

Resistin overexpression is induced by a β_3 adrenergic agonist in diet-related overweightness

Resistin, a recently discovered 12.5 kDa cysteine rich adipose tissue specific secretory factor, has been suggested as a new hormone involved in insulin resistance, which may link obesity with type-2 diabetes (10). This protein released by brown and white adipose tissues appears to participate in the body's ability to respond to insulin together with other molecules secreted by the adipocyte such as free fatty acids, leptin or tumor necrosis factor (1, 4). Circulating resistin levels are increased in diet-induced and genetic models of obesity, while the administration of recombinant resistin to normal mice impairs glucose tolerance and insulin functions (10). However, some of these results have been challenged by a later report, in which it has been shown that resistin expression in fat cell is suppressed in situations of obesity (12). The aim of the current investigation was to evaluate adipocyte mRNA levels in high-fat fed animals (cafeteria diet) and the influence of the treatment with the β_3 -adrenergic agonist tertritolol in overweight animals. In this context, twenty six female rats weighing between 150-175 g were assigned to three groups, which received the experimental diets and intraperitoneal placebo or tertritolol administration for 30 days: control diet and placebo (n=10), cafeteria fed and placebo (n=8) and cafeteria fed animals treated with the β_3 adrenergic agonist (n=8). All procedures were performed according to national and institutional guidelines for animal care and use at the University of Navarra. The extraction of total RNA and semiquantitation by reverse-transcription polymerase chain reaction (RT-PCR) were carried out as previously described (3, 7). The primer to amplify resistin DNA was: Upper

5' CAGAAGGCACAACCGTCA-3 and Lower 5' TCAGGAACCAACCGCAG-3 (Annealing, 58 °C; elongation, 72 °C and cycles, 32 °C), which was designed using the nucleotide sequence reported by KIM *et al* (6) and the oligo 4.05 Primer Analysis software (Phymouth MN). To ensure the linearity of PCR reactions and to validate the cDNA quantification, adequate controls and standard curves were performed as reported elsewhere (3). Levels of messenger RNA were measured as the ratio of signal intensity relative to β -actin by densitometric analysis with adequate imaging software (Bio. Red Hercules, CA). Data were analysed using an ANOVA design followed by an appropriate *a posteriori* test (Dunnett T Test: one tail).

High fat feeding significantly ($p < 0.01$) increased body weight in those animals receiving the cafeteria diet and placebo (308.0 ± 7.3 g) as compared to controls (266.3 ± 4.4 g), while the tertritolol treatment to high-fat fed rats significantly ($p < 0.05$) prevented the excessive weight gain induced by the cafeteria regime (284.1 ± 7.6 g). Also, fat depots (perirenal, periovaric and abdominal regions) were influenced ($p < 0.01$) by the dietary and pharmacological conditions (control diet: 7.0 ± 0.7 g; cafeteria diet: 20.8 ± 2.2 g, and cafeteria diet with β_3 adrenergic treatment: 12.6 ± 2.0 g).

Interestingly (Fig. 1), the resistin mRNA levels (arbitrary units) in adipose tissue, which remained unaffected in control and overweight animals receiving the cafeteria diet, were doubled by the β_3 agonist administration.

Resistin and RELMs (resistin-like molecules) constitute a new family of tissue-specific signaling molecules (11) with a

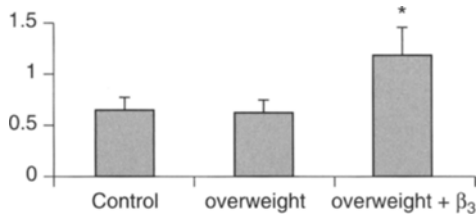


Fig. 1. Resistin mRNA expression levels (arbitrary units) in white adipose tissue as affected by the diet and β_3 adrenergic administration. * $p < 0.05$ vs. control.

restricted distribution in rodent and human tissues. Resistin mRNA levels were markedly increased during 3T3-L1 and primary cell differentiation into adipocytes, whose expression appears to be under a tight nutritional and hormonal regulation; however, intriguing outcomes have been observed in obese animals, where some authors (10) have found an increase in circulatory levels of resistin, while others reported a severe suppression of the adipose tissue resistin expression (12). Our data revealed no changes in resistin mRNA levels in cafeteria fed animals, which may be explained by the fact that gene expression trends and some gene products secretion may follow different patterns (2), but also by the dietary and experimental conditions (timing, diet composition, strain resistance to obesity, etc.). In an earlier study, we found a dual time-dependent effect of a high energy yielding diet on the expression of some adipogenesis related genes such a PPAR γ and aP2 (7).

Compounds with affinity for β_3 adrenoceptors have shown antidiabetic and antiobesity properties (8). In this context, it is reported apparently for the first time, that β_3 adrenoceptor stimulation in diet-induced obese animals produces an

increase in resistin gene expression. This finding is in agreement with data concerning other antidiabetic compounds such as PPAR γ agonists, which increase resistin expression in WAT. Also, a recent report using BRL-35135 reveals that RELM α mRNA was increased in db/db mice (9).

Further studies are needed to determine the biological functions, the mode of action and regulation of resistin (1, 4, 5) as well its possible role as effector of insulin resistance in obesity; however, this factor appears to be involved in adipose metabolism control with potential implications in the energy balance.

Key words: Resistin, Obesity, Adrenoceptor, Cafeteria diet.

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Martínez, J. A., Margareto, J., Marti, A., Milagro, F. I., Larrarte, E. and Moreno Aliaga, M. J.

Dpt. Physiology and Nutrition, University of Navarra. 31008 Pamplona. Spain.

Correspondence to: J. A. Martínez (e-mail: jalfmtz@unav.es).