

Cross-Sectional Evidence of a Signaling Pathway from Bone Homeostasis to Glucose Metabolism

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Context: Preclinical studies suggested the existence of a signaling pathway connecting bone and glucose metabolisms. Supposedly leptin modulates osteocalcin bioactivity, which in turn stimulates insulin and adiponectin secretion, and β -cell proliferation.

Objective: The objective of the investigation was to study the reciprocal relationships of adiponectin, leptin, osteocalcin, insulin resistance, and insulin secretion to verify whether such relationships are consistent with a signaling pathway connecting bone homeostasis and glucose metabolism.

Design: This was a cross-sectional analysis.

Setting: The study was conducted with community-dwelling volunteers participating in the Baltimore Longitudinal Study of Aging.

Participants: Two hundred eighty women and 300 men with complete data on fasting plasma adiponectin, leptin, and osteocalcin, oral glucose tolerance test (plasma glucose and insulin values available at $t = 0, 20,$ and 120 min), and anthropometric measures participated in the study.

Main Outcome Measures: Linear regression models were used to test independent associations of adiponectin, osteocalcin, and leptin with the indices of insulin resistance and secretion. The expected reciprocal relationship between different biomarkers was verified by structural equation modeling.

Results: In linear regression models, leptin was strongly associated with indices of both insulin resistance and secretion. Both adiponectin and osteocalcin were negatively associated with insulin resistance. Structural equation modeling revealed a direct inverse association of leptin with osteocalcin; a direct positive association of osteocalcin with adiponectin; and an inverse relationship of osteocalcin with insulin resistance and adiponectin with insulin resistance and secretion, which is cumulatively consistent with the hypothesized model.

Conclusions: Bone and glucose metabolisms are probably connected through a complex pathway that involves leptin, osteocalcin, and adiponectin. The clinical relevance of such a pathway for bone pathology in diabetes should be further investigated. (*J Clin Endocrinol Metab* 96: E884–E890, 2011)

The skeleton has traditionally been studied for its structural support role, which is essential for locomotion and maintenance of standing posture and for being the major site of calcium and phosphorus deposit. More recently, evidence has emerged suggesting that bone tissue

also participates in regulating energy metabolism by secreting osteocalcin, which influences glucose homeostasis and fat mass.

Preclinical studies have shown that osteocalcin, produced by osteoblasts, stimulates β -cell proliferation and

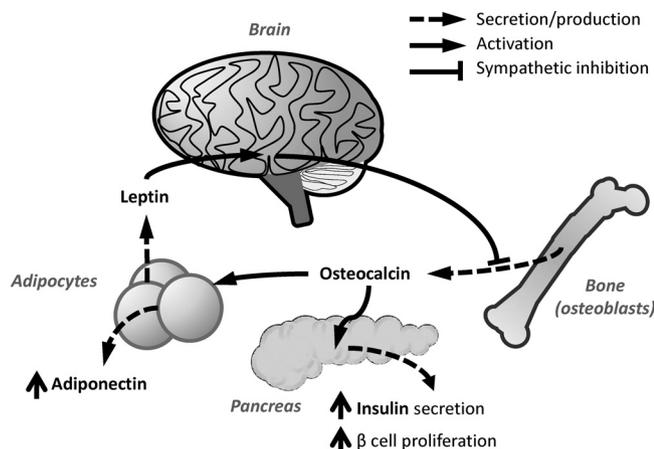


FIG. 1. Integrated physiological model adapted from Confavreux *et al.* (5).

insulin production and enhances adiponectin production from adipocytes (1, 2). Adiponectin, in turn, regulates energy homeostasis by suppressing hepatic gluconeogenesis, stimulating fatty acid oxidation in liver and skeletal muscle and enhancing glucose uptake in skeletal muscle (3). Ducy *et al.* (4) found evidence that leptin, through a hypothalamic relay, regulates osteoblast function.

