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Effect of Intravenous Glucose Tolerance Test on Bone Turnover Markers in Adults with Normal Glucose Tolerance

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ACDEF 1 **Shou-Kui Xiang**
AB 1 **Jing-Bo Wan**
DF 1 **Xiao-Hong Jiang**
BC 2 **Yong-Hua Zhu**
BD 2 **Jin-Hong Ma**
AEFG 1 **Fei Hua**

1 Department of Endocrinology, Third Affiliated Hospital of Soochow University, Changzhou, Jiangsu, P.R. China
2 Department of Clinical Laboratory Medicine, Third Affiliated Hospital of Soochow University, Changzhou, Jiangsu, P.R. China

Corresponding Author: Fei Hua, e-mail: czhuafei@vip.sina.com
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Background: It is well known that enteral nutrients result in acute suppression of bone turnover markers (BTMs), and incretin hormones are believed to play a significant role in this physiological skeletal response. However, there is limited research exploring the impact of parenteral nutrients on BTMs. Our aim was to assess the influence of intravenous glucose on BTMs in adults with normal glucose tolerance (NGT).





Material/Methods: We conducted 1-h intravenous glucose tolerance test (IVGTT) in 24 subjects with NGT. Blood samples were collected before and 5, 10, 15, 20, 30, 60 min after administration of glucose, then serum levels of bone formation marker procollagen type I N-terminal propeptide (P1NP) and resorption marker C-terminal cross-linking telopeptides of collagen type I (CTX) were measured.

Results: During IVGTT, the fasting CTX level fell gradually and reached a nadir of 80.4% of the basal value at 60 min. Conversely, the fasting P1NP level decreased mildly and reached a nadir of 90.6% of the basal value at 15 min, then gradually increased and reached 96.6% at 60 min. The CTX-to-P1NP ratio increased slightly and reached a peak of 104.3% of the basal value at 10 min, then fell gradually and reached a nadir of 83% at 60 min.

Conclusions: Our study indicates that intravenous glucose results in an acute suppression of BTMs in the absence of incretin hormones. The mechanism responsible for this needs further investigation.

MeSH Keywords: **Bone Remodeling • Bone Resorption • Glucose Tolerance Test**

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Background

Bone is continuously remodeled throughout life in order to meet the functional demands of its physiological and mechanical environment. Preservation of bone mass and structure is of great importance and is maintained by a balance between osteoblastic bone formation and osteoclastic bone resorption. This dynamic process of bone remodeling during adult life reflects the circulating concentrations of bone turnover markers (BTMs). Bone formation can be assessed by measuring the concentration of procollagen type I N-terminal propeptide (P1NP), which is produced by osteoblasts and is released into the circulation during bone formation. Conversely, C-terminal cross-linking telopeptides of collagen type I (CTX) is a well-accepted marker of bone resorption, reflecting the degradation of type I collagen by osteoclasts to produce amino-terminal and carboxy-terminal fragments [1].

Diurnal variation in bone turnover is responsive to eating. There is an acute suppression of bone resorption *in vivo*, which occurs within hours of the ingestion of glucose, protein, fat, and mixed food [2–5]. Bone formation is also influenced, but it seems to be less responsive to nutrients than resorption [3,5]. The regulation of bone turnover in response to nutrient intake may be explained by changes in the secretion of several incretin hormones [6], including glucagon-like peptide-1 (GLP-1), GLP-2, and glucose-dependent insulinotropic peptide (GIP).

In healthy subjects, both subcutaneous and intravenous injections of GLP-2 have been shown to induce reduction of CTX [7,8]. Four-month treatment with GLP-2 in postmenopausal women significantly increased hip bone mineral density (BMD) with a reduction in serum CTX [9]. Nissen et al. found GIP infusion reduced bone resorption in humans using glucose clamp technique [10]. Iepsen et al. showed that GLP-1 receptor agonist treatment increased bone formation and prevented bone loss in weight-reduced obese women [11]. The above findings support the role of incretin hormones in nutrient-dependent regulation of bone metabolism.

