EXTENDED REPORT

Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis

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Background: Leptin regulates food intake and modulates immunity and inflammation. A positive feedback mechanism has been described between tumour necrosis factor (TNF) and leptin, and it has been suggested that leptin potentiates inflammation in patients with rheumatoid arthritis (RA).
Objective: To assess whether inflammation correlates with leptin concentrations in patients with RA, and whether anti-TNF treatment modulates leptin concentrations in these patients.
Methods: Leptin, IL6 and CRP were measured (at baseline and after 2 weeks of treatment) in the blood of 31 patients with RA starting either anti-TNF treatment or placebo, and in 18 healthy controls.
Results: In patients with RA, plasma leptin concentrations at baseline correlated inversely with the degree of inflammation as assessed by C reactive protein (CRP; $r_s^2 = 0.21$, $p<0.01$) or interleukin (IL) 6 concentrations ($r_s^2 = 0.22$, $p<0.008$). Mean (SD) leptin concentrations did not differ between patients with RA and controls (6.0 (4.6) *v* 4.2 (2.8) ng/ml in men; 15.1 (7.9) *v* 13.4 (5.2) ng/ml in women). Short course anti-TNF treatment for 2 weeks did not modify leptin concentrations, despite significant reduction of CRP and IL6.
Conclusion: A significant inverse correlation between inflammation and leptin concentrations was found in patients with active RA, although plasma leptin concentrations did not significantly differ from those in healthy controls. This suggests that active chronic inflammation may lower plasma leptin concentrations. Two weeks' treatment with anti-TNF did not change plasma leptin concentrations and longer treatment may be needed to see an effect on leptin.

Leptin was initially described as a hormone that regulates food intake and energy balance.[1] Later, it became apparent that leptin has an important role in regulating neuroendocrine and immune functions. Leptin and its receptors (OB-R) share structural and functional similarities with cytokines of the interleukin (IL) 6 family and their receptors.[2] During acute inflammation, proinflammatory cytokines increase circulating leptin concentrations,[3] and leptin, in turn, potentiates cytokine release from monocytes/macrophages.[4] In addition, leptin stimulates T cell mediated immunity and induces the proliferation and differentiation of haematopoietic cells.[3] Regulation of immune functions in humans is strongly sustained by the increased incidence of severe infections in subjects with genetic leptin deficiency[5] and by the deficiencies of the immune system during starvation and malnutrition, when concentrations of leptin are low.[3]

Rheumatoid arthritis (RA) is a chronic inflammatory condition characterised by polyarthritis and high concentrations of proinflammatory cytokines such as tumour necrosis factor α (TNFα), IL1β, IL6, IL8, and interferon γ, especially in the synovial fluid but also in the circulation. A dual effect of inflammation on leptin production has been suggested. On the one hand, a positive feedback between leptin and proinflammatory cytokines has been reported,[4] and immunised leptin deficient mice (ob/ob) were shown to develop less severe arthritis than control mice.[6] Recently, the relation between leptin and arthritis was further supported by studies showing that human chondrocytes express the leptin receptor OB-Rb and, when acting together with interferon γ, leptin stimulated nitric oxide production in the joint cavity.[7] This suggests that leptin may be directly implicated in the pathogenesis of RA. On the other hand, results of studies assessing leptin concentrations in patients with RA have been controversial.[8–12] Additionally, it has been suggested that chronic inflammation down modulates leptin production, which in turn may lead to an impaired antimicrobial defence.[13]

Our study aimed at investigating circulating leptin concentrations in a group of patients with RA and at assessing whether leptin concentrations correlate with systemic inflammation. In addition, we were interested to determine whether anti-TNF treatment modulates plasma leptin concentrations, as TNF has been shown to stimulate leptin production directly.

PATIENTS AND METHODS

Patients and controls

We analysed samples from 31 patients with active RA (mean age 61, M:F = 11:20) included in a phase I, double blind, placebo controlled clinical study of monotherapy with the humanised anti-TNF antibody adalimumab (Humira, Abbott Laboratories) monotherapy at our centre. Patients fulfilled the 1987 American College of Rheumatology criteria for RA, had active disease as defined by a Disease Activity Score >3.2 at baseline, and underwent a washout period for disease modifying antirheumatic drugs (DMARDs) of at least 3 weeks before the start of the study. Stable dosages of non-steroidal anti-inflammatory drugs and prednisone (<10 mg/day) were allowed during the study. Eighteen healthy controls (mean age 38.4, M:F = 9:9) were also included in this study. Because of the differences in leptin concentrations between men and women and because the ratio of men to women differed in the patient (M:F = 1:1.8) and control (M:F = 1:1) groups, we divided each group according to sex before comparing them. Body mass index

