Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 46, No. 6, 2005
ISSN 0735-1097/05/$30.00

CORRESPONDENCE

Research Correspondence

Release of C-Reactive Protein in Response to Inflammatory Cytokines by Human Adipocytes: Linking Obesity to Vascular Inflammation

To the Editor: Obesity, the most common nutritional disorder in industrialized countries, is associated with increased cardiovascular mortality and morbidity (1). C-reactive protein (CRP), an acute-phase protein and an important predictor of future cardiovascular events in apparently healthy men and women (2), has been thought to be synthesized in the liver following stimulation by cytokines, such as interleukin (IL)-1-beta, IL-6, and tumor necrosis factor (TNF)-alpha (3). Recently, however, the extrahepatic synthesis of CRP was found to occur under similar proinflammatory conditions (4). There is also some evidence for the presence of the CRP message in human adipose tissue (5). In the present study, we investigated whether CRP is produced by cells in adipose tissue in response to inflammatory stimuli using an in vitro model and whether this phenomenon might be modulated using anti-inflammatory drugs.

Primary cultures of human adipocytes from adipose tissue were performed as previously described (6), and the isolated adipocytes were incubated under the conditions required for each particular experiment. C-reactive protein levels in the cell supernatants were measured using an enzyme-linked immunoadsorbent assay specific for human CRP. The minimum concentration detected by the assay was 1.6 ng/ml. All experiments were performed in duplicate. Adipocytes were cultured in tubes, and cells from different donors were used for each experiment. The cells were incubated with recombinant human IL-1-beta (25 ng/ml), IL-6 (10 ng/ml), resistin (100 ng/ml), adiponectin (1 μg/ml), or leptin (40 ng/ml). For the modulation experiments, at the same time of stimulation, cells were incubated with vehicle (dimethylsulfoxide), aspirin (5 μmol/l), troglitazone (10 μmol/l), or fluvastatin (5 μmol/l). Doses and timing were chosen on the basis of findings from previous experiments. After 48 h, the culture supernatants were concentrated and assayed for CRP levels. Data are presented as the mean value ± SD and were analyzed using one-way analysis of variance followed by the Scheffe test for multiple comparisons. Statistical significance was indicated at the level $p < 0.05$.

Figure 1A shows a representative experiment ($n = 4$) of CRP production by adipocytes following treatment with inflammatory cytokines. The incubation of adipocytes with IL-1-beta or IL-6 resulted in more than doubling of CRP production compared with the production in unstimulated cells ($p < 0.05$). Adipocytes treated with either adiponectin and leptin did not produce CRP compared with unstimulated cells. Finally, resistin induced an almost three-fold increase in CRP production ($p < 0.01$). In order to mimic more pathophysiological conditions, we used combinations of the active stimuli together ($n = 3$), and the results are shown in Figure 1B. Combination of IL-1-beta and IL-6 induced an almost three-fold increase in CRP production ($p < 0.01$). The addition of resistin led to an even larger increase in CRP production ($p < 0.01$). Figure 2 shows the effect of fluvastatin, troglitazone, and aspirin on the production of CRP. Treatment with fluvastatin or troglitazone led to a significant, but not complete, inhibition of CRP release from adipocytes ($p < 0.05$). Finally, a larger, but still not complete, modulation of CRP release from adipocytes was observed after treatment with aspirin ($p < 0.05$).

In the present study, we showed for the first time the production of a major acute-phase protein, CRP, by adipocytes isolated from human adipose tissue in response to inflammatory cytokines, thereby suggesting a new link between obesity and vascular inflammation.

