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Human resistin promotes macrophage lipid accumulation

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To the Editor:

The gene encoding resistin/FIZZ3 (found in inflammatory zone 3), which is denoted by the gene symbol *RETN* in humans and *Retn* in mice, belongs to a new gene family of small cysteine-rich secretory proteins, called ‘resistin-like molecules’, originally discovered in a search for adipocyte-derived molecules linking obesity and insulin-resistant diabetes [1]. In rodents, resistin is derived largely from adipose tissue. Hyperresistinaemia impairs glucose tolerance and induces insulin resistance in mice; mice deficient in resistin (*Retn* [−/−]) [2], or expressing a dominant negative form of this molecule [3], are protected from obesity-associated insulin resistance.

By contrast, in humans, expression of *RETN*, which shows significant sequence and tissue divergence from its murine counterpart, is highly localised to bone marrow cells, upregulates during monocyte–macrophage differentiation [4], and triggers inflammatory and proliferative responses in vascular cells [5]. Plasma levels of resistin correlate with markers of inflammation, and are predictive of coronary atherosclerosis in humans [6]. Human carotid endarterectomy samples stain positively for resistin protein, and patients with premature coronary artery disease have higher serum levels of resistin than normal controls [7].

This letter is the first report of a potentially pro-atherogenic metabolic role for human resistin/FIZZ3, demonstrating that human recombinant resistin promotes glucose-dependent lipogenesis and lipid accumulation in differentiated human macrophages derived from the leukaemic (THP-1) monocytic cell line. The effects of resistin on macrophage triglyceride accumulation are

enhanced by high concentrations of glucose and blocked by activators of 5'-AMP-activated protein kinase (the atheroprotective adipokine adiponectin [gAcrp30], and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside [AICAR], which is taken up by cells and metabolised to form the 5'-AMP analogue ZMP).

Monocytes (THP-1) (2×10^6) were differentiated into macrophages by the addition of 250 nmol/l phorbol 12-myristate 13-acetate for 5 days, and maintained in RPMI 1640 medium containing Ultraglutamine (Cambrex Bioscience, Wokingham, UK), supplemented with foetal bovine serum (10%, v/v), HEPES (10 mmol/l), sodium bicarbonate (0.075%, w/v) and penicillin/streptomycin (1,000 U/ml).

Exogenous human recombinant resistin (Biogenesis, Poole, UK) (0–200 ng protein/ml; 0–10 nmol/l of dimeric protein) was added (24 h) in serum-free RPMI 1640, to THP-1 macrophages. This ‘adipo’ cytokine is certified as containing <0.1 ng endotoxin per microgram recombinant human resistin. Addition of polymyxin B at concentrations 100-fold higher than the concentration of endotoxin contaminant did not block the described effects of resistin (below). Where indicated, RPMI 1640 medium was supplemented with glucose to a final concentration of 30 mmol/l. In selected experiments, RPMI 1640 medium, formulated without glucose, was supplemented with glucose to a final concentration of 1 mmol/l. gAcrp30, a 16.6 kDa (145 amino acid) recombinant protein corresponding to the globular fraction of adiponectin (BioCat, Heidelberg, Germany), and AICAR (dissolved in DMSO vehicle), were added to culture medium at the concentrations indicated.

Oligonucleotide primers (MWG Biotech, Ebersberg, Germany) were designed against the open reading frames of *RETN* (forward 5'-ATGAAAGCTCTCTGTCTCCTCC-3' and reverse 5'-TCAGGGCTGCACACGACAGC-3') and *GAPDH*, a ‘housekeeping’ enzyme (forward 5'-CCCTT CATTGACCTCAACTACATG-3' and reverse 5'-AGT CTTCTGGGTGGCAGTGATGG-3'). RT-PCR reactions using ReadyToGo RT-PCR beads (GE Healthcare, Bucks, UK), 100 pmol of each primer and 2 µg RNA template

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were performed. Reverse transcription occurred at 45°C for 30 min, followed by 3 min at 94°C and 40 cycles at 94°C (40 s), 62°C (40 s) and 72°C (90 s) for *RETN*; for *GAPDH*, the PCR conditions were 94°C for 5 min, followed by 40 cycles at 94°C (1 min), 57°C (1 min) and 72°C (1 min). PCR products were separated by agarose gel (1%) electrophoresis, and compared with a 1 kb hyperladder. Levels of resistin protein in tissue culture medium were determined by commercial ELISA (BioVendor Laboratory Medicine, Brno, Czech Republic). Macrophage lipids were extracted using hexane:isopropanol (3:2, v/v), dried under N₂, and resuspended in isopropanol. Total triglyceride and cholesterol mass were measured using Infinity reagents (from Alpha Laboratories, Eastleigh, UK); cellular protein levels were determined, after solubilisation in 0.5 mol/l

NaOH, using the bicinchoninic acid assay (Pierce; supplied by Perbio Science, Cramlington UK). Results are expressed as microgram lipid per milligram cell protein. Significant ($p < 0.05$) differences were determined using repeated-measures ANOVA and a Tukey–Kramer multiple-comparison post *t*-test.