It is well known that enteral nutrients result in acute suppression of BTMs, and incretin hormones are thought to play a key role in this physiological skeletal response. However, there is limited research in the literature exploring the impact of parenteral nutrients on BTMs in the absence of incretin hormones. To avoid the interaction between the ingested glucose and incretin hormones, the glucose load is often administered through an intravenous catheter using the intravenous glucose tolerance test (IVGTT). Here, our aim was to explore the effect of IVGTT on BTMs in adults with normal glucose tolerance (NGT).

Material and Methods

Subjects

We enrolled a total of 25 healthy adults (10 females, 15 males) aged 25–35 years. All participants signed an informed consent. The exclusion criteria were diseases or medication known to affect bone metabolism. This study was approved by the local ethics committee of the Third Affiliated Hospital of Soochow University.

Study design

All 25 participants underwent anthropometric measurements to determine weight and height, from which body mass index (BMI) was calculated. Blood pressure (BP) was measured in the right arm using a mercury sphygmomanometer, and a 75-g oral glucose tolerance test (OGTT) was performed on all subjects. Fasting plasma glucose (FPG) and 2-h postchallenge plasma glucose (2hPG) concentrations were measured. Glucose intolerance was categorized according to the American Diabetes Association criteria [12]. On a different day, those with normal glucose tolerance (NGT) underwent an IVGTT (0.3 g/Kg) using 50% glucose solution. Blood samples were collected before and 5, 10, 15, 20, 30, 60 min after administration of glucose to measure glucose, insulin, and BTMs levels.

Assays

All biochemical markers were measured at the same time point using the same reagent kits by the same technician, following both the manufacturer-provided operating processes and specialized assay laboratory quality control procedures. Plasma glucose was determined using the glucose oxidase method and an autoanalyzer (Beckman Coulter AU5800; Tokyo, Japan). Serum levels of alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase, creatinine, uric acid, urea nitrogen, calcium, phosphorus, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were also examined (Beckman Coulter AU5800; Tokyo, Japan).

Serum concentrations of insulin, CTX, and P1NP were measured using a computer-controlled automatic analyzer (Roche cobas e 601; Mannheim, Germany) for chemiluminescence workstation with insulin, β -Crosslaps, and total P1NP Elecsys reagent kits supplied by Roche Diagnostics (Mannheim, Germany). The intra- and inter-assay coefficients of variation (CV) were 2.3% and 4.1% for insulin, 2.4% and 3.6% for CTX, 2.1% and 3.4% for P1NP, respectively.

Bone mineral densities (BMDs) of the lumbar spine and femur were determined by dual-energy X-ray absorptiometry (Hologic, Bedford, MA, USA).

Statistical analysis

Continuous variables were presented as the mean \pm SD. The Kolmogorov-Smirnoff test was used to test the normality of the data. The response of BTMs during IVGTT is expressed as a percentage of baseline (time 0 min). Within-group changes in BTMs were evaluated by one-way ANOVA for repeated measures followed by Tukey's post hoc test. A *P* value <0.05 was considered significant. The statistical analysis was performed using SPSS 19.0 for Windows.

Results

Baseline clinical characteristics

Of the 25 participants screened, 1 had impaired glucose tolerance (IGT) and 24 had NGT. The subject with IGT was excluded from the IVGTT and analysis. The baseline anthropometric parameters, biochemical indices, and BMD data from these NGT subjects are shown in Table 1. The male and female subjects were similar in age and BMI. Female subjects had significantly increased fasting insulin and 2hPG compared to male subjects. However, serum CTX levels were significantly lower in

Table 1. General characteristics of male and female subjects with NGT.

