Disentangling the relationship between bone turnover and glucose homeostasis: A prospective, population-based twin study

Christine Dalgård a,b, Morten S. Hansen c,d, Sören Möller e, Kirsten O. Kyvik a,e, Morten Frost c,d,e,*

a The Danish Twin Registry, Department of Public Health, University of Southern Denmark, Denmark
b Environmental Medicine, Department of Public Health, University of Southern Denmark, Odense, Denmark
c Department of Endocrinology, Odense University Hospital, Denmark
d Department of Clinical Research, University of Southern Denmark, Denmark
e Open Patient data Explorative Network (OPEN), Odense University Hospital and Department of Clinical Research, University of Southern Denmark, Denmark

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ABSTRACT

Background: Biochemical markers of bone turnover are lower in patients with type 2 diabetes, which may be explained by genetic variants being associated with type 2 diabetes and bone turnover as well as environmental factors. We hypothesized that bone turnover markers associate with and predict changes in glucose homeostasis after control for genetics and shared environment.

Methods: 1071 healthy, non-diabetic (at baseline, 1997–2000) adult mono- and dizygotic twins participating in the prospective study GEMINAKAR were reassessed between 2010 and 2012 with clinical evaluation, biochemical tests and oral glucose tolerance test. Fasting bone turnover markers (CTX, P1NP and osteocalcin) were measured. The association between bone turnover, glucose homeostasis and the ability of bone turnover markers to predict changes in glucose homeostasis were assessed in cross-sectional and longitudinal analyses. Analyses were performed both at an individual level and adjusted for shared environmental and genetic factors.

Results: Glucose levels increased with age, and 33 (3%) participants had developed type 2 diabetes at follow-up. In women, bone turnover markers increased with age, whereas for men only osteocalcin increased with age. Bone turnover markers were not associated with fasting glucose, insulin, or HOMA-IR at baseline or follow-up before or after adjustment for age, sex, BMI, smoking, and use of medication at baseline. Variation in bone turnover markers was mainly explained by unique environmental factors, 70%, 70% and 55% for CTX, P1NP and osteocalcin, respectively, whereas additive genetic factors explained 7%, 13% and 45% of the variation in CTX, P1NP and osteocalcin.

Conclusions: Bone turnover markers were not associated with baseline plasma glucose levels and did not predict changes in glucose homeostasis. Variation in bone turnover markers is mainly explained by environmental factors, however, compared to CTX and P1NP, genetic factors have a larger impact on osteocalcin levels.

1. Introduction

The skeleton has emerged as an endocrine organ, which regulates phosphate metabolism through secretion of fibroblast growth factor-23 (Bhattacharyya et al., 2012) and suppresses appetite and nutrient intake by secretion of lipocalin 2 (Mosialou et al., 2017). In addition, preclinical studies have suggested that bone regulates glucose homeostasis through secretion of undercarboxylated osteocalcin (ucOC), which stimulates β-cell proliferation and insulin secretion and sensitivity (Lee et al., 2007; Ferron et al., 2010; Rached et al., 2010). Insulin signalling in osteoblasts promotes osteoclast activity, which leads to increased bone resorption, higher serum ucOC and improved glucose metabolism (Ferron et al., 2010), thus integrating bone metabolism and glucose homeostasis. Osteoblasts may develop insulin resistance, which associates with a decrease in the secretion of bioactive osteocalcin and lower insulin sensitivity (Wei et al., 2014), indicating that decreasing osteocalcin levels could impair glucose homeostasis. Because ucOC promotes insulin-stimulated glucose uptake in muscle, decreased levels could lead to insulin resistance (Lin et al., 2019).

