**Bone markers sclerostin, osteoprotegerin, and bone-specific alkaline phosphatase are related to insulin resistance in children and adolescents independent from their association with growth and obesity**

***Supporting information***

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**Table S1. Phenotype characterization of the cohort of children and adolescents**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Whole group (n)** | **BMI categories** | | | |
|  | **Normal weight** | **Overweight** | **Obese** | **p (ANOVA)** |
| **Age (years)** | 10.3 ± 3.6 (1325) | 10 ± 3.7 (1020) | 10.9 ± 3.0 (99) | 11.3 ± 3.0 (206) | **<0.001** |
| **Pubertal category\*** | 0.6 ± 0.6 (1242) | 0.5 ± 0.6 (962) | 0.8 ± 0.7 (95) | 0.8 ± 0.7 (185) | **<0.001** |
| **Height-SDS** | 0.21 ± 1.01 (1322) | 0.07 ± 0.95 (1017) | 0.56 ± 1.00 (99) | 0.73 ± 1.06 (206) | **<0.001** |
| **BMI-SDS** | 0.37 ± 1.22 (1322) | -0.17 ± 0.75 (1017) | 1.57 ± 0.18 (99) | 2.47 ± 0.44 (206) | **<0.001** |
| **Percentage of body fat (%)** | 20.2 ± 10.8 (295) | 15.8 ± 7.2 (228) | 29.0 ± 5.0 (16) | 36.9 ± 6.7 (51) | **<0.001** |
| **Systolic blood blood pressure SDS** | 0.20 ± 0.74 (1307) | 0.16 ± 0.73 (1003) | 0.24 ± 0.85 (98) | 0.36 ± 0.70 (206) | **<0.001** |
| **Diastolic blood pressure SDS** | 0.35 ± 0.56 (1307) | 0.31 ± 0.58 (1003) | 0.40 ± 0.55 (98) | 0.51 ± 0.46 (206) | **<0.001** |
| **total cholesterol (mmol/L)** | 4.2 ± 0.7 (1257) | 4.2 ± 0.7 (1002) | 4.3 ± 0.7 (90) | 4.3 ± 0.7 (165) | 0.569 |
| **HDL cholesterol (mmol/L)** | 1.6 ± 0.4 (1259) | 1.6 ± 0.4 (1003) | 1.4 ± 0.3 (90) | 1.3 ± 0.3 (166) | **<0.001** |
| **triglycerides (mmol/L)** | 0.8 ± 0.5 (1256) | 0.7 ± 0.4 (1000) | 0.9 ± 0.4 (90) | 1.1 ± 0.6 (166) | **<0.001** |
| **HbA1c (%) (mmol/mol)** | 5.0 ± 0.3 (31.1 ± 3.3) (1250) | 4.9 ± 0.3 (30.1 ± 3.3) (1008) | 5.0 ± 0.2 (31.1 ± 2.2) (87) | 5.0 ± 0.3 (31.1 ± 3.3) (155) | **<0.001** |
| **Fasting serum glucose(mmol/L)** | 4.7 ± 0.4 (1250) | 4.7 ± 0.4 (997) | 4.8 ± 0.4 (91) | 4.9 ± 0.3 (162) | **<0.001** |
| **2h oGTT glucose(mmol/L)** | 5.9 ± 1.1 (246) | 5.4 ± 1.0 (106) | 5.9 ± 1.1 (18) | 6.3 ± 1.0 (122) | **<0.001** |
| **TyG** | 7.9 ± 0.5 ( 1241) | 7.8 ± 0.4 (993) | 8 ± 0.4 (89) | 8.2 ± 0.5 (159) | **<0.001** |
| **Fasting serum insulin (pmol/L)** | 71.1 ± 51.6 (490) | 49.9 ± 27.5 (335) | 96.7 ± 46.4 (28) | 121.2 ± 63.2 (127) | **<0.001** |
| **2h oGTT insulin (pmol/L)** | 568.0 ± 606.0 (239) | 300.1 ± 218.9 (104) | 662.1 ± 635.4 (17) | 790.5 ± 731.1 (118) | **<0.001** |
| **INSmax** | 1063.9 ± 805.5 (233) | 638.1 ± 368.7(104) | 1314.7 ± 983.1 (16) | 1420.3 ± 885.4 (113) | **<0.001** |
| **HOMA-IR** | 2.2 ± 1.7 ( 474) | 1.6 ± 0.9 (323) | 3.1 ± 1.5 (28) | 3.9 ± 2.0 (123) | **<0.001** |
| **WBISI-Matsuda** | 4.9 ± 3.4 ( 219) | 7 ± 3.7 (101) | 3.7 ± 2.1 (15) | 3 ± 1.8 (103) | **<0.001** |
| **AUCINS/AUCGLU** | 95.1 ± 67.7 ( 218) | 61.9 ± 33.7 (100) | 122.8 ± 90.3 (15) | 123.4 ± 74.4 (103) | **<0.001** |
| **Sclerostin (pg/mL)** | 69.6 ± 31.3 (1323) | 66.1 ± 29.5 (1019) | 72.0 ± 33.6 (98) | 85.5 ± 33.8 (206) | **<0.001** |
| **Osteoprotegerin (pg/mL)** | 308.7 ± 81.6 (1325) | 313.2 ± 82.3 (1020) | 294.1 ± 88.9 (99) | 293.3 ± 71.8 (206) | **0.001** |
| **Bone alkaline phosphatase (ng/mL)** | 40.9 ± 18.5 (1324) | 40.3 ± 18.1 (1019) | 42.5 ± 18.4 (99) | 42.8 ± 20.3 (206) | **0.145** |

