels may increase the diagnostic value of the conventional fasting indices, especially in females, and as such they always have to be considered to be a useful tool for IR diagnosis as part of the routine laboratory assessments.

5.2. Bone – energy homeostasis axis

In most studies the positive effect of T2DM on BMD and the frequency of osteoporosis decreases or ceases if data are adjusted with BMI, meaning that this protective effect is likely due to the increased body weight. The increased bone density in T2DM is indeed the consequence of increased BMI according to our results; diabetes per se is rather a risk increasing than protective factor, especially in the female population. Additionally, blood sugar was associated with bone state only in elderly male population and BMI only showed positive correlation with T score in females. Furthermore osteopenia was significantly more frequent in early postmenopausal diabetic women than in the NGT group despite increased BMI, which was not observed in the male population. Our results drew the attention to the following notions: 1. Basic gender difference may exist in the bone – energy homeostasis relationship 2. Diabetes for bone loss may be considered rather a risk increasing than protective factor.

Since our preliminary clamp results have found gender specific signs in the bone metabolism – energy homeostasis relationship, and recent data have been reported about the close association between OCN and testosterone in male mice, in our next study we analyzed

the gender specific correlations of OCN being the most important humoral link between bone and insulin / glucose metabolism.

We have found that even following the adjustment with confounding factors, higher OCN values were associated with higher adiponectin levels in female subjects similar to earlier studies, moreover the adjustment with adiponectin resulted in lost correlation between OCN and fasting glucose, which supports the mediating role of adiponectin in some but not all of the metabolic effects of OCN in females. Feature selection analysis showed that OCN is independently associated with adiponectin, age, and three of the IVGTT glucose values. This is in accordance with the study of Kanazawa et al. who found a significant positive association of serum osteocalcin and adiponectin levels only in postmenopausal women, but not in men however is in contrast with other findings where the significant association between OCN and adiponectin was independent of sex.

Our study confirms the association of serum OCN with improved metabolic state (after correction of age and FSH) but the mechanism of action may differ significantly between sexes. In contrast to female subjects, no sign of an 'OCN–adiponectin axis' was detected in men; i.e., no correlation was found between adiponectin and serum OCN levels. Recently, it was demonstrated that osteoblasts may induce testosterone production by the testes, though ovarian oestrogen production in females is unaffected. Analysis of cell-specific loss- and gain-of-function models has revealed that OCN regulates the expression of enzymes required for testosterone synthesis in a cAMP response element-binding protein-dependent manner, thus promoting germ cell survival. Our results show that adiponectin is not associated with OCN levels in male subjects; however, increasing OCN levels were indeed associated with higher testosterone values, independent of age, weight, adiposity and HbA1c. When data were adjusted for testosterone levels, some of the significant correlation between OCN and insulin sensitivity, leptin and BMI disappeared. Correlations with whole body glucose uptake, BFP, waist circumference, and triglyceride values were not affected. Feature selection analysis showed that in men OCN is associated with testosterone, BMI, BFP, M1, M3, OGTT 90^{th} min insulin and glucose, and leptin levels. These results might suggest a partial role of testosterone in the metabolic connections of OCN in males, although because the data were cross-sectional, the cause–causality relationship needs to be further clarified.

6. Summary

Between 2005 and 2010 we have carried out approximately 300 hyperinsulinemic normoglycemic clamp studies mostly on normal glucose tolerant and prediabetic patients including drug-naive type 2 diabetic patients at the time of the clamp. In our first study we have assessed the diagnostic value of simple fasting and OGTT derived insulin sensitivity indices and we found that the HOMA model which is used on an everyday basis in clinical practice does not always predict clamp measured insulin sensitivity precisely. OGTT derived insulin sensitivity indices may be a better alternative. In Hungary this was the first large sample sized clamp study which specifically addressed this issue. Using simple liver function test (like ALT) to improve the value of the HOMA model might be helpful, although because of the gender specific nature of the insulin resistance driven pathogenesis of NAFLD described by us first in detail, may only be a valuable approach in women according to our findings. The results of a large sample sized epidemiologic study in 'Balaton Upper-land' carried out between 2003 and 2005 including more than 6000 subjects, drew our attention to the 'Janus faced' nature of the relationship between type 2 diabetes and bone metabolism. Moreover, we have found important new data about possible gender differences in this respect which was also confirmed by our preliminary clamp data. In our further clamp studies, we have proven that in women an 'OCN–adiponectin–glycemic state axis' versus an 'OCN–testosterone–insulin resistance axis' in men exist which draws the attention to the gender specific nature of the bone – energy homeostasis association.

Future directions include the possible further discovery of multiple sides of the bone – energy homeostasis which will enable us to understand clinically meaningful associations in this respect. This may help us in understanding insulin resistance pathogenesis and the prevention of diabetes linked bone loss, which would have a great impact on the diagnosis, prevention and the individualized therapy of some of the most burdening chronic diseases in today's health care systems.

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