Clinical investigations based on histomorphometric assessments of bone tissue showed decreased bone turnover in type 1 and type 2 diabetic (T1D and T2D) patients (Krackauer et al., 1995; Manavalan et al., 2012),
and these observations are supported by lower levels of markers of bone formation and resorption in both conditions as well as in patients with mitochondrial diabetes (Starup-Linde et al., 2014; Langdahl et al., 2017). While osteocalcin was reported to be inversely associated with fasting glucose levels in T2D patients (Kanazawa et al., 2009) and the risk of development of T2D in patients with high risk of cardiovascular disease (Diaz-Lopez et al., 2013), markers of bone formation and resorption were not associated with insulin sensitivity assessed using hyperinsulinemic, euglycaemic clamps, insulin secretion determined using an intravenous glucose tolerance test or incident increases in fasting glucose, insulin levels or insulin resistance (HOMA) in healthy, non-diabetic men (Frost et al., 2018). Hyperinsulinaemia with concomitant euglycaemia has limited (Frost et al., 2018; Ivaska et al., 2015) or no (Clowes et al., 2002; Basu et al., 2011) liminon inhibitory effect on levels of bone resorption markers and does not influence bone formation markers in non-diabetic adults (Ivaska et al., 2015; Clowes et al., 2002; Basu et al., 2011).

Clinical investigations of the relationship between bone turnover evaluated using biochemical markers and glucose homeostasis are challenging as these investigations may be confounded by several factors related to the study population including body composition, comorbid diseases and treatments. Several genetic variants known to regulate bone formation e.g. Wnt-pathway genes such as Wnt5b and TCF7L2, are also associated with T2D (Sladek et al., 2007; Kanazawa et al., 2004). In addition, prospective studies of the association between bone turnover in healthy individuals and incidental changes in glucose homeostasis demand substantial study populations with extensive observation time.

Therefore, in order to determine if lower bone turnover associates with compromised and future impairment of glucose homeostasis as indicated in preclinical and some clinical studies, we conducted cross-sectional and longitudinal investigations of the relationship between biochemical markers of bone turnover and glucose homeostasis with fasting glucose levels as the primary outcome in healthy, non-diabetic adult mono- and dizygotic twins. In addition, as genetics, ageing, and environment are expected to influence bone turnover, we estimated the genetic and environmental contributions to the longitudinal changes in bone turnover markers.

2. Materials and methods

2.1. Design, setting, and participants

Between 1997 and 2000, 2585 twin pairs born between 1931 and 1982 were identified in the population-based Danish Twin Registry and invited for participation in a prospective study entitled GEMINAKAR that aims at delineating factors associated with an increased risk of development of insulin resistance, abdominal adiposity, and cardiovascular disease as previously described (Benyamin et al., 2007; Schousboe et al., 2003). In brief, participants were invited to one of two national centres for interview and characterisation of the metabolic phenotype at baseline. Individuals with known diabetes or cardiovascular disease, pregnant and breast-feeding women, disorders impairing possibilities of performing a maximal bicycle test, and incomplete twin-pairs (e.g. one twin not consenting to participation) were excluded from the investigation at baseline. A total of 1512 twins (756 pairs) including 445 dizygotic (DZ) and 311 monozygotic (MZ) twin pairs were included in baseline investigations. Among 1435 individuals (alive and living in Denmark) invited for a reassessment between 2010 and 2012, a total of 1139 (equalling 79%) completed the clinical assessment. Physical examinations including measurement of height, weight and blood pressure, and blood samples (after 10-12 h of fasting) were collected and stored at −80 °C until analysis both at baseline and follow-up. Furthermore, a 75-gm oral glucose tolerance test (OGTT) with blood sampling at 0, 30, and 120 min was performed at baseline but not at follow-up. For the present study, the population was limited to 1071 twins who were without screen-detected diabetes (FPG ≥ 6.1 mmol/L or 2-h plasma glucose ≥11.1 mmol/L (Alberti and Zimmet, 1998)) based on the OGTT at baseline and had complete measures of BTM.

The regional Health research ethical committee (baseline, S-VF-19970271; follow-up, S-20090065) and the SDU Research & Innovation Organization (17/38416) in compliance with the General Data Protection Regulation approved the investigation, which was performed in accordance with the principles of the Helsinki Declaration. All participants provided informed consent after having received both written and oral information about the study.

