Mod Rheumatol (2007) 17:385–390
DOI 10.1007/s10165-007-0605-8

ORIGINAL ARTICLE

Norio Komai · Yoshitaka Morita · Takeo Sakuta
Atsunori Kuwabara · Naoki Kashihara

Anti-tumor necrosis factor therapy increases serum adiponectin levels with the improvement of endothelial dysfunction in patients with rheumatoid arthritis

Received: February 16, 2007 / Accepted: May 30, 2007

Abstract Lower adiponectin levels in circulation are shown to be associated with endothelial dysfunction, which is a crucial feature in the evolution of atherosclerosis. The aim of our study is to evaluate the effect of anti-tumor necrosis factor (TNF) therapy on adiponectin levels with endothelial function and arterial stiffness. Fifteen Japanese patients with rheumatoid arthritis (RA) received infusions with infliximab (3 mg/kg) at weeks 0, 2, and 6. Serum concentrations of adiponectin, endothelial function, and pulse wave velocity (PWV) were measured before each infusion. Endothelium-dependent vasodilatation and endothelium-independent vasodilatation were evaluated as forearm blood flow response to reactive and nitroglycerin-induced hyperemia using strain-gauge plethysmography. Endothelium-dependent vasodilatation was significantly improved at 2 weeks and 6 weeks by treatment with infliximab. PWV remained unchanged. Anti-TNF therapy significantly increased serum adiponectin levels at 2 weeks and 6 weeks. The adiponectin levels were positively correlated with the endothelium-dependent vasodilatation, and negatively with the disease activity score of 28 joints. Our study shows a short-term efficacy of infliximab on adiponectin levels and endothelial dysfunction of patients with RA, and provides additional evidence to support the regulatory role of TNF-α on the expression of adiponectin in vivo.

Key words Adiponectin · Anti-TNF therapy · Endothelial function · Infliximab · Rheumatoid arthritis

N. Komai · Y. Morita (✉) · T. Sakuta · A. Kuwabara · N. Kashihara
Division of Nephrology and Rheumatology, Department of Internal Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki 701-0192, Japan
Tel. +81-86-462-1111; Fax +81-86-462-1199
e-mail: morita@med.kawasaki-m.ac.jp

Introduction

Adiponectin is an adipocyte-specific secretory protein abundantly present in circulation. It is associated with lipid and glucose metabolism as well as the pathogenesis of atherosclerosis.[1] Higher adiponectin levels are linked with a lower risk of myocardial infarction.[2,3] Earlier studies have suggested a hypothesis that adiponectin may affect endothelial function. It is reported that decreased adiponectin levels in hypertensive patients are correlated with endothelial dysfunction,[4] which is considered to represent the earliest stage of atherosclerosis.[5] Moreover, endothelial function is shown to be impaired in adiponectin-deficient mice.[4]

Rheumatoid arthritis (RA) patients with high inflammatory activity have depressed endothelial function.[6–8] Such findings are important, because coronary artery disease has been recognized as the major cause of excess morbidity and mortality in patients with RA.[9–11] Recent evidence indicates that vascular dysfunction in RA may occur early in disease. Clinically, it is reported that risk of coronary heart disease in RA patients precedes the American College of Rheumatology (ACR) criteria-based diagnosis of RA.[12] Furthermore, we have shown that endothelial function is depressed in a rat model of arthritis 3 weeks after the onset of disease.[13]

It has been demonstrated earlier that anti-tumor necrosis factor (TNF) therapy improves endothelial dysfunction in RA patients.[8,14] However, the effect of anti-TNF might differ in a study population with different genetic factors. Indeed, endothelial dysfunction in RA patients is reported to be associated with certain HLA-DRB1 genotypes.[7] Therefore, it is important for us to assess the effect of anti-TNF on endothelial function in Japanese patients with RA. In this study, we have evaluated the short-term effect of anti-TNF therapy on serum adiponectin levels as well as endothelial function in Japanese patients with RA. The study also included pulse wave velocity (PWV) as the marker of arterial stiffness.

