CORTICAL BONE STRUCTURE AND MECHANICAL PROPERTIES IN CART-DEFICIENT MICE

INTRODUCTION
CART (cocaine-amphetamine related transcript) is a neuropeptide expressed at high levels in the hypothalamus. CART expression in the hypothalamus is upregulated by the hormone leptin, a cytokine-like hormone produced by fat cells. It has been shown that mice lacking leptin have high bone mass in the spine, suggesting that leptin may play a role in regulating bone metabolism. Recently it was found that CART-deficient mice have low bone mass in their spine, suggesting that the effects of leptin on bone mass in the axial skeleton may be mediated at least in part by CART signaling, operating through a central, neuroendocrine pathway. It has been proposed that while leptin may increase bone resorption by stimulating RANK-ligand expression in osteoclasts, leptin can also inhibit osteoclast activity by increasing CART expression, which decreases RANK ligand expression by osteoclasts.

The effects of CART deficiency on bone mass have so far only been examined in trabecular bone of the vertebrae. As noted above, CART deficiency results in low trabecular bone mass, but cortical bone may not respond to the loss of CART signaling in the same manner as trabecular bone. For example, although mice lacking leptin show high bone mass in the spine, cortical bone of the spine and limb is decreased in leptin-deficient ob/ob mice compared to normal mice. We tested the hypothesis that CART deficiency decreases cortical bone mass, density, and strength by examining femora of adult CART-deficient mice. It was predicted that bone mineral content, density, femoral fracture strength, and stiffness would be lower in CART-deficient mice than normal mice, due to a significant increase in bone resorption.

METHODS
Male mice, 6 months of age, were used for this study. The sample size includes 18 CART-deficient (CART−/−) mice on a C57BL6 background and 18 wild-type (CART+/-) C57BL6 mice. Mice were euthanized by CO2 overdose, weighed, and the left hindlimb dissected free and stored at −20°C for mechanical testing. The right hindlimb was fixed for 24 hours in neutral buffered formalin and then stored in 70% ETOH prior to histological sections stained for osteoclast activity show few osteoclasts along the endosteal surface of the proximal tibia in wild-type mice and in mice lacking CART, and no significant differences in osteoclasts per surface (N.Oc/B.Pm) between groups (P=.94).

RESULTS
Results indicate that CART-deficient mice do not differ significantly from normal controls in whole-femur BMC (P=.09), BMD (P=.19), midshaft cortical bone thickness (P=.67), or midshaft cortical bone area (P=.59; Fig. 1). Mechanical testing data show no differences (P>.05) in ultimate force (Fig. 2), energy to fracture, stiffness, or intrinsic properties such as ultimate stress, ultimate strain, or modulus. Histological sections stained for osteoclast activity show few osteoclasts along the endosteal surface of the proximal tibia in wild-type mice and in mice lacking CART, and no significant differences in osteoclasts per surface (N.Oc/B.Pm) between groups (P=.94).

DISCUSSION
Mice lacking leptin are known to show high bone mass in the spine but low bone mass in the femur. Our data suggest that the central, neuroendocrine regulation of bone mass via CART signaling may be restricted primarily to trabecular bone in the spine. Mice lacking CART show normal cortical bone thickness and osteoclast number in the hindlimb, whereas mice lacking leptin show decreased cortical bone area and thickness in the femur. These data suggest that peripheral circulating leptin may directly stimulate endosteal and periosteal bone formation in the limb, whereas central leptin signaling and hypothalamic CART expression may play a more significant role in regulating bone resorption and osteoclast activity in the axial skeleton.

REFERENCES

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52nd Annual Meeting of the Orthopaedic Research Society
Paper No: 1662