2.2. Biochemical tests

Plasma glucose was measured using the hexokinase/G-6-PDH principle (Architect, Abbott, Lake Forest, IL, USA) and serum insulin was analyzed using a time-resolved fluorimmunoassay (Perkin-Elmer Life Sciences, Turku, Finland) at baseline and follow-up. Homeostatic model of insulin sensitivity (HOMA-IR) was calculated as follows: HOMA-IR = (insulin (pmol/l) × 0.167) × Glucose (mmol/l)/22.5. HbA1c was measured at follow-up using a fully automated glycohaemoglobin analyzer (Tosoh HLC-723 G8) with CV < 2.4% and < 1.8% at low (34 mmol/L) and high levels (82 mmol/L), respectively.

Thawed blood samples collected at baseline and follow-up were used for measurement of bone turnover markers (BTM). A fully automated immunoassay system (ISYS, Immunodiagnostic Systems Ltd., Boldon, England) was used for the analyses, and tests were performed in a single run with the same batch of the reagents/assay. Serum Procollagen type I amino-terminal propeptide (P1NP), C-telopeptide of type I collagen (CTX) and total osteocalcin (OC) were measured using the chemiluminescence method, with the following intra- and inter-assay coefficients of variation (CV): P1NP 3% and 5–8% (normal range in men and women 27.7–127.6 μg/L), CTX <5% and 7–10%, (men 115–748 ng/ L, premenopausal women 112–738 ng/L, postmenopausal women 142–1351 ng/L) and OC: 3% and 6%–9% (normal range in men and women 10.4 to 45.6 mg/L).

2.3. Assessment of T2D

At follow-up, incident T2D was defined as self-reported diabetes, FPG ≥ 7.0 mmol/L, HbA1c ≥ 6.5% (48 mmol/mol) or self-reported use of antidiabetic medicine.

2.4. Statistics

Population characteristics are presented as mean (SD), median or frequencies (%), whatever appropriate. The difference in bone turnover markers between sexes was tested using unpaired t-test or Kruskall Wallis test, as appropriate. We used multiple linear and logistic regression models to assess the association between bone turnover markers at baseline and indicators of insulin sensitivity with fasting glucose as the primary outcome and insulin and HOMA-IR as secondary outcomes at baseline and follow-up. Associations between BTMs and risk of T2D at time of follow-up were explored in exploratory analyses. Models were adjusted by sex, age, BMI, smoking, and use of any kind of medication using robust standard errors to consider the clustered data structure of the twin pairs. We also tested for sex interaction in the associations between bone turnover markers and indices of glucose metabolism in the performed regression models. Furthermore, we used fixed-effects models with twins nested within pairs to control for genetic as well as environmental factors shared within families, e.g. prenatal environment and early life postnatal factors shared within twin pairs (within families). Finally, we used conditional logistic regression models for matched data restricted to twin pairs discordant for T2D at follow-up and stratified by twin zygosity. In these analyses, the within MZ-pair analyses adjusts for genetic and early shared family environment and we adjusted for BMI, smoking, and use of medication (any kind) at
baseline as age and sex were adjusted for by design.

We calculated the heritability of the bone turnover markers at baseline and the longitudinal change in each phenotype (bone turnover marker = bone turnover markers\_followup - bone turnover markers\_baseline). This was done using the classical univariate twin modelling, hence the variation in the phenotypes was partitioned into components representing latent additive (A) and dominant genetic (D) factors and shared (C) and non-shared environmental (E) factors. These components can be identified by comparing the phenotypic similarity of monozygotic (MZ) twins with that of dizygotic (DZ) twins. MZ twins are 100% genetic identical, DZ twins are 50% identical like ordinary siblings. We fitted full ACE/ADE models and we also fitted nested models by dropping the C or the A component (AE or CE model). Goodness-of-fit was assessed by AIC (the Akaike Information Criterion).

