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# RELATIONSHIP BETWEEN CIRCULATING OSTEOCALCIN AND INDICES OF LIPID AND CARBOHYDRATE METABOLISM IN YOUNG WOMEN WITH OVULATORY MENSTRUAL CYCLES

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## Abstract

**Introduction:** There are data indicating that low plasma levels of osteocalcin are observed in obesity, type 2 diabetes and/or metabolic syndrome. In addition, long-term treatment of wild-type mice with osteocalcin significantly decreases detrimental effects of a high-fat diet. Thus, it has been postulated that bone turnover expressed as plasma osteocalcin concentrations has a potential to affect at least carbohydrate and lipid metabolism.

**Objective:** To evaluate the relationship between circulating osteocalcin and selected indices of lipid and carbohydrate metabolism in young, healthy women with physiological levels of circulating sex hormones.

**Methods:** Seventeen young women with physiological levels of 17β-estradiol and progesterone between days 5-8 and 19-22 of the menstrual cycle were selected for analysis of plasma osteocalcin, glucose, free fatty acids, glycerol and insulin.

**Results:** Neither anthropometric nor biochemical variables differ with respect to cycle phase. For collected data of both cycle phases circulating osteocalcin was inversely and significantly correlated with plasma glucose ($P < 0.03$), FFA ($P < 0.006$) and insulin ($P < 0.04$). In consequence significant and inverse relationship was noted between plasma levels of osteocalcin and calculated index of insulin sensitivity – QUICKI-FFA ($P < 0.001$).

**Conclusions:** Our data suggest that in healthy young women with physiological levels of ovarian hormones circulating osteocalcin contributes to the regulation of indices of carbohydrate and lipid metabolism, and in consequence affects calculated index of insulin sensitivity (QUICKI-FFA).

***Key words:*** *women, metabolic variables, osteocalcin*

## Introduction

It is well documented that excessive energy intake, sedentary lifestyle, and poor dietary habits increase the risk of insulin resistance and its adverse health consequences [1,2].

Moreover, there are many studies indicating adverse effects of age-related decline in sex hormone secretion on whole body insulin sensitivity both in male and female subjects [3,4]. In turn, there are data suggesting a beneficial effect of sex hormones administration on insulin sensitivity in postmenopausal women and middle-aged men [5].

Recent findings have revealed that whole body insulin sensitivity is subjected to even more complicated regulation due to the action of osteocalcin - a non-collagenous, vitamin K-dependent protein produced by osteoblasts and involved in bone mineralization and calcium homeostasis [6]. Much of the newly synthesized osteocalcin is incorporated into bone matrix but it is also secreted into bloodstream. On the other hand, during bone resorption osteocalcin is degraded, however, the majority enters circulation.

In consequence, circulating osteocalcin should be considered as a marker of bone turnover rather than bone formation [7].

On the other hand, animal *in vitro* studies have demonstrated that circulating osteocalcin regulates the expression of the insulin genes and β cell proliferation but also induces adiponectin expression in white adipocytes [8]. In addition, long-term treatment of wild-type mice with osteocalcin significantly decreases detrimental effects of a high-fat diet. Thus, it has been postulated that bone turnover expressed as plasma osteocalcin concentrations has a potential to affect at least carbohydrate and lipid metabolism.

This assumption has been supported by human data indicating that low plasma levels of osteocalcin are observed in obesity, type 2 diabetes and/or metabolic syndrome [9,10]. Moreover, decreased circulating osteocalcin has been found to contribute to cardiovascular mortality and premature cardiac infraction [11].

However, the above studies concerned subjects with health problems. Thus, the present study was undertaken to evaluate the relationship between osteocalcin and metabolic variables young, healthy

non-obese women**.** Furthermore, to rule out negative metabolic effects of disturbed ovarian hormone secretion exclusively subjects with ovulatory menstrual cycles were included in the study.