Materials and methods

Patients

Fifteen Japanese RA patients with high-disease activity despite treatment with methotrexate (≤10 mg/week) and prednisolone (≤10 mg/day) were included in this study. Patients having active infection, congestive heart failure, demyelinating disease, or malignancies were excluded from the study.[15] None of the participants had a history of cardiovascular disease. The patients' profile was as follows: age 50 ± 3, sex (men/women) 2/13, height 157.4 ± 2.4 cm, weight 53.9 ± 2.9 kg, body mass index 21.4 ± 3.5 kg/m^3, and disease duration 10.0 ± 2.3 years. All patients received infusions with infliximab (3 mg/kg) at weeks 0, 2, and 6. A venous blood sample of the patients was drawn before each infusion of infliximab. The clinical status was evaluated by the disease activity score of 28 joints (DAS28).[16] Endothelial function and PWV in the patients were assessed before each infusion.

Assessment of endothelial function and arterial stiffness

We measured forearm blood flow (FBF) by strain-gauge plethysmography (EC6 model; D.E. Hokanson, Bellevue, WA, USA). Endothelium-dependent vasodilatation and endothelium-independent vasodilatation were evaluated as FBF response to reactive and nitroglycerin-induced hyperemia as described previously with minor changes.[17,18] The pressure of the collecting cuff over the left upper arm was set at 50 mmHg, whereas occlusion pressure of the wrist cuff was 200 mmHg. After that, the upper arm was compressed by inflating a pneumatic tourniquet to a pressure of 250 mmHg for 5 min. After cuff deflation, the maximum FBF was measured as the postischemic vasodilator response to reactive hyperemia. After that, 0.3 mg nitroglycerin was administered sublingually, and a third measurement was performed to measure maximum FBF as nitroglycerin-induced hyperemia. PWV was determined using model FCP-4731 (Fukuda Denshi, Tokyo, Japan) as described,[17,18] which allowed online pulse wave recording and automatic calculation.

Measurement of adiponectin

Serum concentrations of adiponectin were determined by the quantitative sandwich enzyme-linked immunosorbent assay (ELISA; Otsuka Pharmaceutical, Tokushima, Japan).

Statistical analysis

Data were expressed as the mean ± SEM of the indicated number of samples studied. The Wilcoxon signed rank test was used to analyze matched pairs.

Results

Clinical and biochemical characteristics

Table 1 shows clinical and biochemical characteristics of the studied patients. The levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and DAS28 showed high disease activity of the patients. A significant decrease of CRP and ESR was observed at 2 weeks and 6 weeks ($P < 0.01$, each) after treatment with infliximab. The DAS score was also significantly improved at 2 weeks and 6 weeks ($P < 0.01$, each). The serum HDL-cholesterol levels were modestly but significantly ($P < 0.05$) increased at 6 weeks. The total cholesterol tended to increase, although the difference did not reach a statistically significant level. The blood pressure, LDL-cholesterol, and triglyceride levels did not change after the treatment.

To confirm whether the effect of anti-TNF on the increase in serum HDL-cholesterol levels continued for a longer time, we examined blood tests at 6 months and 12 months after treatment with infliximab. The serum HDL-cholesterol levels remained higher at 6 months (64.1 ± 5.1 mg/dl, $P < 0.05$) and at 12 months (61.5 ± 4.1 mg/dl, $P < 0.01$) when

Table 1. Clinical and biochemical characteristics of the studied patients treated with infliximab

	0 weeks	2 weeks	6 weeks
SBP (mmHg)	127.9 ± 5.6	124.1 ± 4.8	119.3 ± 4.9
DBP (mmHg)	75.4 ± 3.6	76.1 ± 3.9	73.8 ± 3.4
Total cholesterol (mg/dl)	181.5 ± 11.7	189.7 ± 11.8	202.6 ± 11.2
HDL-cholesterol (mg/dl)	49.4 ± 3.1	51.1 ± 5.5	57.5 ± 5.1*
LDL-cholesterol (mg/dl)	107.3 ± 7.4	107.23 ± 9.4	107.3 ± 11.1
Triglycerides (mg/dl)	88.3 ± 5.7	84.6 ± 6.4	90.2 ± 6.1
CRP (mg/dl)	3.40 ± 0.65	0.99 ± 0.29**	1.09 ± 0.31**
ESR (mm/h)	62 ± 11	31 ± 4**	29 ± 4**
DAS28	5.07 ± 0.20	3.36 ± 0.27**	3.05 ± 0.26**

