Sclerostin and its associations with energy metabolism in children and adolescents

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Background

The recent evidence has shown that the skeleton can in turn affect carbohydrate metabolism. Bone-derived osteocalcin, which is a specific marker of bone formation, predominantly synthesized by osteoblasts, is pharmacologically active on glucose and fat metabolism [Convareux, 2009]. The results of studies on animals revealed that mice lacking the osteoblast-secreted molecule osteocalcin display decreased β-cell proliferation, glucose intolerance and insulin resistance. Removing one *Osteocalcin* allele from OST-PTP-deficient mice corrects their metabolic phenotype. Ex vivo, osteocalcin can stimulate *CyclinD1* and *Insulin* expression in β-cells and *Adiponectin*, an insulin-sensitizing adipokine, in adipocytes; in vivo osteocalcin can improve glucose tolerance. By revealing that the skeleton exerts an endocrine regulation of sugar homeostasis this study expands the biological importance of this organ and our understanding of energy metabolism [Lee, 2007].

Another osteoblast-secreted molecule – sclerostin is a potent antagonist of the Wnt/β-catenin pathway, which is a major regulator of bone mass. Increased bone formation through this pathway results from both the expansion of osteoprogenitor cells as well as reduced apoptosis of mature osteoblasts [Baron 2007, Kirshan 2006]. Its biological importance is underlined by clinical observations in subjects with sclerosteosis and van Buchem disease, two genetic disorders with impaired sclerostin production and markedly increased bone mass [Staehling-Hampton 2002, Baleman 2002]. A neutralizing antibody to sclerostin has recently shown to increase bone formation and to also reduce bone resorption both in mice [Li, 2009] and humans [Padhi, 2011]. Fascinatingly, attenuation of Wnt-mediated transcription, resulting from an autosomal-dominant missense mutation in LRP6, a coreceptor for the Wnt-signaling pathway, has been linked recently genetically not only to premature osteoporosis, but also to coronary artery disease as well as several features of the metabolic syndrome including hyperlipidemia, hypertension, and diabetes. It was hypothesized that antagonism of Wnt signaling by oxidative stress with increasing age may be a common molecular mechanism contributing to the development not only of involutional osteoporosis, but several pathologies such as atherosclerosis, insulin resistance, and hyperlipidemia. [Manolagas, 2007]. An increased sclerostin levels were found in adult patients with type 2 diabetes mellitus (DM) and atherosclerotic diseases [Morales-Santana S, 2013].

Objective

The aim of the study was to to analyze associations between serum level of sclerostin and as well other bone-related molecules as adipokines and some markers of glucose and lipid metabolism in children and adolescents.

Methods

Fifty-seven patients, 40 with type 1 diabetes mellitus (T1DM) and 17 with obesity, treated in the Department of Pediatric and Adolescent Endocrinology and 11 control, healthy age- and BMI-matched children were included in the study during years 2012-2014. The Local Ethical Committee approved the study. All participants and their parents gave their written, informed consents.

Patients with T1DM, 19 girls and 21 boys, mean age 12.4±2.7 yrs, body mass index (BMI) 20.2±3.8 kg/m^2 were treated with an intensive insulin therapy method. In 32 patients insulin analogs were administered, among which were 17 patients who were treated with CSII. In 8 patients, regular and NPH insulin were administered. The mean daily insulin dose of the entire group of patients reached 0.8±0.57 IU/kg b.w. The mean diabetes duration was 4.2±2.9 years.

Seventeen **obese children**, 9 girls and 8 boys, mean age 11.3±3.8 yrs, BMI 27.2±7.3 kg/m^2 were included into the study. Obesity was defined as at or above 95th percentile of the sex-specific BMI for age growth charts.

Control group consisted of eleven, healthy children, mean age 11.5±5.0 yrs, BMI 19.0±2.8 kg/m^2.

Methods

Anthropometrical measurements

Height was measured to the nearest millimeter using a rigid stadiometer. Weight was measured unclothed to the nearest 0.1 kg using a calibrated balance scale. Reference data for Polish Children were used [Palczewska, 1999]. Body mass index (BMI) will be calculated as weight in kilograms (kg) divided by the square of height in meters (m^2).

Material

Fasting blood samples for measurement of bone derived sclerostin, osteocalcin (OC) and Receptor Activator of Nuclear Factor NF-κB ligand (RANKL), fat tissue-derived leptin and adiponectin, as well as vitamin D, lipid profile, glucose, C-peptide, insulin, and HbA1c concentrations were taken from the antecubital vein at 8.00 AM. HbA1c, glucose, and lipids levels were measured at once. After clotting, blood samples were centrifuged. Serum was stored in -80°C until the time of measurement of the rest parameters.

Biochemical methods

Serum levels of: sclerostin (Biomedica), osteocalcin (DiaSource), RANKL (Biomedica), leptin (DiaSource), adiponectin (DiaSource), insulin (DiaSource), and C-peptide (Diasource) were measured by ELISA methods. 25(OH)D3 was measured with HPLC. HbA1c was measured by standardized ISCC method. Serum glucose level was measured by dry chemistry, and lipids with an enzymatic method (routine chemistry method).

Statistical analysis

Statistical analysis was performed using the Statistica software package. In statistical analysis ANOVA with post-hoc Turkey test, and multiple regression analysis were used.