## Materials and methods

### *Participants*

The participants were recruited among female students on the basis of advertisements in student dormitories and by word of mouth. All the subjects were healthy non-smokers not taking any medication on a regular basis. They were advised to follow their habitual diet throughout the study and to refrain from physical activity at least 48 h before blood sampling. All the subjects gave their written consent prior to participation and the procedures of this study were approved by the local ethics committee.

The prospective subjects agreed to monitor their basal body temperature (BBT) for six months before the study. Plasma 17β-estradiol and progesterone concentrations were determined twice per cycle – between days 5 and 8 and again between days 19 and 22 of the cycle. In 17 women with biphasic BBT pattern and undoubtedly physiological levels of ovarian hormones other biochemical variables were measured [12]. In all participants weight and height were measured using standard procedures.

### *Blood sampling and biochemical analysis*

The subjects were asked to take their last meal or snack no later than 10:00–10:30 p.m. on the day preceding blood sampling. Fasting venous blood was drawn into tubes with anticoagulant using disposable needles and syringes under aseptic conditions between 8:00 and 9:00 a.m. Plasma and erythrocytes were separated by centrifugation (15 min/3000 rpm, 4°C) and plasma was stored at –70°C until analysis.

Plasma glucose was measured at 500 nm using the GOD-PAP method, plasma free fatty acids (FFA) were determined at 550 nm and plasma glycerol at 520 nm. All assays were performed using Randox commercial kits (Randox Laboratories Ltd., United Kingdom). The coefficients of variation (CV) for the above variables did not exceed 5.3%. Plasma 17β-estradiol and progesterone were assayed using RIA methods and Orion Diagnostica (Finland) kits. Intra- and inter-assay coefficients of variation (CV) were 3.5% and 7.9% for 17β-estradiol and 4.3% and 5.0% for progesterone. Osteocalcin, and insulin were measured using the IRMA method and commercial kits (Cis bio International, France for osteocalcin and Orion Diagnostica, Finland for insulin). Respective intra- and inter-assay coefficients of variation was 2.8% and 5.2% for osteocalcin and 6.8% and 9.3% for insulin. The sensitivity of the methods were as follows: 20 pmol for 17β-estradiol, 0.2 nmol/l for progesterone, 4.9 ng/ml for osteocalcin, and 1 mU/ml for insulin. All assays were run in duplicate.

According to Perseghin et al. [13] incorporation of fasting FFA level into QUICKI- improves correlation with the clamp-based index of insulin sensitivity and its discriminatory power in case of mild insulin resistance. This is why QUICKI-FFA was used in the current study and calculated according to the following formula:

QUICKI – FFA = 1/[log glucose (mg/dl)] + [log insulin (μU/ml)] + [log FFA (mmol/l)]

### *Statistical analysis*

Data distribution was evaluated using the Shapiro-Wilk test. The Student test for paired data or the Wilcoxon matched-pairs test were used for the comparison of data with normal and skewed distribution, respectively. Before calculation of Pearson coefficients all data except of glycerol were logarithmically transformed. All calculations were performed using Statistica v. 7.1 (StatSoft, Inc., USA). Values were reported as means ± SD. Statistical significance was set at $P < 0.05$.

## Results

No differences in subjects' anthropometric characteristics were noted between days 5-8 and 19-22 of the menstrual cycle (Table 1). Plasma levels of 17β-estradiol and progesterone between days 19-22

Table 1. *Anthropometric and hormonal characteristics of the subjects (mean ± SD)*

| Variables | Days 5-8 | Days 19-22 |
|---|---|---|
| Age (years)* | 20.7 ± 1.1 | – |
| Weight (kg) | 62.3 ± 8.9 | 62.7 ± 9.0 |
| Height (cm) | 171.8 ± 7.2 | 172.0 ± 7.3 |
| BMI | 21.1 ± 1.9 | 21.2 ± 1.9 |
| 17β-estradiol (pmol/L) | 222.2 ± 109.4 | 521.4 ± 130.9[a] |
| Progesterone (nmol/L) | 2.3 ± 0.6 | 41.4 ± 12.6[a] |
| Cycle length (days)^ | 27.3 ± 1.6 | – |