Data are indicated as the mean ± SEM ($n = 15$)
SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, disease activity score of 28 joints
* $P < 0.05$, ** $P < 0.01$, compared with 0 weeks

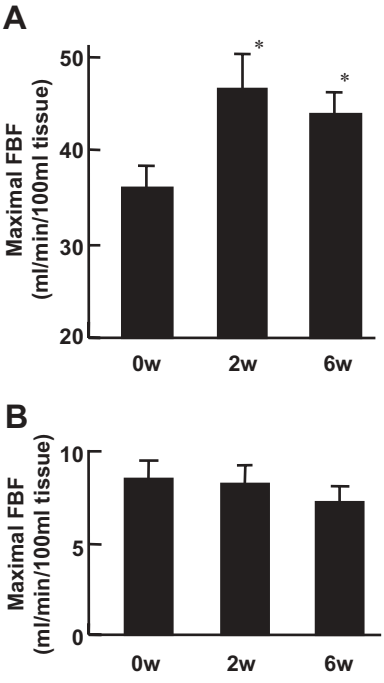

Fig. 1. Endothelium-dependent vasodilatation is increased in patients with rheumatoid arthritis (RA) by treatment with infliximab. Endothelial function was assessed in RA patients before and after the treatment with infliximab. Endothelium-dependent vasodilatation (**A**) and vasodilatation-independent vasodilatation (**B**) were evaluated as forearm blood flow response to reactive and nitroglycerin-induced hyperemia using strain-gauge plethysmography. Data are indicated as the mean ± SEM ($n = 15$). *$P < 0.05$, compared with 0 week

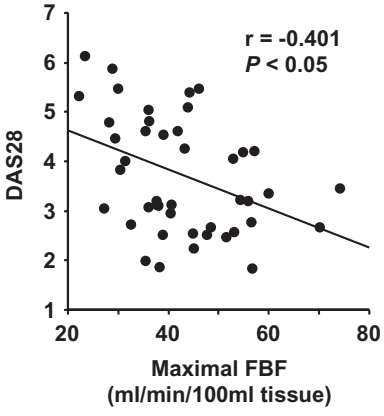

Fig. 2. Negative relationship between endothelium-dependent vasodilatation and the disease activity score of 28 joints (DAS28)

compared with the level before the treatment (49.4 ± 3.1 mg/dl). LDL-cholesterol and triglyceride levels did not change at 6 months and 12 months.

Endothelial function and arterial stiffness

We evaluated the endothelium-dependent vasodilatation and endothelium-independent vasodilatation in RA patients before and after treatment with infliximab. Endothelium-dependent vasodilatation and endothelium-independent vasodilatation were evaluated as FBF response to reactive and nitroglycerin-induced hyperemia using strain-gauge plethysmography. The maximal FBF response to reactive hyperemia in the patients before treatment with infliximab was 36.0 ± 2.2 ml/min/100 ml tissue. A significant increase was observed at 2 weeks (46.6 ± 3.7, $P < 0.01$) and 6 weeks (43.7 ± 2.5, $P < 0.05$; Fig. 1A). The endothelium-dependent vasodilatation was negatively correlated with the DAS score ($r = –0.401$, $P < 0.05$; Fig. 2). This indicates that endothelial dysfunction in RA patients is associated with the disease activity. The FBF with nitroglycerin-induced hyperemia was not improved by the treatment (Fig. 1B). PWV as the marker of arterial stiffness remained unchanged (Fig. 3).

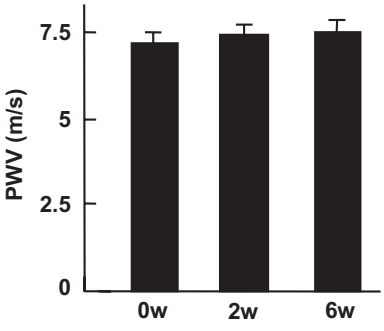

Fig. 3. Infliximab does not affect arterial stiffness in RA patients. Pulse wave velocity was measured in RA patients before and after the treatment with infliximab. Data are indicated as the mean ± SEM ($n = 15$)