	Male	Female	<i>P</i> value
N	14	10	–
Age (years)	29.2 \pm 2.6	29.1 \pm 3.3	0.878
BMI (Kg/m ²)	22.2 \pm 2.8	22.1 \pm 1.3	0.873
SBP (mmHg)	119 \pm 8	116 \pm 7	0.364
DBP (mmHg)	74 \pm 6	70 \pm 5	0.146
FBG (mmol/L)	4.35 \pm 0.32	4.42 \pm 0.27	0.602
2hPG (mmol/L)	5.84 \pm 0.91	6.14 \pm 0.83	<0.001
Insulin (mIU/L)	6.18 \pm 2.24	13.44 \pm 2.84	<0.001
Alanine transferase (IU/L)	23.5 \pm 8.9	14.6 \pm 5.8	0.011
Aspartate transferase (IU/L)	20.5 \pm 3.0	16.1 \pm 3.1	0.002
Alkaline phosphatase (IU/L)	68.6 \pm 11.2	61.4 \pm 8.5	0.102
Calcium (mmol/L)	2.40 \pm 0.16	2.35 \pm 0.12	0.362
Phosphorus (mmol/L)	1.09 \pm 0.08	1.12 \pm 0.17	0.563
Uric acid (umol/L)	311.4 \pm 51.3	251.1 \pm 42.3	0.006
Urea nitrogen (mmol/L)	3.68 \pm 0.60	3.46 \pm 0.86	0.464
Creatinine (umol/L)	82.8 \pm 7.8	62.2 \pm 4.6	<0.001
Triglycerides (mmol/L)	1.65 \pm 0.72	1.33 \pm 0.58	0.254
Total cholesterol (mmol/L)	4.19 \pm 0.59	3.87 \pm 0.34	0.131
HDL cholesterol (mmol/L)	1.16 \pm 0.22	1.28 \pm 0.19	0.147
LDL cholesterol (mmol/L)	2.37 \pm 0.41	2.04 \pm 0.25	0.063
CTX (pg/ml)	550.3 \pm 120.5	426.3 \pm 115.7	0.019
P1NP (ng/ml)	52.1 \pm 8.4	56.7 \pm 27.3	0.561
L1–4 BMD (g/cm ²)	0.93 \pm 0.14	1.00 \pm 0.11	0.142
Femoral neck BMD (g/cm ²)	0.83 \pm 0.15	0.79 \pm 0.15	0.512
Total hip BMD (g/cm ²)	0.96 \pm 0.16	0.87 \pm 0.16	0.187

Data are presented as the mean \pm SD.

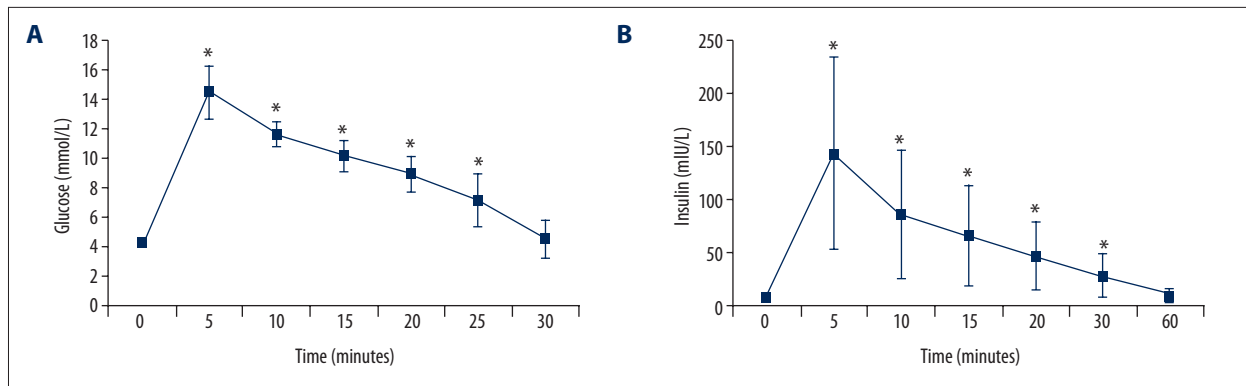


Figure 1. Absolute values for plasma glucose (A) and insulin (B) during the IVGTT. Data are presented as mean \pm SD. * $P < 0.05$, comparison with baseline.