In an attempt to connect these preclinical findings in a unique physiological paradigm, Confavreux *et al.* (5) proposed the notion that bone affects energy metabolism through a signaling pathway that involves osteocalcin, adipokines, and pancreatic β -cells. In particular, they suggested that leptin regulates the functions of β -cells and the metabolisms of skeletal muscle and adipocytes both directly and indirectly by affecting the bioavailability and posttranslational modification of osteocalcin (Fig. 1) (5). In accordance with this hypothesis, cross-sectional studies in humans demonstrated negative associations of osteocalcin with body mass index (BMI), fat mass, and fasting plasma glucose and positive associations with insulin sensitivity and insulin secretion (6–8). However, to our knowledge, the complex interrelationship of leptin, adiponectin, and osteocalcin with markers of glucose metabolism has not been previously investigated.

Using data from the Baltimore Longitudinal Study of Aging (BLSA), we therefore tested the above paradigm in humans, looking at both direct and indirect associations among leptin, adiponectin, and osteocalcin with markers of insulin resistance and β -cell function, using multiple linear regression models and structural equation models.

Patients and Methods

Study population

The BLSA is an ongoing observational study of normative aging in community-dwelling volunteers conducted at and sponsored by the National Institute on Aging since 1958.

Participants undergo medical, physiological, and psychological examinations at regular intervals (9). The BLSA protocol was approved by the Intramural Research Program of the U.S. National Institute on Aging and the Institutional Review Board of the MedStar Health Research Institute (Baltimore, MD). All participants provided informed participation consent at each visit.

We performed a cross-sectional analysis on data from 580 BLSA participants whose latest study visit fell between April 2003 and May 2009 and had all the following measures: fasting plasma adiponectin, leptin, and osteocalcin, and a standard 75-g oral glucose tolerance test (OGTT) with plasma glucose and insulin values at baseline and after 20 and 120 min. OGTT is routinely performed in all BLSA visits after a 10-h overnight fast, and participants on insulin or steroid treatment within 3 months before the study visit are excluded from the OGTT. Subjects using oral hypoglycemic agents were excluded from this analysis.

Measurements

Plasma glucose levels were measured using a glucose analyzer (Beckman Instruments, Brea, CA). Plasma insulin was measured using an ELISA with an interassay variation of 2.6–3.6% and an intraassay variation of 2.8–4.0% (Mercodia, Uppsala, Sweden). Plasma leptin was measured using an ELISA kit with an interassay variation of 2.6–6.2% and an intraassay variation of 2.6–4.6% (Millipore, Billerica, MA). Plasma total adiponectin was measured using a RIA kit with an interassay variation of 6.9–9.3% and an intraassay variation of 1.8–6.2% (Millipore). Plasma total osteocalcin levels were measured by a commercial laboratory (Pacific Biometrics Inc., Seattle, WA) using an ELISA with an interassay variation of 5.4–8.0% and an intraassay variation of 1.8–6.2%.

BMI was calculated as body weight (kilograms)/height² (meters²). To measure insulin resistance, we used an updated version of the homeostatic model assessment of insulin resistance (HOMA2-IR) model with nonlinear solutions pairing fasting plasma glucose and insulin values, developed by Matthews and colleagues (10). HOMA2-IR was used because it also accounted for variations in hepatic and peripheral glucose resistance. β -Cell function was measured using a modified insulin secretion index (ISI) calculated using 20-min, post-OGTT plasma glucose and insulin and fasting insulin based on the index described by Stumvoll *et al.* (11).

