Carboxylation of Osteocalcin Affects Its Association With Metabolic Parameters in Healthy Children

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OBJECTIVE — Osteocalcin (OC), a bone-derived protein, was recently shown to regulate metabolic pathways in mice. Undercarboxylated OC (ucOC), but not carboxylated OC (cOC), increases adiponectin and insulin secretion. It is unclear if carboxylation of OC affects its association with metabolic parameters in humans.

RESEARCH DESIGN AND METHODS — The associations between ucOC, cOC, total and high-molecular-weight (HMW) adiponectin, and insulin secretion (homeostasis model assessment [HOMA]-β) were investigated in a population-based sample of healthy prepubertal children ($n = 103$; 49 boys and 54 girls).

RESULTS — Weight-dependent associations were observed between the different forms of OC and metabolic parameters. Higher cOC was related to lower HMW adiponectin (with a stronger association in leaner children; $P < 0.001$). Higher ucOC-to-cOC ratio was associated with higher HOMA-β ($P < 0.01$) in leaner children and associated with higher HMW adiponectin ($P < 0.001$) in heavier children.

CONCLUSIONS — In a weight-dependent manner, cOC and the proportion of ucOC are differentially related to HMW adiponectin and insulin secretion in healthy children.

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There is feedback between glucose and bone metabolism (1). Adiponectin, a protein secreted by the adipose tissue with insulin-sensitizing and anti-atherosclerotic properties (2), has emerged as an element in the regulation of bone mass (3). Recent studies have closed this feedback by revealing a direct regulation of metabolic pathways by the skeleton through osteocalcin (OC) production (4).

Osteocalcin, an osteoblast product, is the most abundant noncollagenous protein of bone matrix and a long-known parameter of bone formation (5). The protein is subjected to posttranslational carboxylation by a vitamin K–dependent carboxylase to yield carboxylated (cOC) and undercarboxylated (ucOC) molecules. cOC has higher affinity for hydroxyapatite and is thought to be the active form in the bone (5).

Recent studies have disclosed that ucOC, but not cOC, is capable of enhancing adiponectin and insulin secretion in mice (4,6); clinical studies have shown independent associations between circulating total OC and metabolic traits in adult populations (7–10). However, it is currently unclear which of the carboxylated forms of OC is associated with metabolism in humans.

We investigated the clinical associations between both serum ucOC and cOC, total and high-molecular-weight (HMW) adiponectin (because it is unknown if this fraction of the protein is related to serum OC), and insulin secretion (homeostasis model assessment [HOMA]-β) in a population-based sample of healthy children. Our primary hypothesis was that serum ucOC is the preferred molecular form associated with adiponectin and insulin secretion. As a secondary hypothesis, any given association between cOC and metabolic parameters is a reflection of the known regulation of bone mass by metabolism, given that *1*) cOC is the active form in the bone and *2*) cOC does not have metabolic effects in vitro or in vivo.

RESEARCH DESIGN AND METHODS

— Subjects were 103 school-age Caucasian children (49 boys and 54 girls; aged 6.6 ± 0.1 years; supplementary Table 1 in the online appendix, available at http://care.diabetesjournals.org/cgi/content/full/dc09-1837/DC1) consecutively recruited among children seen at the pediatric primary care clinics for well-child checkup visits in Alt Empordà, a region in northern Spain. Inclusion criteria included age between 5 and 9 years and absence of puberty. Exclusion criteria were evidence of acute or chronic illness. The protocol was approved by the regional Institutional Review Board. Informed written consent was obtained from the parents.

Weight and height were measured with a calibrated scale and a Harpenden stadiometer, respectively. Waist circumference was measured at the umbilical level. Blood pressure was measured with an electronic sphygmomanometer. Body composition was assessed by bioelectric impedance (Hydra Bioimpedance Analyzer 4200; Xitron Technologies, San Diego, CA).