Adipose tissue secretes various bioactive substances, generally referred to as adipocytokines, including IL-6, TNF-alpha, leptin, adiponectin, and resistin, that may contribute to obesity-linked metabolic and vascular diseases (7). In addition, obese individuals have high circulating levels of a range of inflammatory markers produced by adipose tissue, including IL-1-beta and IL-6 (8), cytokines responsible for both hepatic and extrahepatic production of CRP (4). In several studies, high plasma levels of this acute-phase protein were strongly associated with obesity and obesity-related diseases (9). There is recent evidence of CRP expression in human adipose tissue as well. Ouchi et al. (5) showed CRP mRNA expression in human adipose. However, they made no attempt to investigate the stimuli able to induce CRP. Here, we found that human adipocytes cultured in vitro produced CRP after exposure for 48 h to inflammatory cytokines, such as IL-1-beta, IL-6, and

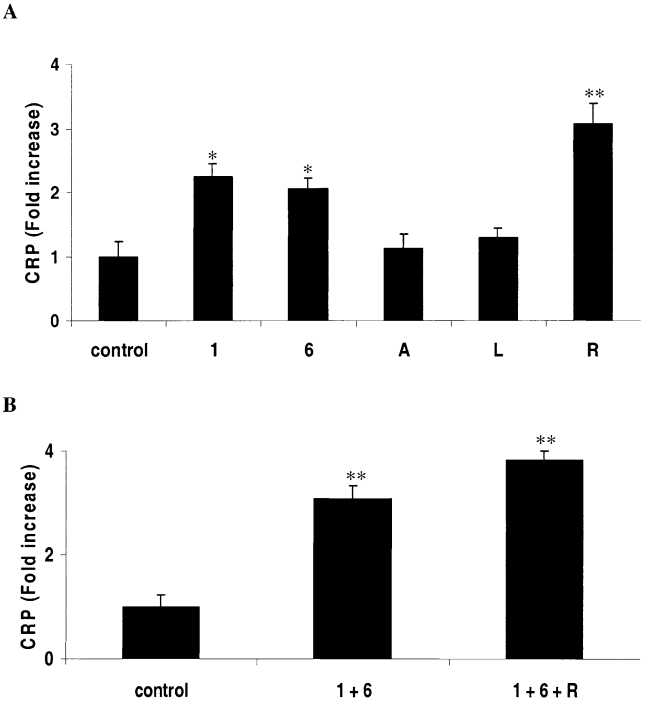

Figure 1. (A) Effect of interleukin (IL)-1-beta (1), IL-6 (6), adiponectin (A), leptin (L), or resistin (R) on C-reactive protein (CRP) production in human adipocytes. **(B)** Effect of combination of stimuli (1 + 6, 1 + 6 + R) on CRP production in human adipocytes. Values are expressed as the "-fold" increase in CRP levels compared with levels in untreated cells, and each **bar** represents the mean ± SD of duplicate determinations. *$p < 0.05$ vs. untreated cells; **$p < 0.01$ vs. untreated cells.

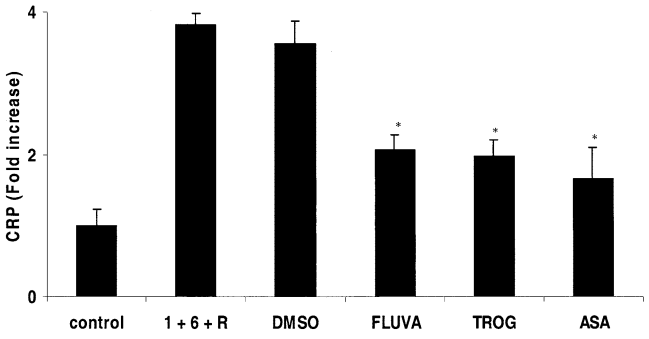

Figure 2. Modulation of C-reactive protein (CRP) synthesis in human adipocytes treated with dimethylsulfoxide (DMSO) (control), fluvastatin (FLUVA), troglitazone (TROG), or aspirin (ASA). Values are expressed as the "-fold" increase in the level compared with the level in untreated cells, and each **bar** represents the mean ± SD of duplicate determinations. *$p < 0.05$ vs. combination of cytokines. 1 + 6 + R = interleukin-1 + interleukin-6 + resistin.

resistin. Interestingly, treatment of adipocytes with two other adipocytokines, adiponectin and leptin, did not lead to CRP production. Furthermore, treatment with several anti-inflammatory drugs shown to be effective in reducing serum CRP levels, such as aspirin, troglitazone, and fluvastatin (10,11), leads to reduction, but not complete inhibition, of CRP release from adipocytes. This might explain in part the beneficial cardiovascular effects of these drugs.

