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# Response to Comment on "Obestatin, a Peptide Encoded by the Ghrelin Gene, Opposes Ghrelin's Effects on Food Intake"

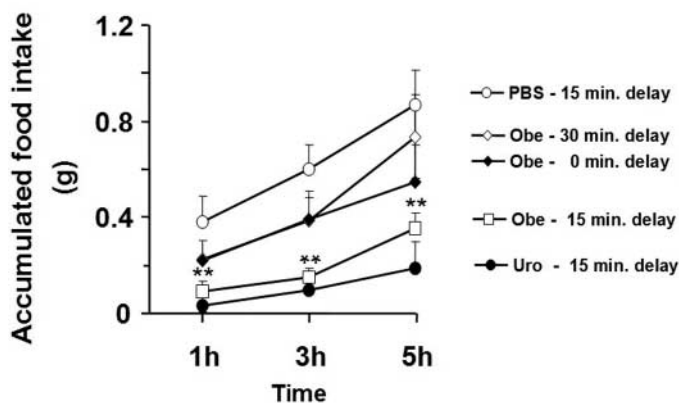
Jian V. Zhang,<sup>1</sup> Cynthia Klein,<sup>1</sup> Pei-Gen Ren,<sup>1</sup> Stefan Kass,<sup>2</sup> Luc Ver Donck,<sup>2</sup> Dieder Moechars,<sup>2</sup> Aaron J. W. Hsueh<sup>1\*</sup>

We cannot reproduce our original findings on obestatin binding and activation of GPR39 receptors in vitro. However, we can reproduce our original findings on the in vivo effects of obestatin in mice (decreases in food intake, gastric emptying responses, and body weight gain) under precise experimental conditions. Further studies are needed to reveal the exact relation between obestatin and the G protein-coupled receptor GPR39.

Based on a bioinformatic prediction of a hormone flanking the mature ghrelin peptide, we purified obestatin from the

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**Fig. 1.** Obestatin suppression of food intake in mice: effects of delay in food replacement. Adult C57BL6 male mice were housed individually. Before intraperitoneal treatment with obestatin (Obe, 750 nmol/kg body weight) or urocortin (Uro, 50 nmol/kg body weight; Phoenix Pharmaceuticals, Inc., Belmont, California), mice were deprived of food for 16 hours with free access to water. Food pellets were made available to animals at 0, 15, or 30 min after hormone injection. Food intake was measured by placing preweighed pellets in the cage and weighing uneaten pellets at 1, 3, and 5 hours after food replacement.  $n = 8$  mice per group.

rat stomach and custom-synthesized the peptide to demonstrate its biological activities in vivo and in vitro (1). Following the publication of these findings, our collaborators indicated that the initial batch of obestatin (GL Biochem, Ltd., Shanghai, China) contained impurities. We subsequently used purified obestatin from other sources (Penta Biotech, Fremont, California, and Global Peptide, Ft. Collins, Colorado) (2) and could not repeat the binding of iodinated obestatin

to tissue homogenates or recombinant GPR39 receptors. In addition, purified obestatin does not stimulate cAMP (cyclic adenosine monophosphate) and SRE (serum-responsive element) responses by cells expressing recombinant GPR39. These findings are consistent with the findings of Chartrel *et al.* (3) and two recent reports (4, 5). Because up to four iodine molecules can be incorporated into the obestatin peptide during iodination (6), we hypothesize that purified obestatin lost bioactivity after iodination, whereas our initial contaminated peptide retained some bioactivity.

We note that the phenotypes of GPR39 mutant mice are at least partially consistent with the role of GPR39 as the obestatin receptor (7).

We repeated our original in vivo findings (decreases in food intake, gastric emptying responses, and body weight gain) using purified obestatin from different sources (Penta Biotech or Global Peptide). As shown in Fig. 1, an exact time course (15 min) of food replacement delay after obestatin administration is essential to demonstrate the suppressive effect of this hormone with a short circulating half-life (8, 9). The apparent short half-life of obestatin could contribute to the observed suppression of food intake by some laboratories (9–11) but not others (12–15). Since publication of our report (1), obestatin has been shown to suppress drinking responses (16), improve memory (11), decrease growth hormone secretion in vivo (9), regulate sleep (17), activate cortical neurons (18), and stimulate proliferation of retinal pigment epithelial cells (19). Further studies are needed to reveal the exact role of obestatin as a metabolic and brain/gut hormone.

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