Statistical analyses were performed with Stata version 16 (StataCorp, College Station, TX) and R (version 3.3.1), and all statistical tests were 2-sided with \( P < 0.05 \) indicating significance.

3. Results

A total of 1071 twin individuals were free from diabetes at baseline, had available blood samples from both baseline and follow-up and were therefore included in the study. Baseline mean age of men and women were 38.7 (10.7) and 37.9 (10.2) years, respectively. Further clinical and biochemical characteristics are shown in Table 1. Compared with women, men had a higher BMI and fasting glucose concentration at baseline.

3.1. Bone turnover markers

Bone turnover markers CTX, P1NP, and osteocalcin were significantly higher in men than women both at baseline and time of follow-up. While all markers increased during the study in women, reductions in both CTX and P1NP but not osteocalcin were observed in men (Table 1).

3.2. Association between bone turnover markers and indices of glucose homeostasis

Baseline bone turnover markers were not associated with fasting glucose, insulin, or HOMA-IR at baseline or follow-up (all \( P > 0.1 \), Table 2). During a mean of 12 years of follow-up, 33 (3.0%) including 19 male (58%) new cases of T2D were ascertained, with similar follow-up participants with and without incident T2D. In adjusted logistic regression models, none of the bone turnover markers were associated with incident T2D (Table 3), nor did interaction models suggest different associations between bone turnover markers and glucose homeostasis between men and women (\( P \) interaction > 0.05; data not shown). Furthermore, neither the fixed effects models nor the conditional logistic regression models considering genetic and familial confounding factors revealed associations between BTM and the indices of glucose homeostasis or T2D at follow up (results not shown).

3.3. Genetic and environmental factors and their relationship with bone turnover markers and changes in bone turnover markers

ACE/ADE models were fitted for the baseline concentration as well as changes in each bone turnover marker. The genetic modelling analyses demonstrated that for the best fitting models additive genetic factors explained 0%, 0% and 79% of the variance in CTX, P1NP and osteocalcin, respectively, at baseline while the unique environmental factors explained 0%, 0% and 79% of the variance in CTX, P1NP and osteocalcin (Table 4). The best fitting models for changes in P1NP and osteocalcin estimated moderate additive genetic influence (30% and 35%, respectively) while non-shared environmental factors were more

| Table 1 |
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| Characteristics and bone turnover markers in 1071 twins without diabetes at baseline from the GEMINAKAR cohort. |
| Total (N = 1071) | Women (N = 574) | Men (N = 497) | p-Value$^a$ |
| Age, y | 38.3 (10.4) | 37.9 (10.2) | 38.7 (10.7) | 0.21 |
| BMI, kg/m2 | 24.4 (3.5) | 24.0 (3.7) | 25.0 (3.0) | < 0.01 |
| Smokers, N (%) | 320 (30%) | 168 (29%) | 152 (31%) | 0.71 |
| Use medication, N (%) | 214 (20%) | 166 (28.9%) | 48 (9.7%) | < 0.01 |
| Optical, ng/ml | 0.37 [0.24-0.56] | 0.29 [0.20-0.46] | 0.45 [0.30-0.68] | < 0.01 |
| CTX, ng/L | 0.027 [-0.128-0.164]$^b$ | 0.077 [-0.063-0.257] | -0.035 [-0.21-0.069] | < 0.01 |
| P1NP, ng/mL | 55.7 [42.4-74.2] | 51.0 [39.5-68.3] | 60.8 [46.3-81.4] | < 0.01 |
| Osteocalcin, ng/ml | -3.9 [-18.7-9.3]$^b$ | 2.25 [-15.2-18.1] | -7.7 [-22.7-1.8] | < 0.01 |
| Glucose, mmol/L | 23.3 [18.1-31.2] | 22.3 [17.4-29.9] | 24.4 [18.6-33.2] | < 0.01 |
| HOMA-IR | 0.2 [-5.4-5.6] | 1.6 [-5.0-8.6] | 1.3 [-6.1-2.9] | < 0.01 |
| Glucose, mmol/L | 4.7 [4.4-5.0] | 4.6 [4.4-4.9] | 4.8 [4.5-5.1] | < 0.01 |
| HOMA-IR | 0.8 [0.5-1.2]$^b$ | 0.8 [0.5-1.1] | 0.9 [0.5-1.2] | 0.01 |
| Insulin, pmol/L | 34 [25-46] | 34 [25-46] | 33 [24-44] | 0.15 |
| Insulin, pmol/L | 3 [-9-17]$^b$ | 2 [-11-16] | 5 [-7-18] | 0.01 |
| HOMA-IR | 1.18 [0.84-1.6] | 1.19 [0.84-1.6] | 1.16 [0.84-1.59] | 0.79 |
| HOMA-IR | 0.32 [-0.18-0.94]$^b$ | 0.25 [-0.22-0.84] | 0.42 [-0.15-1.04] | 0.01 |
| HbA1c, mmol/L | 34.4 (3.9) | 34.4 (3.8) | 34.3 (4.0) | 0.37 |
| Follow-up | 5.3 (0.4) | 5.3 (0.3) | 5.2 (0.4) | 0.35 |