In conclusion, our study demonstrates that human adipocytes can produce CRP under the stimulation of several proinflammatory cytokines; moreover, CRP production may be modulated by selected pharmacologic intervention. The mechanism(s) underlying these findings are not fully defined, and further studies are needed in this area.

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doi:10.1016/j.jacc.2005.06.017

REFERENCES

1. Grundy SM. Obesity, metabolic syndrome, and coronary atherosclerosis. Circulation 2002;105:2696–8.
2. Ridker PM, Rifai N, Rose L, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557–65.
3. Moshage HJ, Roelofs HM, van Pelt JF, et al. The effect of interleukin-1, interleukin-6 and its interrelationship on the synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes. Biochem Biophys Res Commun 1988;155:112–7.
4. Calabro P, Willerson JT, Yeh ET. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation 2003;108:1930–2.
5. Ouchi N, Kihara S, Funahashi T, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003;107:671–4.
6. Ding ST, Smith EO, McNeel RL, et al. Modulation of porcine adipocyte beta-adrenergic receptors by hormones and butyrate. J Anim Sci 2000;78:927–33.
7. Guerre-Millo M. Adipose tissue and adipokines: for better or worse. Diabet Metab 2004;30:13–9.
8. Yudkin JS, Kumari M, Humphries SE, et al. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000;148:209–14.
9. Visser M, Bouter LM, McQuillan GM, et al. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131–5.
10. Haffner SM, Greenberg AS, Weston WM, et al. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. Circulation 2002;106:679–84.
11. Ridker PM, Rifai N, Pfeffer MA, et al. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1999;100:230–5.

Letters to the Editor

Physiological Mechanisms of Atrially Induced Heart Rate Turbulence

We read with interest the study by Vikman et al. (1) and are pleased that this respectable group shares our interest in atrially induced heart rate turbulence. Although we understand the reasoning behind the suggestion that "vagal inhibition in response to premature atrial excitation is absent, or even that transient enhancement of vagal outflow occurs before atrial fibrillation," we would like to offer the investigators an alternative explanation for their findings.

We all would surely agree that less effective ventricular contraction and compensatory pause after premature beats is responsible for transient hemodynamic deficit, missed baroreflex afferent input, and early vagal inhibition. This mechanism is applicable for both ventricular and atrial premature complexes (APCs). Autonomic modulation of sinus nodal discharge after APCs may explain values of turbulence onset (TO) ≤ 0. When TO >0 is found, the underlying mechanism has to be different. The mechanism suggested by Vikman et al., namely the temporary direct suppression of sinus node automaticity, is certainly plausible. This phenomenon, which may mask (or overwhelm) the autonomic component of early acceleration, has been previously demonstrated (2).

Because of a missing relationship between TO and APC prematurity, Vikman et al. rejected the hypothesis that sinus resetting is the predominant factor influencing the temporal changes of TO. However, this lack of correlation seems to us compatible rather than incompatible with sinus nodal resetting. Autonomically mediated TO should be positively related to the APC coupling interval, whereas TO mediated by direct suppression of sinus node automaticity should be negatively related to the APC coupling interval. Thus, coexistence of both mechanisms may effectively offset the relationship between TO and APC prematurity.

Although TO and turbulence slope after ventricular premature complexes and turbulence slope after APCs reflect heart rate vagal modulation, this is not the case for TO after APCs (3). We have not found any significant relationship between atrial TO and other previously established surrogates of heart rate vagal modulation in large Holter databases (Wichterle et al., unpublished data, 2005).

We thus wonder whether Vikman et al. (1) would agree that their finding might be interpreted as either 1) temporal decrease of vagal modulation, or 2) temporal change of APC prematurity (which cannot be exactly assessed from Holter recording) and/or site of origin of APCs—all factors potentially facilitating sinus node resetting. We believe that the latter possibility might be more probable because none of the other investigated indices of autonomic modulation (including