Data are expressed as mean ± SD. Significant differences (p<0.05) are marked in bold. Abbreviations: body mass index (BMI), insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3), 120-minute values of glucose during a 75g oral glucose-tolerance test (2h oGTT glucose value); product of triglycerides and fasting glucose (TyG), 120-minute values of insulin during a 75g oral glucose-tolerance test (2h oGTT insulin value); Peak insulin level during an oGTT (INSmax); Homeostatic model assessment - insulin resistance (HOMA-IR); whole body insulin sensitivity index (WBISI Matsuda). ratio of areas under the curve for insulin and glucose levels during a 75g oral glucose-tolerance test (AUCINS/AUCGLU); \*Pubertal category: 0=prepubertal, 1=pubertal, 2=postpubertal (adolescent).

**Table S2. Selected insulin secretion and insulin resistance indices**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Index** | **Description** | **Calculation** | **Units** | **References** |
| **INS0** | Fasting serum insulin | INS0 | INS (pmol/l) | Hanson RL et al. Am J Epidemiol 2000;151:190-198 |
| **HOMA-IR** | Homeostatic model assessment - insulin resistance | G0\*INS0/22.5 | G (mmol/L); INS (μU/mL) | Matthews DR et al. Diabetologia 1985;28:412-419 |
| **TyG** | Product of fasting triglycerides and glucose levels | ln(TG\*INS0+logG0] | G (mg/dL); TG (mg/dL) | Simental-Mendia LE et al. Metab Syndr Relat Disord 2008;6:299-304 |
| **INS120** | 120-minute insulin during an oGTT | INS120 | INS (pmol/l) | Hanson RL et al. Am J Epidemiol 2000;151:190-198 |
| **INSmax** | Peak insulin level during an oGTT | INSmax | INS (pmol/l) | Reaven GM et al. J Clin Endocrinol Metab 1993;76:44-48 |
| **AUCINS/ AUCGLU** | Ratio of areas under the curve for insulin and glucose levels during an oGTT | AUCINS/AUCGLU | G (mmol/L); INS (pmol/L) | Retnakaran R et al. Diabet Med 2009;26:1198-1203 |
| **WBISI Matsuda** | Whole body insulin sensitivity index | 10000/SQRT | G (mg/dL); INS (μU/mL) | Matsuda M et al. Diabetes Care 1999;22:1462-1470 |
| ((G0\*INS0)\* |
| (Gmean\*INSmean)) |

Abbreviations: glucose (G), fasting glucose (G0), insulin (INS), TG – triglycerides, Gmean – mean glucose levels during an oral glucose-tollerance test (oGTT), INSmean – mean insulin levels during an oral glucose-tollerance test.

**Table S3. Linear multiple regression analyses for sclerostin, osteoprotegerin and co-factors**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **Parameter** | **ΔR2** | **β±SEM** | **p value** |
|
| **Dependent variable: sclerostin (R2 = 0.03; P < 0.001; n = 1237)** | | | | |
| **1** | BMI SDS | 0.027 | 0.14 ± 0 | **<0.001** |
| **2** | Height SDS | 0.006 | 0.08 ± 0 | **0.004** |
| **Dependent variable: osteoprotegerin (R2 = 0.04; P < 0.001; n = 1237)** | | | | |
| **1** | Age | 0.032 | -0.13 ± 0 | **0.003** |
| **2** | BMI SDS | 0.008 | -0.07 ± 0 | **0.012** |
| **3** | Height SDS | 0.001 | -0.04 ± 0 | 0.218 |
| **4** | Sex | 0.001 | 0.04 ± 0 | 0.199 |
| **5** | Pubertal status | 0.001 | -0.05 ± 0.01 | 0.259 |
| **Dependent variable: B-ALP (R2 = 0.19; P < 0.001; n = 1238)** | | | | |
| **1** | Age | 0.116 | -0.22 ± 0.04 | **<0.001** |
| **2** | Height SDS | 0.035 | 0.18 ± 0.03 | **<0.001** |
| **3** | Sex | 0.031 | -0.17 ± 0.03 | **<0.001** |
| **4** | Pubertal status | 0.008 | -0.15 ± 0.04 | **<0.001** |
| **5** | BMI SDS | 0.001 | 0.04 ± 0.03 | 0.161 |