Values are mean (SD), median [25-75 percentiles], or frequency (%).$^a$ Differences between sex; Kruskal-Wallis rank-sum test.$^b$ p < 0.05. Difference between baseline and follow up values; Wilcoxon signed rank test.
twins without diabetes at baseline from the GEMINAKAR cohort. The relative proportion of variance in bone turnover markers differed between menopausal women (N = 267) and non-menopausal women (N = 307). A decrease in the proportion of additive genetic factors corresponds to an increase in the proportion explained by environmental factors (Supplemental Table 1). In men, additive genetic factors appeared not to influence the variation in bone turnover markers at baseline but the majority at time of follow-up (Supplemental Table 2a). Because genetic factors were expected to influence the variation in markers, data were reassessed. Although exclusion of two outliers uncovered that 35% of the variation in CTX was explained by additive genetic factors, both P1NP and osteocalcin remained primarily explained by environmental factors (Supplemental Table 2b).

### 4. Discussion

This study shows that commonly used biochemical markers known to reflect bone turnover including CTX, P1NP and osteocalcin were not associated with measures of glucose homeostasis, nor were these markers associated with changes in glucose homeostasis or development of T2D in this 12-year follow-up study in Danish twins. Collectively, these findings are not supporting a connection between biochemical markers of bone formation or resorption and glucose homeostasis with or without adjustment for the impact of shared environment and genetics.

The integration of bone and glucose homeostasis has gained substantial support from preclinical studies showing that ucOC is a regulator of insulin secretion and sensitivity as well as insulin-stimulated glucose uptake in muscle. Clinical studies corroborating these data are lacking in part because assays determining levels of bioactive ucOC have been unavailable (Liu et al., 2016). However, it has been reported that reduced levels of osteocalcin associate with plasma glucose (Kindblom et al., 2009; Kanazawa et al., 2011; Saleem et al., 2010; Yeap et al., 2010; Movahed et al., 2012), lower insulin sensitivity (Saleem et al., 2010; Fernandez-Real et al., 2009; Pittas et al., 2009; Lee et al., 2012) and presence of T2D (Kindblom et al., 2009; Movahed et al., 2012; Im et al., 2008) in cross-sectional studies. Importantly, similar associations between OC as well as alkaline phosphatase and T2D were reported, indicating that osteocalcin may not have an independent effect on presence of T2D (Movahed et al., 2012) whereas absence of an association between osteocalcin and fasting glucose level was observed in postmenopausal women (Lee et al., 2012). Importantly, although the majority of these studies indicate favourable associations between
osteocalcin and glucose homeostasis, osteocalcin levels may not associate with incident changes in glucose levels or development of T2D. Thus, Hwang et al. (2012) reported an inverse association between osteocalcin and fasting glucose levels but observed no connection between osteocalcin levels and development of T2D among >1200 non-diabetic men during an observation time of 8.4 years. Yeo et al. (2015) revealed an association between uOC and reduced risk of T2D, however, a slightly weaker association was identified between levels of CTX but not P1NP and presence of T2D. In addition, undercarboxylated osteocalcin was associated with improvement in insulin sensitivity in a 2-year follow-up study. Jointly, these studies indicate that osteocalcin and undercarboxylated osteocalcin could emerge as a predictor of T2D. However, most of these data are based on cross-sectional investigations, effectively inhibiting opportunities of inferring causality. The cross-sectional and prospective data from our investigation showed that neither osteocalcin nor any of the other markers of bone turnover predicted changes in glucose homeostasis or indeed development of T2D.