Statistical analysis

All glucose metabolism indices and biochemical variables showed highly skewed distributions, and therefore, they were presented as median (interquartile range) and

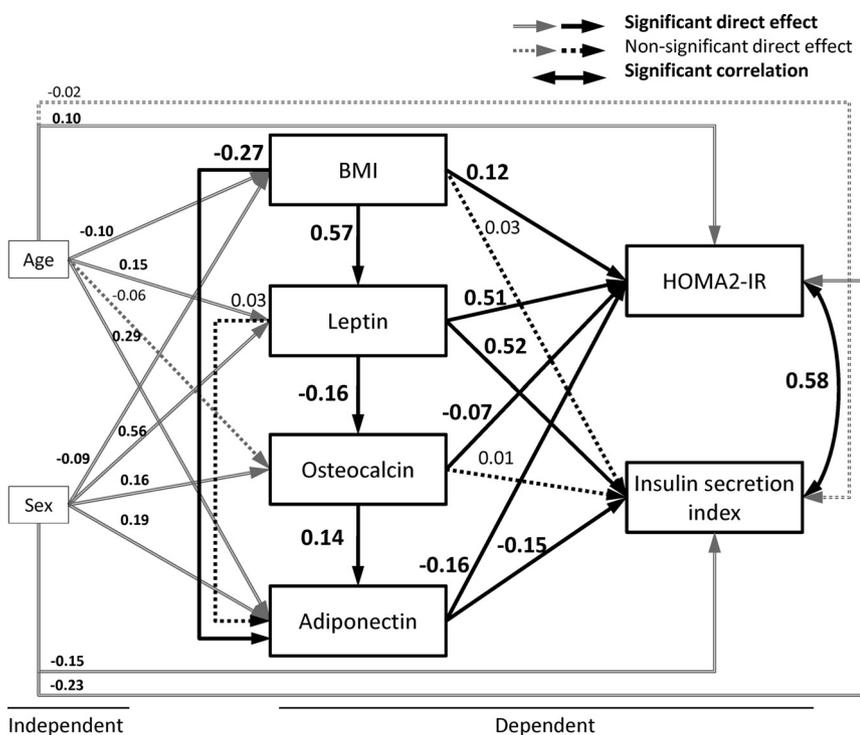


FIG. 2. The structural equation model fits the underlying data ($\chi^2 = 2.67, df = 2, P = 0.263$). Standardized regression coefficients are at the base of each arrow; bolded coefficients are statistically significant ($P < 0.05$) and correspond to thickened solid lines. Sex is coded 0 for men and 1 for women.

\log_{10} transformed for analyses. Student’s *t* tests were performed for baseline comparisons between women and men. Multiple linear regression models were used to regress HOMA2-IR and ISI onto adiponectin, osteocalcin, and leptin after adjusting for age, sex, and BMI. F tests were used to compare fit (R^2) between nested models.

We used structural equation modeling (SEqM) to further test the hypothesized interrelationships among age, sex, BMI, leptin, osteocalcin, adiponectin, HOMA2-IR, and ISI, according to a predefined interpretative model, as shown in

Fig. 2. A χ^2 test was used to determine the measures of fit for the SEqM comparing degrees of freedom (*df*) with the χ^2 value. A value greater than the level of significance $\alpha = 0.05$ ($P > 0.05$) indicates that the covariance matrix hypothesized *a priori* is consistent with the observed covariance matrix. This model was then cross-validated using a bootstrapping method described by Bollen and Stine (12), whereby χ^2 values generated from random samples are compared with the naïve model χ^2 . $P < 0.05$ was used to determine statistical significance. All regression coefficients presented are standardized to make them comparable. Analyses were performed using SPSS and Amos (version 17.0; SPSS Inc., Chicago, IL).

Results

Study population characteristics

Table 1 summarizes the characteristics of the 580 BLSA participants included in this study. Compared with men, women had statistically significant lower weight, BMI, fasting plasma glucose, and 20-min post-OGTT plasma glucose but higher leptin, osteocalcin, and adiponectin. Age, fasting plasma insulin, 20-min post-OGTT plasma insulin and ISI were comparable between men and women.

Table 2 summarizes the multiple linear regression models estimating the relationship of adiponectin, osteocalcin, and leptin with HOMA2-IR (*left*) and ISI (*right*), adjusting for age, sex, and BMI.