Independent variables for the model: sex, age, BMI SDS, and pubertal status.

Abbreviations: body mass index (BMI); standard deviation score (SDS).

**Table S4. Linear multiple regression analyses for height, sclerostin and osteoprotegerin**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **Parameter** | **ΔR2** | **β±SEM** | **p value** |
|
| **Dependent variable: height SDS (R2 = 0.17; P < 0.001; n = 1211)** |  |  |  |  |
| **1** | BMI SDS | 0.087 | 0.22 ± 0.03 | **<0.001** |
| **2** | IGF1\_SDS | 0.039 | 0.25 ± 0.03 | **<0.001** |
| **3** | Bone alkaline phosphatase | 0.028 | 0.15 ± 0.03 | **<0.001** |
| **4** | Sex | 0.011 | -0.12 ± 0.03 | **<0.001** |
| **5** | Osteoprotegerin | 0.003 | -0.08 ± 0.03 | **0.003** |
| **6** | Sclerostin | 0.004 | 0.07 ± 0.03 | **0.012** |

Independent variables for the model: sex, age, BMI SDS, pubertal status, IGF1 SDS, IGFBP3 SDS, sclerostin, osteoprotegerin, and bone alkaline phosphatase. The significant correlations (p<0.05) are marked in bold. Abbreviations: body mass index (BMI); standard deviation score (SDS); insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3).

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**Figure S1. Diurnal variation of bone markers.** Profiles of diurnal variation of serum levels of bone markers were done by hourly sampling of serum aliquots over 24h of 4 probands and are shown as mean and SEM. Times of meals and period with lights off are indicated by dotted lines and shaded area. Paired t-test was performed for assessment of serum levels during the light period (0800 to 2200) and light off period (2200 to 0700).



**Figure S2. Effect of exogenous stimuli on bone markers.** A) oGTT. Eleven lean probands underwent standardized oGTT with 75g Glucose. Course of serum levels of bone markers is indicated in mean±SEM. (B-D) Serum levels are given before (baseline), immediately after (0) and every 30 min following a bout of acute strenuous exercise in 4 adult probands. Statistical analysis was performed by ANOVA repeated measures test applying Dunnett´s post test with 0 as reference.

