could be intraindividual variations and the moderate reproducibility of PEP.^{9, 10}

Therefore, besides the use of a placebo group, a valid and reproducible test is needed for follow-up evaluation of RP trials.

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Unlike ghrelin, obestatin does not exert any relevant activity in chondrocytes

Rocio Lago, Rodolfo Gomez, Carlos Dieguez, Juan J Gomez-Reino, Francisca Lago, Oreste Gualillo

Obestatin is a 23 amino acid amidated peptide identified as the product of posttranslational cleavage of the preproghrelin,¹ the polypeptide precursor of ghrelin. Obestatin has been reported to have actions opposite to ghrelin,² but its ghrelin antagonist properties are still unclear and controversial. As our group has identified ghrelin as a peptide synthesised and secreted by chondrocytes, with significant biological activity on cartilage cell metabolism,³ it was conceivable that obestatin might exert some physiological action on chondrocytes. These cells are emerging as a local producer and target of several endocrine factors including ghrelin³ and leptin.^{4, 5} It is of note that the obestatin co-joined hormone ghrelin has been revealed as a convergent factor between bone metabolism and energy homeostases.^{6, 7} So, it was plausible to verify the presence of obestatin in chondrocytes and to study the potential physiological effects of this recently discovered peptide.

According to our results, specific enzyme immunoassay (EK03190; Phoenix Pharmaceuticals Inc., Belmont, California, USA, linear range 0.23–2.37 ng/ml) is able to detect immunoreactive obestatin in the supernatants of cultured murine ATDC5 chondrocytes (0.543 ± 0.032 ng/ml), suggesting that chondrocytes have been equipped with the biochemical machinery in charge of the posttranslational process of the preproghrelin peptide by generating both ghrelin and obestatin. In contrast to ghrelin,³ obestatin binds weakly to chondrocytes, and quantitative analysis of obestatin binding sites indicates the presence of less than 1000 binding sites per cell. Next, we assessed the effect of obestatin on chondrocyte metabolic activity by means of MTT colorimetric assay. These experiments have been performed in undifferentiated ATDC5 murine cells, but were further confirmed in differentiated mature ATDC5 cells,⁸ as well as in the immortalized human chondrocyte cell line, C28-I2 (a

kind gift of Dr Mary B. Goldring, Hospital for Special Surgery, New York, USA).⁹ Obestatin-stimulated chondrocytes showed a significant decrease in their metabolic activity (fig 1). Intriguingly, this effect was similar, and not opposite, to that exerted by ghrelin,³ suggesting that obestatin is able to modulate the mitochondrial respiratory chain or oxidative burst as ghrelin did, excluding in this way any kind of antagonism between ghrelin and obestatin.

To exclude any potential cytotoxic effect of obestatin, we analysed the chondrocyte cell cycle by propidium iodide staining flow cytometry and determined lactate dehydrogenase levels in supernatants of human cultured chondrocytes stimulated or not with obestatin. No difference between controls and obestatin-treated cells exists, suggesting that obestatin, per se, exerts no cytotoxic effect.

Obestatin, unlike ghrelin, was unable to modulate significantly either fatty acid or glucose uptake, both macronutrients that serve as precursors for eicosanoids and glycoaminoglycans. Moreover, obestatin had no effects on the quantitative expression of matrix metalloproteases 3 and 9, as well as on enzymes involved in cellular stress such as lactate dehydrogenase, alkaline phosphatase and nitric oxide synthase type 2.

In conclusion, our study suggests that obestatin, unlike ghrelin, plays a marginal role in the regulation of chondrocyte metabolism. We cannot exclude the possibility that the unique indicative response of obestatin as a downmodulator of the chondrocyte respiratory rate might be linked to the induction, or repression, of genes whose roles in chondrocyte physiology have yet to be defined. The identification of factors involved in obestatin response may thus provide the basis for future studies on the molecular effects of this novel member obtained by posttranslational processing from the same ancestor peptide that generates ghrelin.

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Obestatin does not modulate:

- Cell cycle
- LDH levels
- Alkaline phosphatase
- Fatty acid uptake
- Glucose uptake
- Matrix metalloproteases 3 and 9
- Nitric Oxidase synthase type2

Figure 1 Obestatin inhibits chondrocyte metabolic activity, evaluated by the MTT colorimetric method, in a dose-dependent manner. *** $p < 0.001$. As a positive control, metabolic activity was evaluated in the presence of 5% (vol/vol) fetal bovine serum (FBS). LDH, Lactate dehydrogenase.

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Endothelial nitric oxide synthase +894 polymorphism is associated with recurrent salivary gland swelling and early onset in patients with primary Sjögren's syndrome

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Primary Sjögren's syndrome (SS) is a chronic autoimmune disease that affects primarily the salivary and lacrimal glands. Nitric oxide (NO) has been implicated in the pathogenesis of SS and raised nitrite concentrations have been observed in the saliva of patients with SS.¹ NO is a molecule that plays a key role in many physiological and pathological processes. It is produced *in vivo* from the amino acid L-arginine by a family of nitric oxide synthases (NOS), of which three isoforms have been identified, i.e. neuronal, endothelial and inducible.² Endothelial nitric oxide synthase (eNOS) is a constitutively expressed isoform of NOS. The eNOS gene entails

several polymorphisms, of which for example the +894 T>G polymorphism is of functional significance, the T allele having been associated with high NO production.³

We sought to establish whether the eNOS +894 polymorphism is associated with susceptibility to primary SS or to the salivary manifestations of the disease, and after informed consent analysed the genetic polymorphism of eNOS (+894T>G, rs1799983) in 64 (62 female, two male) patients

Abbreviations: eNOS, Endothelial nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; SS, Sjögren's syndrome