TABLE 1. Characteristics of study population

Characteristic	Total	Men	Women	P
n	580	300	280	
Age (yr)	69 (59–78)	71 (60–79)	66 (59–76)	0.119
Weight (kg)	76.5 (65.8–87.3)	83.0 (75.2–93.3)	67.0 (59.7–78.4)	<0.001
BMI (kg/m ²)	25.9 (23.6–29.5)	26.5 (24.4–29.5)	25.3 (22.5–29.5)	0.045
OGTT and metabolism indices				
Fasting glucose (mmol/liter)	5.0 (4.7–5.3)	5.1 (4.8–5.4)	4.8 (4.6–5.2)	<0.001
20-min glucose (mmol/liter)	7.2 (6.4–8.1)	7.3 (6.6–8.2)	7.1 (6.3–7.9)	0.026
Fasting insulin (pmol/liter)	49.9 (33.6–76.1)	51.7 (33.7–75.4)	48.6 (33.4–77.7)	0.644
20-min insulin (pmol/liter)	264.7 (167.7–404.7)	246.3 (144.9–390.8)	291.2 (184.6–414.5)	0.064
HOMA2-IR	0.93 (0.63–1.41)	0.97 (0.63–1.41)	0.91 (0.62–1.43)	0.007
ISI	1844 (1592–2176)	1791 (1552–2181)	1889 (1629–2176)	0.099
Hormones				
Adiponectin (μg/ml)	11.9 (7.1–19.2)	10.1 (5.7–17.1)	14.3 (8.8–21.2)	<0.001
Osteocalcin (ng/ml)	15.6 (11.4–20.9)	14.9 (11.0–19.7)	16.2 (11.8–22.0)	0.048
Leptin (ng/ml)	13.1 (6.7–26.1)	8.8 (4.9–14.8)	22.5 (12.0–39.7)	<0.001

Data presented as median (25th to 75th percentiles) unless specified otherwise. P is for comparisons between men and women.

TABLE 2. Multiple linear regressions evaluating the association of insulin resistance (HOMA2-IR) and β -cell function (ISI) with adiponectin, osteocalcin, and leptin

Variables	HOMA2-IR			ISI		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Adiponectin	−0.17 ^a	−0.15 ^a	−0.16 ^a	−0.14 ^b	−0.14 ^b	−0.14 ^a
Osteocalcin		−0.11 ^b	−0.07 ^c		0.05	0.01
Leptin			0.51 ^a			0.52 ^a
Model adjusted R ²	0.23 ^a	0.24 ^b	0.35 ^a	0.14 ^a	0.14	0.25 ^a

All models adjusted for age, sex, and BMI. Standardized β -coefficients are presented. HOMA2-IR, ISI, adiponectin, osteocalcin, and leptin were log₁₀ transformed for analysis.

^a $P < 0.001$.

^b $P < 0.01$.

^c $P < 0.05$.

Multiple linear regression models

Adiponectin, osteocalcin, and leptin were independently and significantly associated with HOMA2-IR

After adjusting for age, sex, and BMI, adiponectin was inversely associated with HOMA2-IR (model 1; $P < 0.001$). When osteocalcin was included in the model, both osteocalcin and leptin were independent, significant correlates of HOMA2-IR (model 2; $P < 0.001$ and $P < 0.01$, respectively). Osteocalcin has a small but significant contribution to the explained variance of HOMA2-IR. When leptin, adiponectin, and osteocalcin were all included in the age- and sex-adjusted model, they were all independently and significantly associated with HOMA2-IR (model 3). Of note, the addition of leptin substantially attenuated the association of BMI with HOMA2-IR, reducing the size of the standardized regression coefficient by more than 70%.

Leptin and adiponectin, but not osteocalcin, are independently associated with insulin secretion index

After adjusting for confounders (age, sex, and BMI), adiponectin significantly correlated with ISI (model 1; $P < 0.01$), but osteocalcin was not a significant correlate of ISI (models 2 and 3). Leptin was significantly and positively correlated with ISI (model 3; $P < 0.001$) and singly improved model fit by 79%. Again, the introduction of leptin in the model reduced the size of the standardized regression coefficient for BMI by more than 94%.