Fasting serum glucose, lipids, and immunoreactive insulin were assayed as de-

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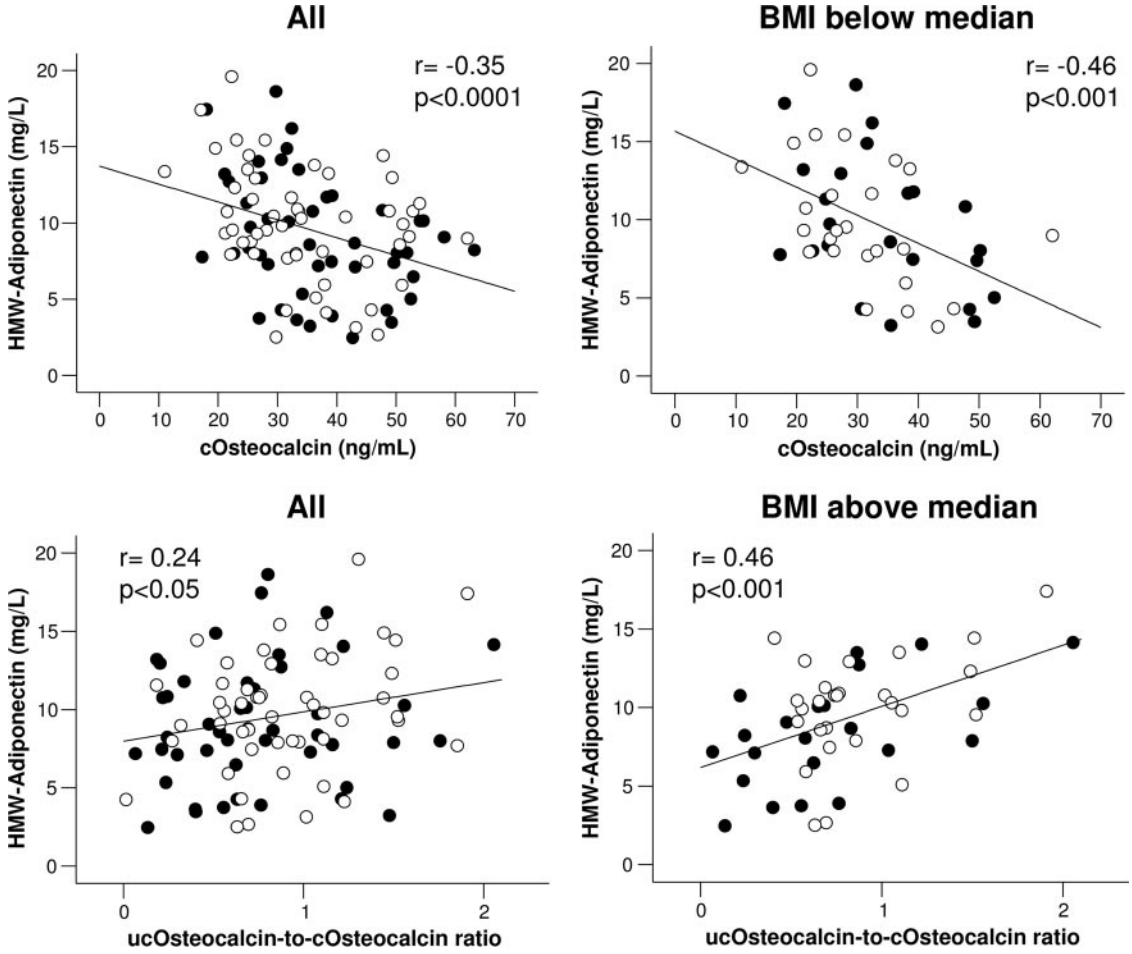