**Table S5. Linear multiple regression analyses for selected parameters of glucose and insulin metabolism with sclerostin and osteoprotegerin**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **Parameter** | **ΔR2** | **β±SEM** | **p value** |
| **Independent variables for all models: sex, age, BMI SDS, height SDS, pubertal status, HbA1c, B-ALP, sclerostin, and osteoprotegerin** | | | | |
| **Dependent variable: fasting glucose (R2 = 0.18; P < 0.001; n = 1172)** | | | | |
| **1** | age | 0.137 | 0.42 ± 0.03 | ***<0.001*** |
| **2** | B-ALP | 0.023 | 0.13 ± 0.03 | ***<0.001*** |
| **3** | sex | 0.014 | -0.13 ± 0.03 | ***<0.001*** |
| **4** | BMI SDS | 0.010 | 0.10 ± 0.03 | ***<0.001*** |
| **Dependent variable: 2h oGTT glucose (R2 = 0.12; P < 0.001; n = 235)** | | | | |
| **1** | BMI SDS | 0.110 | 0.32 ± 0.06 | ***<0.001*** |
| **2** | B-ALP | 0.018 | 0.17 ± 0.06 | **0.027** |
| **Dependent variable: TyG (R2 = 0.14; P < 0.001; n = 1163)** | | | | |
| **1** | BMI SDS | 0.068 | 0.21 ± 0.03 | ***<0.001*** |
| **2** | Pubertal category | 0.047 | 0.17 ± 0.04 | ***<0.001*** |
| **3** | Sclerostin | 0.010 | 0.12 ± 0.03 | ***<0.001*** |
| **4** | sex | 0.010 | 0.08 ± 0.03 | 0.004 |
| **5** | B-ALP | 0.006 | 0.09 ± 0.03 | 0.002 |
| **6** | age | 0.002 | 0.07 ± 0.04 | 0.096 |
| **Dependent variable: fasting insulin (R2 = 0.60; P < 0.001; n = 461)** | | | | |
| **1** | BMI SDS | 0.395 | 0.60 ± 0.03 | ***<0.001*** |
| **2** | age | 0.180 | 0.39 ± 0.05 | ***<0.001*** |
| **3** | sex | 0.011 | 0.14 ± 0.03 | ***<0.001*** |
| **4** | B-ALP | 0.019 | 0.15 ± 0.03 | ***<0.001*** |
| **5** | Pubertal category | 0.003 | 0.08 ± 0.05 | 0.058 |
| **Dependent variable: 2h oGTT insulin (R2 = 0.40; P < 0.001; n = 228)** | | | | |
| **1** | BMI SDS | 0.255 | 0.50 ± 0.05 | ***<0.001*** |
| **2** | age | 0.080 | 0.33 ± 0.06 | ***<0.001*** |
| **3** | Sclerostin | 0.031 | 0.13 ± 0.06 | **0.018** |
| **4** | sex | 0.014 | 0.14 ± 0.06 | **0.014** |
| **5** | B-ALP | 0.011 | 0.11 ± 0.06 | 0.064 |
| **6** | Osteoprotegerin | 0.006 | 0.09 ± 0.06 | 0.112 |
| **Dependent variable: INSmax (R2 = 0.37; P < 0.001; n = 222)** | | | | |
| **1** | BMI SDS | 0.283 | 0.52 ± 0.05 | ***<0.001*** |
| **2** | age | 0.075 | 0.32 ± 0.06 | ***<0.001*** |
| **3** | B-ALP | 0.016 | 0.14 ± 0.06 | 0.019 |
| **4** | Sclerostin | 0.009 | 0.09 ± 0.06 | 0.094 |
| **5** | sex | 0.005 | 0.07 ± 0.06 | 0.183 |
| **Dependent variable: HOMA-IR (R2 = 0.55; P < 0.001; n = 445)** | | | | |
| **1** | BMI SDS | 0.400 | 0.61 ± 0.03 | ***<0.001*** |
| **2** | age | 0.138 | 0.33 ± 0.05 | ***<0.001*** |
| **3** | sex | 0.005 | 0.09 ± 0.03 | ***0.005*** |
| **4** | B-ALP | 0.007 | 0.10 ± 0.04 | **0.007** |
| **5** | Pubertal category | 0.002 | 0.08 ± 0.05 | 0.133 |
| **Dependent variable: AUCINS/AUCGLU (R2 = 0.44; P < 0.001; n = 207)** | | | | |
| **1** | BMI SDS | 0.271 | 0.5 ± 0.05 | ***<0.001*** |
| **2** | age | 0.128 | 0.4 ± 0.06 | ***<0.001*** |
| **3** | Sclerostin | 0.030 | 0.15 ± 0.05 | ***0.004*** |
| **4** | B-ALP | 0.011 | 0.15 ± 0.06 | **0.012** |
| **5** | sex | 0.017 | 0.14 ± 0.06 | **0.012** |
| **Dependent variable: WBISI Matsuda (R2 = 0.61; P < 0.001; n = 208)** | | | | |
| **1** | BMI SDS | 0.424 | -0.64 ± 0.04 | ***<0.001*** |
| **2** | age | 0.147 | -0.44 ± 0.05 | ***<0.001*** |
| **3** | B-ALP | 0.026 | -0.2 ± 0.05 | ***<0.001*** |
| **4** | sex | 0.014 | -0.12 ± 0.05 | **0.009** |
| **5** | Sclerostin | 0.008 | -0.09 ± 0.04 | 0.046 |

Excluded were underweight individuals. The significant correlations (p<0.05) are marked in bold, and significant correlations after the Bonferroni correction (p<0.006) and marked both in bold and in Italic. Abbreviations: body mass index (BMI); standard deviation score (SDS); 120-minute values of glucose during a 75g oral glucose-tolerance test (2h oGTT glucose value); product of triglycerides and fasting glucose (TyG),120-minute values of insulin during a 75g oral glucose-tolerance test (2h oGTT insulin value); Peak insulin level during an oGTT (INSmax); Homeostatic model assessment - insulin resistance (HOMA-IR); ration of areas under the curve for insulin and glucose levels during a 75g oral glucose-tolerance test (AUCINS/AUCGLU); whole body insulin sensitivity index (WBISI Matsuda).