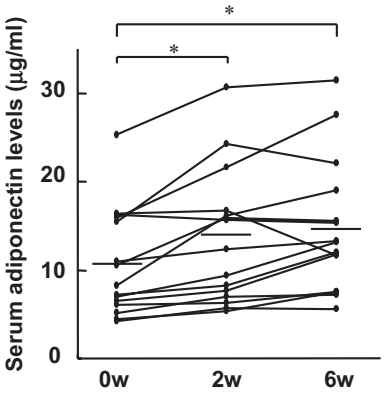

Fig. 4. Serum adiponectin concentration is increased in RA patients by treatment with infliximab. Serum levels of adiponectin in RA patients ($n = 15$) before and after the treatment with infliximab were determined by enzyme-linked immunosorbent assay. *$P < 0.01$, compared with 0 week

Adiponectin levels

We examined whether treatment with infliximab affects serum levels of adiponectin in RA patients. Serum adiponectin levels were increased at 2 weeks (13.0 ± 1.8 μg/ml, $P < 0.01$) and 6 weeks (15.0 ± 2.6 μg/ml, $P < 0.01$) when compared with the control (10.5 ± 1.2 μg/ml; Fig. 4). The serum levels of adiponectin were positively correlated with the endothelium-dependent vasodilatation ($r = 0.451$,

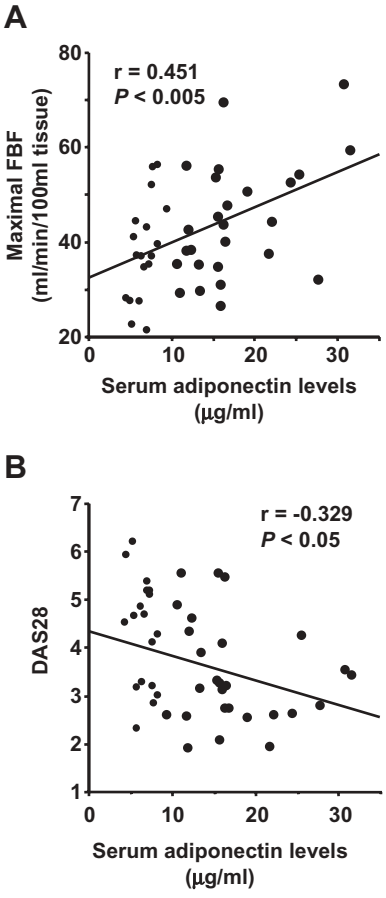

Fig. 5. **A** Positive relationship between serum adiponectin levels and the endothelium-dependent vasodilatation. **B** Negative relationship between serum adiponectin levels and the DAS28 score

$P < 0.005$), and negatively correlated with the DAS28 ($r = -0.329$, $P < 0.05$; Fig. 5). The adiponectin levels tended to be negatively correlated with CRP levels ($r = -0.207$), although the difference is not statistically significant. No correlation was found between the adiponectin and ESR levels.

Discussion

The earlier studies have demonstrated that infliximab improves endothelial dysfunction in RA patients.[8,14] In the present study, we show that the vascular protective effect of anti-TNF therapy is also seen in Japanese patients. Our study also demonstrates that serum levels of adiponectin are increased by treatment with infliximab. Mounting evidence indicates that adiponectin has antiatherogenic properties.[1] The expression of adiponectin is reduced in insulin resistance, diabetes, and the metabolic syndrome. Adiponectin knock-out mice develop diet-induced insulin resistance as well as injury-induced neointimal thickening.[4] Elevated plasma adiponectin is shown to suppress the development of atherosclerosis in apolipoprotein E-deficient mice.[19] Therefore, through its effect of increasing the adiponectin levels, anti-TNF therapy may well benefit patients with RA.

Our finding is consistent with earlier reports, which indicate the regulatory role of TNF-α on expression of adiponectin. Expression of adiponectin by human adipose tissue is inversely correlated with TNF-α expression.[20] Furthermore, treatment of human adipocytes with TNF-α is shown to decrease the in vitro production of adiponectin.[21] TNF-α is highly expressed in RA patients. Accordingly, it is plausible to consider that the overproduced TNF-α suppresses expression of adiponectin in vivo.