Results

Serum levels of sclerostin, OC, RANKL, and adiponectin did not differ among three groups: patients with T1DM, obese patients, and healthy ones. There were significant differences regarding leptin, C-petide, fasting serum glucose (FG), HbA1c, LDL-cholesterol levels, and HDL-cholesterol/Total Cholesterol ratio among the examined groups. Leptin and C-peptide concentrations were significantly higher in obese children than in patients with T1DM and in controls (p<0.001, p<0.0001, respectively). FG and HbA1c were significantly higher in patients with T1DM than obese and healthy ones (p<0.001) (Table 1). HDL-cholesterol level, and HDL-cholesterol/Total Cholesterol ratio were the highest in healthy ones (p<0.0001, p<0.001, respectively) and LDL-cholesterol level was the highest in obese patients (p<0.001) (Table 2).

Table 1.
The mean values (±SD) of sclerostin, osteocalcin (OC), Receptor Activator of Nuclear Factor NF-κB ligand (RANKL), leptin, adiponectin, C-peptide, HbA1c, fasting serum glucose (FG), and vitamin D concentration in patients with T1 DM, in obese patients and in controls.

Group	Sclerostin [pmol/l]	OC [ng/ml]	RANKL [pmol/l]	Leptin [ng/ml]	Adipo-nectin [ug/ml]	C-peptide [ng/ml]	Insulin [uIU/ml]	HbA1c [%]	FG [mmol/l]	25(OH)D$_3$ [ng/ml]
T1 DM	34.8±8.8	26.3±16.6	24.9±43.8	2.2±3.3	11.6±4.4	0.54±0.2	-	7.7±1.6	7.9±2.9	20.4±9.4
Obese	40.2±9.9	28.9±17.4	44.3±51.6	8.1±5.9	6.2±5.3	1.52±0.6	15.6±4.5	5.2±0.2	4.4±0.5	17.2±9.2
Control	34.1±6.9	21.7±15.9	28.2±40.7	2.4±0.9	7.7±3.3	0.96±0.3	11±5.8	5.4±0.1	4.4±0.6	19.2±9
P	NS (0.19)	NS	NS	<0.001	NS	<0.0001	NS	<0.001	<0.001	NS

Results

Table 2.
Differences in lipid profile (mean±SD data are presented) among the groups of patients with T1 DM, obese patients and controls.

Group	Chol [mmol/l]	TGL [mmol/l]	HDL [mmol/l]	LDL [mmol/l]	HDL/TC [%]
T1 DM	4.43±0.7	1.01±0.7	1.57±0.3	2.36±0.7	35.97±7.8
obese	4.67±1.1	1.5±1.0	1.18±0.3	3.04±0.8	26.0±7.2
control	3.94±0.8	1.02±0.5	1.42±0.3	2.04±0.6	37.3±10.1
p	0.07	0.07	<0.0001	<0.001	<0.001

In multiple regression analysis sclerostin was positively related to OC and negatively related to HbA1c level (p < 0.001, p = 0.04, respectively) in the whole group. The linear associations between sclerostin and OC and between sclerostin and HbA1c levels are presented in Figures 1 and 2 (Figure 1 and 2).

Figure 1
The linear association between sclerostin and osteocalcin in children and adolescents adolescents

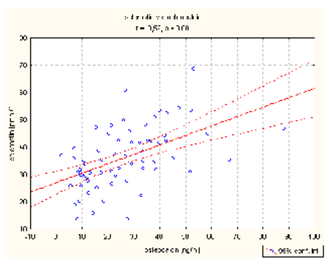
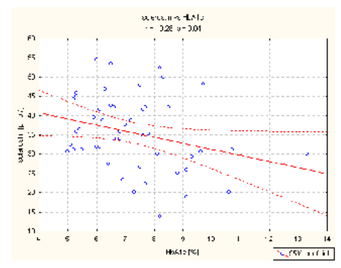


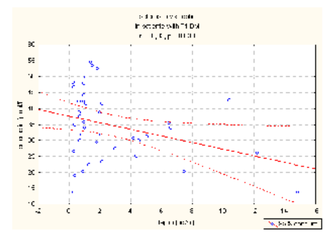
Figure 2
The linear association between sclerostin and HbA1c level in children and



Type 1 DM patients

In the group of patients with T1DM the partial regression coefficient of sclerostin for OC was strong (r = 0.62, p < 0.001). Moreover in that group, multiple regression analysis showed that sclerostin was negatively related to as well HbA1c as leptin levels (p=0.036, p=0.038, respectively). The linear association between sclerostin and leptin is presented in Figure 3 [Figure 3].

Figure 3
The linear association between sclerostin and leptin in children and adolescents with type 1 diabetes mellitus



Obese patients

In obese patients multiple regression analysis did not find any relationships between sclerostin and other parameters. C-peptide levels was strongly positively related to BMI in that group (p=0.0004).

Control group

In the control group sclerostin was positively related to C-peptide level (p=0.02). Moreover C-peptide was positively related to BMI and RANKL in healthy ones (p=0.020, p=0.0036, respectively).

Conclusions

The results of our study suggest that sclerostin could play an important role in the energy metabolism in children and adolescents. Its action seems to be associated with other bone-derived molecules as osteocalcin, but also fat-derived leptin. Moreover their relationships could be modified in different metabolic stages.

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