Structural equation model

Figure 2 illustrates our hypothesized relations of age, sex, BMI, leptin, osteocalcin, adiponectin, HOMA2-IR, and ISI. The model includes standardized coefficients for each relationship in the analysis; each coefficient represents the change in the variable at the *arrowhead* associated with each SD change in the variable at the *tail of the arrow*. The *arrows* represent the proposed direction of the path relationships, consistent with our *a priori* hypotheses. The results from our

hypothesized associations yielded the following: age was negatively associated with BMI but positively with leptin, adiponectin, and HOMA2-IR; sex (coded 0 for men and 1 for women; the coefficient reflects associations relative to men) was negatively associated with BMI and HOMA2-IR and positively with leptin, osteocalcin, and adiponectin; BMI was positively associated with leptin and HOMA2-IR and negatively with adiponectin; leptin was negatively associated with osteocalcin and positively with HOMA2-IR and ISI; osteocalcin was negatively associated with HOMA2-IR and positively with adiponectin; and finally, adiponectin was negatively associated with HOMA2-IR and ISI ($P < 0.05$ for all associations). As expected, HOMA2-IR and the ISI were significantly correlated. The covariance matrix that resulted from this model was compared with the covariance matrix of the actual data. The results showed $\chi^2 = 2.67$, $df = 2$, $P = 0.236$. The nonsignificant χ^2 test indicated that the proposed model adequately explained the underlying covariance matrix of the actual data. The cross-validation bootstrap after 10 trials of 1000 random samples revealed a χ^2 mean of 2.29, none of which was statistically different from the naïve SEqM χ^2 of 2.670 (P range = 0.291–0.335); the SEqM is therefore evidently reproducible in the general population and not a phenomenon of the study population. The SEqM is consistent with the results of the regression analysis but additionally takes into account the dependency between individual variables that are commonly overlooked by examination of each variable independently; ignoring interdependency may bias the findings.

Discussion

The associations of leptin and adiponectin to glucose and energy metabolism are well established and were confirmed by the results of this study (3, 13, 14). Recent evidence gathered in animal models suggests that osteocalcin, a hormone secreted during bone turnover, exerts

regulatory activity on glucose metabolism. Consistent with this hypothesis, originally proposed by Confavreux *et al.* (5), our study provides empirical evidence for interactions among osteocalcin, leptin, and adiponectin with markers of insulin resistance and insulin secretion in humans. We confirmed this evidence using two parallel statistical analyses based on multiple linear regression models and SEqM (5).

In our population, plasma levels of adiponectin, osteocalcin, and leptin were significantly higher in women, in agreement with other population studies (15–17). Our finding of a negative association of adiponectin with insulin resistance is also consistent with results from other human studies (18, 19). Because both adiponectin receptors AdipoR1 and AdipoR2 are expressed in human β -cells (20), we expected to find an independent correlation between adiponectin and insulin secretion, and our results support this hypothesis. Adiponectin has been shown to augment insulin secretion, at least in rodents (21, 22).

Our finding that leptin has a significant positive correlation with insulin resistance may be explained by concurrent increase in sc fat (which correlates with leptin levels) and intraabdominal fat (which correlates with insulin resistance in obesity) (23). The positive correlation of leptin with insulin secretion found in our study is in agreement with another human study (24). However, this finding is in contrast to rodent studies in which leptin has been shown to inhibit insulin secretion (25, 26), and we are not aware of any evidence in humans supporting those rodent studies.

A novel finding of this study is that osteocalcin was significantly and independently correlated with insulin resistance, whereas it had no evident direct effect on insulin secretion. Cross-sectional studies have generally shown that higher osteocalcin levels correlate with better glucose metabolism: osteocalcin has a negative association with BMI, fasting glucose and insulin, metabolic syndrome and insulin resistance and leptin and is positively correlated with adiponectin (6, 27–29). In a prospective study, higher baseline osteocalcin was associated with lower increase in fasting plasma glucose at 3-yr follow-up (28). Interestingly, our data did not confirm the association between osteocalcin and an index of insulin secretion originally reported by Fernández-Real *et al.* (7). This contrast could be due to the method used to measure insulin secretion; our index derived from OGTT, whereas Fernández-Real *et al.* used *iv* glucose tolerance test.