The improved endothelial function of infliximab in RA patients may in part be mediated by its effect of increasing the adiponectin levels. The earlier studies indicate that adiponectin can directly affect endothelial functions. Adiponectin down-regulates TNF-dependent expression of endothelial adhesion molecules such as ICAM-1, VCAM-1, and E-selectin.[22] It is also shown that the suppressive effect of adiponectin is through inhibition of cAMP-PKA and NF-κB signaling pathways.[23] Adiponectin stimulates in vitro production of nitric oxide, a strong vasodilator, in vascular endothelial cells.[24] Furthermore, in adiponectin-deficient mice, endothelial function is shown to be impaired.[4]

Adiponectin may have a proinflammatory role in arthritis. A recent study shows that adiponectin is present in the synovium of inflammatory joints in RA patients, and is expressed by synovial fibroblasts as well as articular adipocytes.[25] The investigators also demonstrate that adiponectin stimulates the production of the main mediators of destructive arthritis, interleukin (IL)-6 and pro-MMP-1, by synovial fibroblasts.[25] Of note, TNF inhibitor confers inhibitory effects on adiponectin-induced expression of IL-6 and pro-MMP-1.[25] Therefore, we assume that even if synovial expression of adiponectin is increased by anti-TNF therapy, its proinflammatory role in the development of arthritis can be modulated by TNF inhibitors.

Are serum levels of adiponectin lower in RA patients? Are adiponectin levels in RA patients correlated with the disease activity? No study has been previously performed to address these questions. Our results show that serum adiponectin levels are negatively correlated with the DAS28 score. However, our findings need to be viewed with caution, as they result from the analysis of the mixed data before and after treatment with anti-TNF therapy obtained from a small number of patients.

It is now well known that circulating adiponectin levels are lower in patients with diabetes, obesity, or metabolic syndrome. Furthermore, adiponectin levels are affected by multiple factors, including sex, aging, and lifestyle.[1] Women have higher adiponectin levels than men. Some dietary factors, such as soy protein, fish oils, and linoleic acid, are also reported to increase adiponectin levels.[1] Therefore, a further study with large numbers of patients will be required to clarify whether the disease activity in RA patients affect the adiponectin levels.

We have recently shown that the endothelial function is depressed in rat adjuvant-induced arthritis.[13] More recently, we have demonstrated that serum adiponectin levels are lower in rats with arthritis,[26] which is compatible with the

present results observed in RA patients. Reactive oxygen species, which likely contribute to the pathophysiology of endothelial dysfunction, were found to be overproduced in the aorta from arthritic rats.[13] We also revealed that NAD(P)H oxidases are responsible for the vascular oxidative stress.[13,26] TNF-α is one of the important factors to regulate the activity and expression of vascular NAD(P)H oxidases.[27] Thus, it could be speculated that vascular oxidative stress is higher in patients with active RA, and is decreased after infusion of anti-TNF antibodies.

We demonstrated that PWV as a marker of arterial stiffness is not affected by the short-term use of infliximab. This finding is consistent with an earlier report by Australian investigators. They have shown that a 6-week treatment with TNF antagonists (etanercept, adalimumab, or infliximab) does not improve augmentation index, which is another marker of arterial stiffness measured by pulse wave analysis.[28] However, British investigators have recently reported that a 12-week treatment with etanercept, another anti-TNF agent, reduces PWV in RA patients.[29] These discrepancies could be explained by the differences in samples studied, anti-TNF agents, and duration of the treatment.

HDL-cholesterol levels are increased 6 weeks after initiation of infliximab, and continue to increase at 6 months and 12 months. In contrast, there are no significant changes in the LDL-cholesterol and triglyceride levels. These results are supported by the earlier reports with larger numbers of studied patients.[30,31] This effect may also contribute to decrement in the vascular damage in RA, provided that it continues for a longer time.

It has been recently reported that anti-TNF use is associated with a reduced risk of mortality in RA patients.[32] The primary risk reduction was shown to be on cardiovascular disorders.[32] Further studies with long-term investigations are needed to definitively conclude what our results indicate—that anti-TNF therapy has vascular protective effects.

Acknowledgments We are grateful to Ms. Eriko Abe for help with adiponectin ELISA assay.