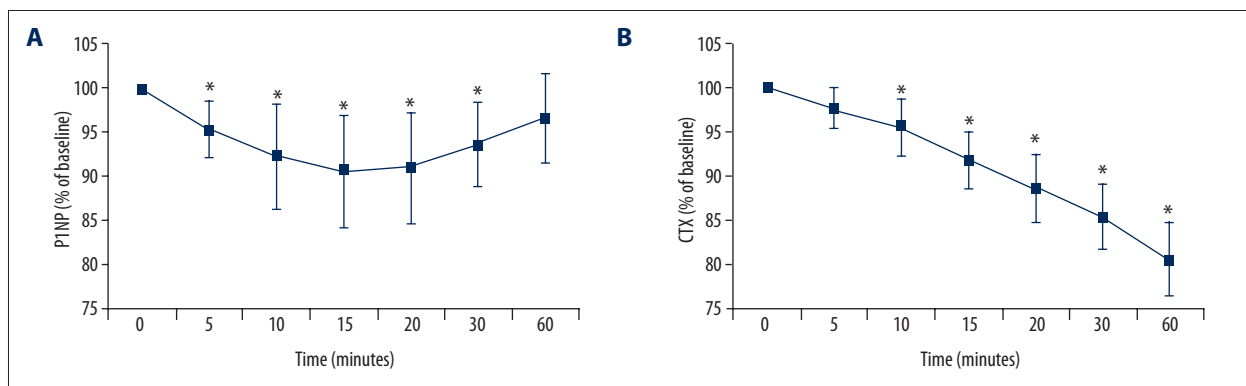


Figure 2. Percentage changes in bone resorption marker CTX (A) and bone formation marker P1NP (B) during the IVGTT. Data are presented as mean \pm SD with the baseline values set at 100%. * $P < 0.05$, comparison with baseline.

female subjects. In addition, there was no significant difference in serum P1NP levels between male and female subjects.

Changes in BTMs during IVGTT

Plasma glucose and insulin response during IVGTT are shown in Figure 1. Changes in BTMs during IVGTT are shown in Figure 2, presented as percentage of the fasting level (baseline). During IVGTT, the fasting CTX level fell gradually and reached a nadir of 80.4% of the basal value at 60 min (Figure 2A). Conversely, the fasting P1NP level fell mildly and reached a nadir of 90.6% of the basal value at 15 min, then gradually increased and reached 96.6% at 60 min (Figure 2B).

Changes in CTX-to-P1NP ratio during IVGTT

To adjust reciprocal modifications in resorption and formation markers, the CTX-to-P1NP ratio was applied [13]. Figure 3 shows the changes in CTX-to-P1NP ratio during IVGTT. It increased slightly and reached a peak of 104.3% of the basal value at 10 min, then fell gradually and reached a nadir of 83% at 60 min.

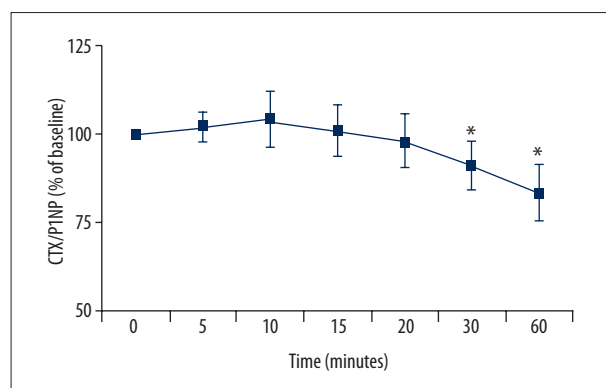


Figure 3. The CTX-to-P1NP ratio changes during the IVGTT. The data are presented as the percent change from baseline values (mean \pm SD), with the baseline values set at 100%. * $P < 0.05$, comparison with baseline.

Discussion

Our study indicates that an acute intravenous glucose load induces a gradual and significant decrease in bone resorption marker CTX in NGT subjects. Conversely, bone formation marker P1NP shows a transient and mild decrease during IVGTT.