Using the multiple linear regression models, we demonstrate that the contributions of adiponectin, osteocalcin, and leptin to insulin resistance were higher when analyzed together than when each association between single hormones was analyzed separately. Adiponectin and os-

teocalcin both have a significant, negative association, whereas leptin has a strong, positive association with insulin resistance. Similarly, using a structural equation model, we demonstrated that the integrated physiology model proposed by Confavreux *et al.* (5) is plausible in humans: leptin, osteocalcin, and adiponectin each exhibit a direct effect on insulin resistance, but leptin also has an indirect effect on insulin resistance through osteocalcin and adiponectin. The negative association between leptin and osteocalcin demonstrated in the SEqM confirm recent data from the literature (8). Furthermore, osteocalcin has an indirect effect on insulin resistance through adiponectin. Results from these two models are, in large part, consistent with the paradigm proposed by Confavreux *et al.* (5): leptin regulates osteocalcin, which in turn modulates β -cell function and peripheral insulin sensitivity indirectly through adiponectin. Apart from the indirect effect of leptin through osteocalcin, leptin has a direct and independent effect in regulating insulin resistance and β -cell function (5). In addition, adiponectin has a direct negative effect in modulation of insulin resistance and β -cell function.

There are several limitations in this study. First, these analyses are cross-sectional and can show only association but not causality. Second, the BLSA population in this study has a median age of 69 yr; therefore, this physiological model should be confirmed in a younger population. Third, total osteocalcin levels were used in our analyses whereas uncarboxylated osteocalcin is the form of the hormone responsible for the metabolic effects in rodent studies (1, 2). However, in humans, uncarboxylated osteocalcin is not associated with insulin resistance, whereas carboxylated and total osteocalcin are inversely associated with insulin resistance in cross-sectional and longitudinal studies (30, 31). This may not be surprising because *ESP*, the gene that encodes osteotesticular protein tyrosine phosphatase, a receptor-like protein-tyrosine-phosphatase responsible for regulating the carboxylation of osteocalcin, is not present in humans (32). Evidence in humans supports that carboxylation of osteocalcin is catalyzed by a vitamin K-dependent carboxylase (33). Fourth, we measured total adiponectin levels in our study. Higher-molecular-weight forms of adiponectin have been suggested by others to be a better indicator of metabolic regulation (34, 35). Fifth, the indices of insulin resistance (HOMA2-IR) and insulin secretion (ISI) are good surrogates for euglycemic-hyperinsulinemic and hyperglycemic clamp procedures respectively (often referred to as the gold standard tests for insulin resistance and β -cell function) but do have limitations for which they may not be sensitive enough to represent the variable of interest. Finally, the SEqM fits the underlying data, but these

results do not reflect the only possible model or prove that the model is correct but indicate only its plausibility. Our SEqM results do, however, offer directional estimations that remain true to the underlying data.

Our findings further support the notion of a physiological pathway that weaves together bone, fat, and energy metabolisms in humans. The identification of osteocalcin, a bone-derived hormone with the potential of acting as an insulin sensitizer, is an exciting discovery. More research is needed to better understand how this novel integrated pathway interacts with other established pathways of glucose regulation and possible clinical applications.

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K.S.G., J.K.N., and R.G.S. contributed equally, researched data, and wrote the manuscript. R.R. contributed to the discussion and review/edited the manuscript. E.J.M. contributed to the discussion and reviewed/edited the manuscript. O.D.C. researched the data. L.F. contributed to the discussion and reviewed/edited the manuscript. J.M.E. contributed to the discussion, researched the data, and reviewed/edited the manuscript. C.W.C. contributed to the researched data and wrote the manuscript.

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