Figure 1—*Correlation graphs of both carboxylated osteocalcin (cOsteocalcin) and undercarboxylated-to-carboxylated osteocalcin (ucOsteocalcin-to-cOsteocalcin) ratio with HMW adiponectin in healthy children* (n = *103) and in subgroups according to a BMI cutoff (below or above the median).* ● *and* ○ *depict boys and girls, respectively.* r *and* P *values are from Pearson analyses.*

scribed (11). Insulin sensitivity and secretion were estimated by the homeostasis model assessment (HOMA–insulin resistance [IR] and HOMA-β [12]). Total and HMW adiponectin (the active fraction of the protein) were measured by sandwich enzyme-linked immunosorbent assays (Linco, St. Charles, MO) (10). Total OC was measured by an enzyme immunological test (Nordic Bioscience Diagnostics, Herlev, Denmark) with a sensitivity of 0.5 ng/ml, and ucOC was measured by a solid-phase enzyme immunoassay (EIA) kit (Glu-OC MK-118; Takara Bio, Otsu, Shiga, Japan) with a sensitivity of 0.25 ng/ml. Coefficients of variation at our laboratory were <6%. Serum cOC was calculated as the difference between total and ucOC.

Statistical analyses using SPSS version 12.0 (SPSS, Chicago, IL) consisted of simple correlation followed by stepwise multiple regression. ucOC-to-cOC ratio, rather than ucOC, was used to correct for the parallel inverse change in cOC. Significance level was set at $P < 0.05$.

RESULTS — Weight-dependent associations were observed between the different forms of OC and metabolic parameters. Higher cOC was related to lower HMW adiponectin (with a stronger association in leaner children; $P < 0.001$; Fig. 1). Higher ucOC-to-cOC ratio in leaner children was associated with higher HOMA-β ($P < 0.01$) and in heavier children associated with higher HMW adiponectin ($P < 0.001$; Fig. 1). These associations were either decreased or absent for total adiponectin.

In multiple regression analyses, both HMW adiponectin (β = −1.04 to −1.32; R^2 = 0.11–0.20) and BMI (β = 3.06, R^2 = 0.07) were independently related to cOC. In similar analyses, ucOC-to-cOC ratio (β = 1.58–3.76; R^2 = 0.04–0.20) was independently related to HMW adiponectin. Nonpredictive variables were sex, fat mass, and HOMA-IR.

Finally, ucOC-to-cOC ratio was independently related to HOMA-β (β = 0.17, R^2 = 0.08). Nonpredictive variables were sex, BMI, and fat mass. This association, however, was apparent in leaner but not in heavier children.

CONCLUSIONS — Our study defines the clinical associations between the different carboxylated forms of OC and metabolic parameters in healthy children.

Recent clinical reports have demonstrated significant associations between circulating total OC and adiponectin in adults (7–10,13). Data regarding the relation to insulin secretion are scarcer (8). Despite the fact that most of these studies did not discern between ucOC and cOC, the associations were assumed as being consistent with the purported role of ucOC regulating adiponectin and insulin secretion (4). Our results support these

findings and those from experimental research (4,6) pointing, for the first time, to our knowledge, to an increase in the relative concentration of ucOC as being associated with both increased HMW adiponectin and insulin secretion in humans.

Our findings also indicate that cOC (the active form in the bone) is related to metabolic parameters in humans. The independent associations between cOC and both HMW adiponectin and BMI fit well with the known regulation of bone mass by metabolic parameters (14), particularly with the known inverse association between adiponectin and bone mass (1). These observations, together with the fact that adiponectin receptors are expressed in osteoblasts (15), support a possible role of HMW adiponectin in the regulation of OC expression and/or carboxylation, thereby opening the perspective for an adiponectin-osteocalcin loop in humans.

Our study finally suggests different priorities in the reciprocal regulation of glucose and bone metabolism depending on the weight status. The abundance of HMW adiponectin in leaner subjects may contribute to the relative osteopenia commonly observed in these subjects. An increase in the relative proportion in ucOC may contribute to improved insulin secretion in leaner subjects and compensate for the decrease in HMW adiponectin in heavier subjects.

In conclusion, in a weight-dependent manner, carboxylation of OC affects its association with metabolic parameters in healthy children.

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