Bjarnason et al. [2] showed that IVGTT induced a significant reduction in serum CTX after 2 h in postmenopausal women. However, their study did not observe acute changes of BTMs within 1 h during IVGTT. In our study, P1NP decreased significantly at 5 min and CTX fell significantly at 10 min during IVGTT in NGT subjects. The changes in CTX-to-P1NP ratio indicate that intravenous glucose produces a greater decrease in bone resorption, thereby changing the bone remodeling balance in favor of bone formation.

Previous studies have shown that a variety of enteral nutrients result in acute suppression of BTMs, and incretin hormones may play a key role in postprandial reduction of BTMs. The observation that oral glucose results in a greater suppression of bone resorption compared with intravenous glucose [2] further supports the existence of an entero-osseous axis. Our study indicates that intravenous glucose load also results in acute suppression of BTMs in the absence of incretin hormones, but the mechanism responsible for this is not known. Possible factors include acute insulin secretion, the direct effect of glucose, or other associated endocrine responses.

Using euglycemic hyperinsulinemic clamp technique, a few studies have assessed the direct effect of insulin on bone metabolism in humans. Basu et al. [14] showed that acute changes in insulin levels did not modulate serum P1NP, CTX, under-carboxylated osteocalcin, or osteoprotegerin levels. Clowes et al. [15] found that a euglycemic, hyperinsulinemic clamp failed to alter serum levels of CTX, PINP, or osteocalcin. Ivaska et al. [16] demonstrated that, after 4-h insulin infusion, there was a small but significant decrease in bone resorption marker CTX. High-dose insulin exposure resulted in a similar, but more pronounced, suppression of serum CTX. In contrast, shorter 2-h insulin infusion did not alter serum CTX, and P1NP remained unchanged during high and low as well as long and short insulin infusions. However, clinical observations in patients with type 1 and type 2 diabetes suggest that insulin may act as an anabolic agent in bone and preserve bone mass in humans [17]. The above findings suggest that insulin does not regulate the acute effect of intravenous glucose on bone resorption, but is likely to have longer-term effects on bone metabolism.

Interestingly, Clowes et al. [15] did find that a hypoglycemic hyperinsulinemic clamp resulted in acute suppression of bone turnover (CTX by 34%, PINP by 15%, and osteocalcin by 5%), suggesting that the acute change in bone turnover is due to direct effects of hypoglycemia on bone cells or counter-regulatory hormones triggered by hypoglycemia. In another study, Clowes et al. [6] showed that there was no significant decrease in bone turnover in response to oral glucose during octreotide

infusion which inhibited the release of gastrointestinal and pancreatic peptides. The result indicates that hyperglycemia itself may not have a direct effect on BTMs. In addition, the role of other associated endocrine responses (such as PTH, cortisol, and growth hormone) during IVGTT in the changes of BTMs remains uncertain and further studies are required.

To maintain a constant bone mass during adult life, bone is remodeled continuously by a coupling between osteoblastic bone formation and osteoclastic bone resorption. Oral nutrient intake results in a marked suppression of bone resorption markers and a moderate suppression of bone formation markers within hours after nutrient ingestion, a phenomenon explained by the uncoupling of the 2 processes postprandially [18]. In our study, we observed a similar phenomenon through the changes in CTX-to-P1NP ratio. This indicates that parenteral nutrients also shift the bone turnover balance in favor of bone formation. Further studies are advocated to elucidate the mechanism of this phenomenon, which may provide new proposals for the treatment of osteoporosis.

The strengths of this study include the homogeneity of the cohort with young age, normoglycemic status, and a very limited number of potential confounding factors that may influence bone turnover. In addition, we showed the continuous change of BTMs within 1 h during IVGTT. Despite its strengths, our study has potential limitations. First, the study was carried out with a small sample of subjects due to the highly laborious and invasive study protocol, and there is a need for studies with broader participation. Second, we did not observe the effect of IVGTT on BTMs over a longer time frame. Thus, no conclusions can be drawn regarding the effect of intravenous glucose on BTMs after 1 h.

Conclusions

Our study indicates that intravenous glucose results in an acute suppression of BTMs in the absence of incretin hormones. The mechanism responsible for this needs further investigation.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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