Differentiated THP-1 macrophages do not appear to express *RETN* mRNA, or secrete resistin into the culture medium, under any of the conditions used in these experiments. Resistin PCR product is amplified from RNA extracted from differentiated human U937 macrophages, but not THP-1 macrophages; by contrast, the *GAPDH* PCR product is amplified in the same sample of RNA from both cell types (Fig. 1a). Further, incubation of THP-1 macrophages in RPMI 1640 medium containing

Fig. 1 **a** RT-PCR products amplified from RNA isolated from human THP-1 or U937 macrophages, using primers designed against *RETN* and the ‘housekeeping’ gene *GAPDH*. –ve the template negative control. The predicted products for *RETN* (326 bp) and *GAPDH* (455 bp) shown were generated in three separate RT-PCR reactions from a typical experiment, and confirmed in a separate independent experiment. **b** Human (THP-1) macrophages were incubated in the presence of human recombinant resistin (0–200 ng/ml) and normal (10 mmol/l; open squares, open circles) or elevated (30 mmol/l; filled squares, filled circles) medium glucose concentrations for 24 h, before extracting cellular lipids and measuring total cellular cholesterol (open squares, filled squares) and triglyceride (open circles, filled circles) mass. Values represent means±SEM of three independent experiments; ** $p < 0.01$ and * $p < 0.05$, values

differing significantly from the control incubation (0 ng resistin/ml and 10 mmol/l glucose); † values differing significantly ($p < 0.05$) from the relevant concentration of resistin at 10 mmol/l glucose. **c**, **d** Human (THP-1) macrophages incubated in the absence (grey columns) or presence (black columns; 50 ng/ml) of human recombinant resistin and the indicated concentrations of adiponectin (gAcrp30) (**c**) or AICAR (**d**) for 24 h, before extracting cellular lipids and measuring total cellular triglyceride content. Values represent the means±SEM of three (**c**) and four (**d**) independent experiments; *** $p < 0.001$ and ** $p < 0.01$, values differing significantly from the control incubation in the absence of resistin; † values that differ significantly ($p < 0.05$) from the test incubation containing resistin (50 ng/ml)

normal (10 mmol/l) or high (30 mmol/l) concentrations of glucose, or in medium supplemented with endotoxin (1 µg/ml), does not result in secretion of resistin protein into the culture medium (<0.1 ng/ml, the lower detection limit of the ELISA used).

Exogenous human recombinant resistin (5–100 ng/ml; 24 h) induces significant increases in macrophage triglyceride mass, maximally up to 25.2 and 49.4% ($p < 0.01$) at 20 ng resistin/ml (Fig. 1b), in the presence of 10 mmol/l or 30 mmol/l glucose, respectively. It is noteworthy that the high glucose concentration does not significantly induce triglyceride accumulation in the absence of resistin (Fig. 1b). Low concentrations of glucose (1 mmol/l) in the medium essentially negate resistin-induced increases in macrophage triglyceride mass (control vs resistin [50 ng/ml]: 83.8 ± 3.2 µg/mg cell protein vs 85.0 ± 4.3 µg/mg cell protein, means $\pm$ SEM [$n = 3$], NS). Resistin also increases macrophage total cholesterol mass, maximally by 23.1% ($p < 0.05$) at 20 ng/ml in the presence of 10 mmol/l glucose (Fig. 1b), but no further increase is seen at higher glucose concentrations (Fig. 1b).

The globular fragment of adiponectin (gAcrp30) and resistin exert mutually antagonistic effects on accumulation of macrophage triglyceride mass, with the highest concentration of gAcrp30 (500 ng/ml) fully antagonising the action of resistin (50 ng/ml) but itself inducing significant increases (28.6%) in triglyceride accumulation (Fig. 1c). The mechanism(s) underlying these effects is not established but is worthy of further investigation. Addition of the AMP kinase activator AICAR (1.0 mmol/l) also significantly antagonised the effects of resistin on triglyceride accumulation, but did not itself alter macrophage triglyceride mass (Fig. 1c).

In summary, we report that resistin, in the absence of native or modified lipoproteins, can induce increases in cholesterol and triglyceride cellular mass in human (THP-1) macrophages. Significantly, resistin causes glucose-dependent triglyceride accumulation by THP-1 macrophages, which does not occur in the absence of

this cytokine. gAcrp30 and AICAR, which are established activators of 5'-AMP activated protein kinase, inhibit triglyceride accumulation due to resistin. The significance of these findings *in vivo* remains to be proven, particularly since our report describes the effects of resistin only under serum-free tissue culture conditions. Nevertheless, our results suggest that resistin may contribute not only to arterial inflammation, but to macrophage accumulation of cholesterol and triglycerides [8] and the marked acceleration of atherogenesis and coronary heart disease in individuals with type 2 diabetes mellitus.

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References

1. Steppan CM, Bailey ST, Bhat S et al (2001) The hormone resistin links obesity to diabetes. *Nature* 409:307–312
2. Banerjee RR, Rangwala SM, Shapiro JS et al (2004) Regulation of fasted blood glucose by resistin. *Science* 303:1195–1198
3. Kim K-H, Zhao L, Moon Y et al (2004) Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity. *Proc Natl Acad Sci USA* 101:6780–6785
4. Patel L, Buckels AC, Kinghorn IJ et al (2003) Resistin is expressed in human macrophages and directly regulated by PPAR γ activators. *Biochem Biophys Res Commun* 300:472–476
5. Verma S, Li SH, Wang CH et al (2003) Resistin promotes endothelial cell activation: further evidence of adipokine–endothelial interaction. *Circulation* 108:736–740
6. Reilly MP, Lehrke M, Wolfe ML et al (2005) Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 111:932–939
7. Burnett MJ, Lee CW, Kinnaird TD et al (2005) The potential role of resistin in atherogenesis. *Atherosclerosis* 182:241–248
8. Fredrickson DS, Goldstein JL, Brown MS (1978) The familial hyperlipoproteinaemias. In: Stanbury JG, Wyngaarden MF, Fredrickson DS (eds) *The metabolic basis of inherited diseases*. McGraw-Hill, New York, pp 604–644