The absence of an association between osteocalcin and glucose homeostasis after 12 years of follow-up indicates that osteocalcin is unsuitable as a biological marker of future long-term changes in glucose homeostasis in healthy, middle-aged men and women. Importantly, participants in our investigation were characterised by being healthy and fairly young, limiting the opportunity to investigate association between bone turnover markers and adverse glucose homeostasis at study entry and risk of development of T2D during the observation period. Therefore, an association with incident T2D was not considered to be a key outcome of this study. Indeed, with only 33 incident cases, the models have reduced predictive power. Furthermore, despite several reports showing comparable links between both osteocalcin and undercarboxylated osteocalcin and glucose homeostasis, we cannot exclude the possibility that assessment of undercarboxylated osteocalcin may have exposed associations with glucose homeostasis. Future studies that include individuals at higher risk of developing T2D are needed to confirm the findings reported here.

Our study population differs from previous reports by the inclusion of twins rather than singletons. Twins diverge from singletons in several ways including different intrauterine conditions and lower birth weight, which is associated with an increased risk of T2D. However, the association between glucose homeostasis and birth weight in twins is explained by genetic and rearing environmental factors (Frost et al., 2012), and the prevalence of T2D is similar in twins and singletons (Petersen et al., 2011), suggesting that observations in the current study population likely pertain to the general population.

The predicted connection between bone turnover markers and glucose homeostasis may be influenced by both genetics and environmental factors. The twin design applied here provides an opportunity to adjust for both factors and to interpret the impact of genetics on the association. Our co-twin control results from the conditional logistic regression support the results from the so-called individual-level analyses, i.e. the multiple logistic regression, and therefore, previous studies suggesting an association between BTM and glucose metabolism may have been confounded by familial factors.

Our study showed that genetics account for a substantially larger part of the variation in the change in osteocalcin levels than changes in P1NP and in particular CTX. Previous studies have reported high heritability of osteocalcin, ranging from 40 to 60% (Kuipers et al., 2012; Garnero et al., 1996; Kelly et al., 1991), which is in line with our findings. The highest level of heritability of osteocalcin is reported in premenopausal and the lowest in postmenopausal women indicating that genetic control of bone formation may change with menopause. Although the genetic contribution to the variation in bone turnover markers diminished in women during the study, the differences in the contribution between menopausal and non-menopausal women were smaller suggesting that the genetic contribution changes with age rather than with menopausal status per se. By contrast, changes in CTX and P1NP levels were mainly influenced by unique environmental factors, which contrasts observations in Australian twins showing that variance in bone turnover markers mainly is explained by shared factors. In part, these dissimilarities may be due to differences in age, size and possibly ethnicity of the study populations. Irrespective, these data reveal that changes in bone turnover markers are differentially regulated, which may influence assessment of these variables in longitudinal studies.

In conclusion, our investigation shows that levels of bone turnover markers and measures of glucose homeostasis are unrelated in middle-aged, healthy male and female twins. Furthermore, neither of the markers proved effective as predictors of changes in glucose homeostasis. Differential effects of genetics and environmental factors on bone turnover markers were observed, and further investigations are needed to elucidate the causes and clinical implications.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Declaration of competing interest

The authors declare that no competing interests exist.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bonr.2021.100752.

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