* At the beginning of the study; ^ registered over six months period; [a]$P < 0.001$

Table 2. *Biochemical variables between days 5-8 and 19-22 of the menstrual cycle in young women (mean ± SD)*

| Variables | Days 5-8 | Days 19-22 |
|---|---|---|
| Osteocalcin (ng/ml) | 14.4 ± 4.1 | 14.5 ± 3.2 |
| Glucose (mmol/L) | 4.6 ± 0.5 | 4.5 ± 0.4 |
| FFA (mmol/L) | 0.312 ± 0.153 | 0.319 ± 0.215 |
| Glycerol (μmol/L) | 80.9 ± 26.0 | 74.2 ± 28.5 |
| Insulin (μU/ml) | 7.7 ± 3.2 | 7.5 ± 2.4 |
| QUICKI-FFA | 0.435 ± 0.072 | 0.437 ± 0.025 |

Table 3. *Pearson correlation coefficients between circulating osteocalcin and other variables in collected data from 5-8 and 19 -22 days of the menstrual cycle*

| Variables | *r* |
|---|---|
| BMI | - 0.110 |
| Glucose (mmol/L) | - 0.390[a] |
| FFA (mmol/L) | -0.470[b] |
| Glycerol (μmol/L) | 0.060 |
| Insulin ((μU/ml) | - 0.370[c] |
| QUICKI-FFA | -0.592[d] |

All data except of glycerol were logarithmically transformed before calculation
[a]$P < 0.03$; [b]$P < 0.006$; [c]$P < 0.04$; [d]$P < 0.001$

were significantly higher vs. days 5-8 ($P < 0.001$). There were no differences in circulating glucose, FFA, glycerol, insulin, osteocalcin and calculated index of insulin sensitivity (QUICKI –FFA) between days 5-8 and 19-22 of the menstrual cycle (Table 2).

For collected data of both menstrual cycle phases circulating osteocalcin was inversely correlated with all tested variables except of glycerol ($P < 0.03$, $P < 0.006$ and $P < 0.04$ for glucose, FFA and insulin, respectively) (Table 3). In consequence, significant and inverse relationship was noted between ostaeocalcin levels in plasma and calculated index of insulin sensitivity (QUICKI-FFA) ($P < 0.001$).

## Discussion

According to reference values included into commercial kits, all variables in the current study were within physiological limits. Thus, our data refer to females with normal ovarian hormones and other variables levels in plasma. Additionally, BMI value indicated that it did not exceeded 25, thus all of our participants were lean. The lack of association between BMI and circulating osteocalcin suggests minor effect of body fat on osteocalcin in lean subjects.

Index of insulin sensitivity QUICKI-FFA in our study did not differ between days 5-8 vs. days 9-22 of the menstrual cycle. However, it is worth noting that data concerning the effects of menstrual cycle phase on insulin sensitivity are equivocal indicating significantly lower or similar insulin sensitivity in the luteal vs. follicular phase of the cycle [14,15].

In addition, circulating osteocalcin in both phases of the cycle are in agreement with other data [16,17].

Moreover, our results concerning osteocalcin effects on glucose plasma levels are in agreement with Kindblom et al. [18] findings in diabetic and non-diabetic elderly subjects.

There are also data suggesting ostecalcin contribution to lipid metabolism. Pittas et al. [19] have found negative action of low circulating osteocalcin on markers of metabolic syndrome. Furthermore, it has been noted that osteocalcin mRNA is expressed in adipose tissue [20] acting together with body fat in metabolism regulation.

Summing up, in our study correlation coefficients between circulating osteocalcin and indices of lipid and carbohydrate metabolism were not strong but significant possibly due to small subject number. However, even taking into account this limitation it seems that in young, lean females with normal menstrual cycles circulating osteocalcin plays an important metabolic role.

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## Declaration of interest

The authors report no conflicts of interest.

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