References

1. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116: 1784–92.
2. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–9.
3. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730–7.
4. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, et al. Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 2003;42:231–4.
5. Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? Circulation 2004;109:II27–33.
6. Bergholm R, Leirisalo-Repo M, Vehkavaara S, Makimattila S, Taskinen MR, Yki-Jarvinen H. Impaired responsiveness to NO in newly diagnosed patients with rheumatoid arthritis. Arterioscler Thromb Vasc Biol 2002;22:1637–41.
7. Gonzalez-Juanatey C, Testa A, Garcia-Castelo A, Garcia-Porrua C, Llorca J, Vidan J, et al. HLA-DRB1 status affects endothelial function in treated patients with rheumatoid arthritis. Am J Med 2003;114:647–52.
8. Hürlimann D, Forster A, Noll G, Enseleit F, Chenevard R, Distler O, et al. Anti-tumor necrosis factor-α treatment improves endothelial function in patients with rheumatoid arthritis. Circulation 2002;106:2184–7.
9. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, et al. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481–94.
10. Del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 2001;44:2737–45.
11. Van Doornum S, Brand C, King B, Sundararajan V. Increased case fatality rates following a first acute cardiovascular event in patients with rheumatoid arthritis. Arthritis Rheum 2006;54: 2061–8.
12. Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, Jacobsen SJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. Arthritis Rheum 2005;52:402–11.
13. Haruna Y, Morita Y, Komai N, Yada T, Sakuta T, Tomita N, et al. Endothelial dysfunction in rat adjuvant-induced arthritis: vascular superoxide production by NAD(P)H oxidase and uncoupled endothelial nitric oxide synthase. Arthritis Rheum 2006;54: 1847–55.
14. Gonzalez-Juanatey C, Testa A, Garcia-Castelo A, Garcia-Porrua C, Llorca J, Gonzalez-Gay MA. Active but transient improvement of endothelial function in rheumatoid arthritis patients undergoing long-term treatment with anti-tumor necrosis factor alpha antibody. Arthritis Rheum 2004;51:447–50.
15. Miyasaka N, Takeuchi T, Eguchi K. Official Japanese guidelines for the use of infliximab for rheumatoid arthritis. Mod Rheumatol 2005;15:4–8.
16. DAS-score NL. Home of the DAS. http://www.das.score.nl/www.das.score.nl/.
17. Komai N, Ohishi M, Morishita R, Moriguchi A, Kaibe M, Matsumoto K, et al. Serum hepatocyte growth factor concentration is correlated with the forearm vasodilator response in hypertensive patients. Am J Hypertens 2002;15:499–506.
18. Komai N, Ohishi M, Moriguchi A, Yanagitani Y, Jinno T, Matsumoto K, et al. Low-dose doxazosin improved aortic stiffness and endothelial dysfunction as measured by noninvasive evaluation. Hypertens Res 2002;25:5–10.
19. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 2002;106:2767–70.
20. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes 2003;52:1779–85.
21. Wang B, Jenkins JR, Trayhurn P. Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-alpha. Am J Physiol Endocrinol Metab 2005;288:E731–40.
22. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100: 2473–6.
23. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296–301.
24. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem 2003;278:45021–6.
25. Ehling A, Schaffler A, Herfarth H, Tarner IH, Anders S, Distler O, et al. The potential of adiponectin in driving arthritis. J Immunol 2006;176:4468–78.
26. Haruna Y, Morita Y, Yada T, Satoh M, Fox DA, Kashihara N. Fluvastatin reverses endothelial dysfunction and increased vascu-

lar oxidative stress in rat adjuvant-induced arthritis. Arthritis Rheum 2007;56:1827–35.
27. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86: 494–501.
28. Van Doornum S, McColl G, Wicks IP. Tumour necrosis factor antagonists improve disease activity but not arterial stiffness in rheumatoid arthritis. Rheumatology 2005;44:1428–32.
29. Maki-Petaja KM, Hall FC, Booth AD, Wallace SM, Yasmin, Bearcroft PW, et al. Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. Circulation 2006;114:1185–92.
30. Vis M, Nurmohamed MT, Wolbink G, Voskuyl AE, de Koning M, van de Stadt R, et al. Short term effects of infliximab on the lipid profile in patients with rheumatoid arthritis. J Rheumatol 2005;32: 252–5.
31. Spanakis E, Sidiropoulos P, Papadakis J, Ganotakis E, Katsikas G, Karvounaris S, et al. Modest but sustained increase of serum high density lipoprotein cholesterol levels in patients with inflammatory arthritides treated with infliximab. J Rheumatol 2006;33: 2440–6.
32. Michaud K, Wolfe F. Reduced mortality among RA patients treated with anti-TNF therapy and methotrexate. Arthritis Rheum 2005;52:S145.