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The relationship between bone turnover and insulin sensitivity and secretion: Cross-sectional and prospective data from the RISC cohort study☆

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ABSTRACT

Bone metabolism appears to influence insulin secretion and sensitivity, and insulin promotes bone formation in animals, but similar evidence in humans is limited. The objectives of this study are to explore if bone turnover markers were associated with insulin secretion and sensitivity and to determine if bone turnover markers predict changes in insulin secretion and sensitivity. The study population encompassed 576 non-diabetic adult men with normal glucose tolerance (NGT; n = 503) or impaired glucose regulation (IGR; n = 73). Baseline markers of bone resorption (CTX) and formation (P1NP) were determined in the fasting state and after a 2-h hyperinsulinaemic, euglycaemic clamp. An intravenous glucose tolerance test (IVGTT) and a 2-h oral glucose tolerance test (OGTT) were performed at baseline, and the OGTT was repeated after 3 years. There were no differences in bone turnover marker levels between NGT and IGR. CTX and P1NP levels decreased by 8.0% ($p < 0.001$) and 1.9% ($p < 0.01$) between baseline and steady-state during the clamp. Fasting plasma glucose was inversely associated with CTX and P1NP both before and after adjustment for recruitment centre, age, BMI, smoking and physical activity. However, baseline bone turnover markers were neither associated with insulin sensitivity (assessed using hyperinsulinaemic euglycaemic clamp and OGTT) nor with insulin secretion capacity (based on IVGTT and OGTT) at baseline or at follow-up. Although inverse associations between fasting glucose and markers of bone turnover were identified, this study cannot support an association between insulin secretion and sensitivity in healthy, non-diabetic men.

1. Introduction

Fracture risk is increased in people with type 2 diabetes (T2D) despite bone mass generally being normal or increased [1]. Clinical studies revealed decreased levels of circulating biochemical markers of bone formation and resorption in individuals with T2D [1] as well as lower bone formation and resorption and lower bone quality in bone biopsies in T2D [2]. The mechanisms behind these changes in bone turnover and the increased risk of fracture in T2D are not fully elucidated.

Inadequate secretion of insulin and insulin resistance are the cornerstones in the development of T2D. Insulin is considered bone anabolic due to stimulatory effects on osteoblast differentiation [3], and mice not expressing the insulin receptor in osteoblasts have low bone mass [4]. Insulin signaling in osteoblasts favours osteoclast bone resorption activity through secretion of osteoprotegerin and, subsequently, generation and release of the undercarboxylated form of osteocalcin, an osteoblast-secreted marker of bone formation, which may stimulate insulin secretion from the pancreatic β-cells [5]. Furthermore, hyperglycaemia impairs osteoblast activity and survival [6–8] and promotes adipogenic rather than osteogenic differentiation of adipose and muscle-derived stem cells [9]. Additionally, gain-and-loss-of-function models of insulin signaling in mice osteoblasts provide evidence that a high fat diet causes insulin resistance in bone, which lowers bone turnover and osteocalcin activity, causing higher bone volume and glucose intolerance in mice [10].

Thus, based on preclinical investigations, insulin levels and beta-cell function as well as insulin sensitivity would be expected to correlate

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with markers of bone formation in humans, but clinical data from non-diabetic individuals remain to be established. Integration of bone and glucose homeostasis in humans is supported by direct associations between total osteocalcin and estimates of insulin secretion and sensitivity based on oral and intravenous glucose tolerance tests (OGTT or IVGTT) [11–14] and inverse associations with plasma glucose in T2D [15], age-related increases in plasma glucose [13], and incidence of T2D [16–18]. Also, markers of bone resorption but not bone formation were inversely associated with the incidence of T2D [17]. However, other investigations have not provided support of associations between total osteocalcin and plasma glucose or incident diabetes [19–22]. Although the increased levels of fasting insulin usually observed in early stages of T2D could promote bone formation and subsequently bone resorption due to coupling of bone formation and resorption, possibly explaining the association between bone mineral density (BMD) and fasting insulin levels observed in some [23,24] but not all epidemiological studies of non-diabetic individuals [25], insulin resistance in bone cells may reduce bone formation and resorption, which are reported to be lower in patients with T2D [1,26]. Corroborating these reports, insulin sensitivity assessed by an IVGTT was inversely associated with BMD in a selected group of non-diabetic, generally obese men with heart disease [27], and homeostasis model assessment of insulin resistance (HOMA-IR) was positively associated with volumetric BMD in postmenopausal, non-diabetic women [28]. While these studies suggest that insulin resistance may increase bone mass, possibly due to lower bone turnover in T2D [26], it remains unknown if insulin sensitivity measured using the gold standard, i.e. the hyperinsulinaemic, euglycaemic clamp and insulin secretion assessed by OGGT or IVGTT, are associated with bone turnover in non-diabetic individuals.

The aim of this study was to investigate the relationship between bone turnover using markers of bone resorption (CTX) and formation (P1NP), and insulin secretion and sensitivity assessed with the hyperinsulinaemic euglycaemic clamp and measures derived from intravenous and oral glucose tolerance tests in clinically healthy, non-diabetic men. Furthermore, we explored if bone turnover was associated with insulin secretion and 3-year changes in insulin secretion and sensitivity.

