Letter to the Editor, concerning: "FGF23-regulated production of Fetuin-A (AHSG) in osteocytes"

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Dear Editor -

As amongst the leading laboratories on fetuin biology (1), we take great interest in novel developments concerning fetuin-A. Fetuin-A expression, regulation and role are issues that we have studied for several decades now (2). Thus, with great interest, we read carefully the article by Mattinzoli et al (3), which describes the expression of fetuin-A (AHSG) by osteocytes and the regulation of AHSG gene expression by FGF23 in osteocytes. In examining the data, we feel that the conclusions drawn by the authors are not sufficiently supported by the experimental findings. Listed specifically below are our comments concerning the experimental data that in our opinion does not support their proposal that AHSG is produced in bone primarily by osteocytes, and that this production is modulated by FGF23.

1) Immunohistochemistry. In Figures 1A, 1B, 1C, 2A, 2B and 2C, the authors point to a weak immunostaining for fetuin-A and FGF23 in osteoblasts, immediately adjacent to intensely stained bone marrow cells (with the latter not discussed). Surprisingly, Fetuin-A staining is absent in the mineralized bone matrix compartment, an absence which contradicts the fact that fetuin-A is well known to be one of the most abundant noncollagenous proteins found in the bone matrix (4-6), accumulating there after arriving there via the circulation after production by hepatocytes in the liver. An alternative explanation of the contradictory staining patterns observed in the present work is that the immunolabeling is not specific for fetuin-A, thus rendering the results and interpretation questionable. Furthermore, the authors present no positive and negative controls for their antibody staining.

2) Fetuin-A expression studies. No attempt was made to compare the supposed osteocyte fetuin-A immunolocalization in bone against the well-established strong hepatic expression of fetuin-A. Without this, the reader cannot judge whether the expression is substantial or spurious, possibly arising from false-positive amplification by the PCR method. Publications using established quantitative analyses like radioactive Northern blots and *in situ* hybridization report liver as the only large organ that expresses substantial amounts of fetuin-A mRNA (2, 8, 9). Like the authors have presented, we too have detected fetuin-A amplicons upon PCR analysis in extrahepatic tissues and cell lines; however, this occurred only after at least 7-8 additional cycles of amplification as compared to liver expression. Since this translates to 100-200-fold less expression, we like others generally regard these tissues as being fetuin-A negative. This is further supported by our detection of false-positive fetuin-A expression in various tissues (including kidney and liver) of fetuin-A knockout mice upon use of Affymetrix DNA arrays, despite the proven fact that these mice lack the fetuin-A AHSG gene. Current amplification techniques have become so sensitive that false-positive reports of expression are becoming pervasive. For this, appropriate controls are necessary, and these are lacking.
3) The mouse fetuin-A primers used for this study,
forward: CACCGAACTTACCACGACCT and