2. Materials and methods

The Relationship between Insulin Sensitivity and Cardiovascular Risk Study (RISC) is a prospective cohort study conducted at 19 European research centres across 14 European countries [29]. Baseline and 3-year follow-up data were included in the present study. In short, between 2002 and 2004, 1556 clinically healthy female and male volunteers aged 29–61 years were recruited from the local community. Individuals being treated for obesity, diabetes, hypertension or lipid disorders were excluded from participation. The exclusion criteria comprised recent weight change (>5 kg) or major surgery, chronic pulmonary and cardiovascular diseases, renal failure including renal transplant, seizure disorders including epilepsy, steroid treatment, and any diagnosis of cancer in the previous 5 years but not osteoporosis or treatment for osteoporosis. After physical examination, biochemical testing and a 75-g 2-h OGTT were performed. Individuals with increased fasting or 2-h glucose levels (≥7 and 11.1 mmol/L, respectively), increased blood pressure (≥140/90 mmHg) or increased lipids (triglyceride ≥4.6 mmol/l and total cholesterol ≥7.8 mmol/L) were excluded [29]. In order to limit the effects of factors known or anticipated to influence bone and glucose homeostasis such as menstrual cycle, only male participants of the RISC study were selected for the present investigation.

2.1. Anthropometrics and lifestyle

Body height was measured using a standard ruler (stadiometer) without shoes. Waist size was measured on bare skin at the smallest point between costal edges and the iliac crest. Body weight and fat free mass (FFM) were measured with participants in light clothes and in the fasting state using a Tanita bioimpedence TBF-300 body composition analyser (Tanita International, United Kingdom). Physical activity was registered by the 7-day International Physical Activity Questionnaire (IPAQ) and used to calculate metabolic equivalent energy expenditure per week. The level of physical activity was explored both as a continuous and a categorical measure as the study population was categorized in three groups based on their level of physical activity (inactive, minimally active and health enhancing physical activity). Smoking status was dichotomized according to whether the participant reported current use of tobacco products.

2.2. Assessment of glucose homeostasis

All participants underwent a 75-g OGTT after an overnight fast, with samples being collected at after 0, 30, 60, 90 and 120 min, at baseline and at 3 years. At baseline, hyperinsulinaemic euglycaemic clamp was performed within one week of the OGTT. During the clamp, insulin was infused at a rate of 240 pmol per min per square meter, and infusion of dextrose (20%) was modified at 5–10 min intervals in order to keep plasma glucose levels within 0.8 mmol/L of 4.5–5.5 mmol/L. To evaluate first phase insulin secretion, an intravenous glucose tolerance test (IVGTT) was performed after the clamp in a subset of the participants (n = 438 men). A weight-adjusted dose of glucose (0.3 g per kg bodyweight) was infused in one minute, and samples were subsequently collected after 2, 4, 6 and 8 min.

2.3. Biochemical tests

Blood samples were separated into serum and plasma and stored at −80 degrees until biochemical tests were performed. Samples were transferred on dry ice between sites and laboratories. Glucose was measured using the glucose oxidase technique (Cobas Integra, Roche) (within- and between assay coefficients of variation: 1.8% and 2.1%). Serum insulin and C-peptide were assessed using a two-sided time-resolved flouroimmunoassay (AutoDELFIA, Insulin Kit, Wallac Oy, Turku, Finland) based on monoclonal antibodies (Within and between assay coefficients of variation: Insulin (normal levels): 4.3% and 3.7%. C-peptide (normal levels): 5.3% and 2.6%). Serum Procollagen type I amino-terminal propeptide (PINP) and C-telopeptide of type I collagen (CTX-1) were measured by the chemiluminescence method in the fasting state and at steady-state of a euglycaemia during the clamp (IDS-iSYS. Within and between assay coefficients of variation (CV): PINP: 7% and 7%. CTX-1: 5% and 18%). Vitamin D was measured using direct competitive electrochemiluminescence immunoassay (COBAS 311, Roche).

2.4. Insulin sensitivity and beta-cell function

Based on the OGTT, participants were classified into two groups, individuals with normal glucose tolerance (NGT) or impaired glucose regulation (IGR), which included individuals with impaired fasting glycaemia (6.1–6.9 mmol/l), impaired glucose tolerance (2-h-OGTT glucose levels between 7.8 and 11.0 mmol/l) and a combination of both. Insulin sensitivity was calculated as the ratio of the average glucose infusion rate during the last 40 min of the 2-h clamp (adjusted for fat-free mass), M, and mean insulin levels during the same time interval (M/I). At both baseline and the 3-year follow-up, insulin sensitivity was assessed using plasma glucose and insulin levels measured during the 2-h-OGTT at baseline and follow-up. We used the oral glucose insulin sensitivity index normalized to lean body mass optimized for the RISC-study (OGIS-RISC), which has been shown to correlate with insulin sensitivity assessed by clamp [30]. Insulin resistance was also assessed using homeostatic model assessment (Fasting plasma glucose – fasting plasma insulin divided by 22.5).