Abbreviations: BMI, body mass index; CRP, C reactive protein; DMARDs, disease modifying antirheumatic drugs; IL, interleukin; RA, rheumatoid arthritis; TNFα, tumour necrosis factor α

Table 1 Evaluation of the inflammatory status, as assessed by levels of CRP and IL6, and plasma leptin concentrations in patients with RA after 2 weeks' anti-TNF treatment (n = 23) or placebo (n = 8)

	Week 0	Week 2	p Value
Leptin (ng/ml)			
Men (n = 10)	6.2 (5.0)	5.7 (3.5)	NS
Women (n = 13)	15.4 (8.7)	16.7 (9.9)	NS
C reactive protein (mg/ml)			
Anti-TNF	86.1 (54.4)	35.4 (35.6)	<0.0001
Placebo	53.7 (49.2)	53.9 (50.0)	NS
Interleukin 6 (pg/ml)			
Anti-TNF	88.3 (60.5)	42.3 (40.7)	<0.001
Placebo	60.0 (59.6)	60.8 (60.2)	NS

Results are given as mean (SD).

administration, whereas no changes were observed with placebo (table 1).

After 2 weeks of anti-TNF treatment, plasma leptin concentrations in patients with RA were similar to those at baseline both in men and women (table 1). Moreover, no significant differences were seen in the placebo treated group.

DISCUSSION

Inflammatory mediators, such as the cytokines TNFα and IL1β, decrease energy intake and may lead to the wasting described in patients with RA. Wasting, in turn, affects the inflammatory response and may suppress cellular immunity. In this complex relationship, leptin is a possible mediator. In this study we show that in patients with RA, both circulating leptin concentrations and leptin adjusted for the BMI, inversely correlated with the inflammatory status of the patients, as assessed by the inflammatory markers CRP and IL6. These results are supported by the observations that long term in vitro stimulation of adipose tissue by TNFα or IL1β inhibits leptin and leptin mRNA production.[15] Similarly, in patients with tuberculosis, another chronic inflammatory condition, inflammation correlates negatively with leptin concentration.[13] In patients with RA, plasma leptin concentrations did not correlate with BMI, suggesting that regulation of leptinaemia in RA is complex, and that weight is not the only major regulator. These facts led us to propose the hypothesis that in RA chronic inflammation, probably through proinflammatory cytokines (for example, TNF, IL1, IL6), is an important determinant of plasma leptin concentration and has an inhibitory effect on leptin production.

In addition, we report that plasma leptin concentrations in patients with RA do not differ from those found in healthy controls. This is in line with two earlier studies.[8 9] In contrast, Bokarewa *et al* found higher plasma leptin concentrations in a group of patients with RA.[11] Theoretically, one would expect increased leptin concentration owing to the proinflammatory status of RA and to the stimulatory activity of TNFα and IL1β on leptin release.[3 15] Similarly, patients with sepsis and those who had had major surgery, two situations also characterised by increased TNFα and IL1β concentrations, had raised serum leptin concentrations.[16] However, as shown above, chronic inflammation in patients with RA had inhibitory effects on leptin concentrations in the blood, in contrast with the acute inflammation of sepsis and surgery. Recently, Harle *et al* found that serum leptin concentrations were almost three times lower in a group of women with RA than in a group of healthy women.[12] In addition, the body compartment in which leptin is measured may be of importance. Although blood concentrations of leptin did not differ significantly between patients with RA and controls, concentrations in the synovial fluid may be of importance.[7 11]

The lack of difference between plasma leptin concentrations in patients with RA and healthy controls may seem in contrast with the inverse correlation of leptin and inflammation in these patients, which suggests that there will be lower leptin concentration in patients with RA. The cause of this discrepancy is probably due to a combination of factors: a significant percentage of the patients with RA did not have very high inflammatory parameters at the time of investigation; the BMI of the patients with RA in our group was slightly higher than that of the control volunteers; and some of the inhibitory effects of chronic inflammation might have been counterbalanced by potential stimulatory actions of acute inflammatory reactions during exacerbations of RA.

We also evaluated a possible correlation of the duration of the disease with plasma leptin concentrations, but no direct relation between these two measures was found. These results are in line with those of Anders *et al*,[8] whereas Bokarewa *et al* showed a gradual increase of leptin concentrations with the duration of RA.[11]

To explain our results better we evaluated the influence of previous treatment with DMARDs on serum leptin concentration in our RA group. We could not establish a relation between leptin concentrations at baseline and this treatment, no matter which type of drug or what dosage was used. Bokarewa *et al* found higher leptin concentrations in a group of patients with RA treated with methotrexate than in a group receiving other DMARDs, but at the same time, these concentrations were similar to those found in a group of patients who were not treated with any DMARDs. In the same study no difference in serum leptin concentrations was found between patients with RA treated and not treated with glucocorticoids.[11] Sulfasalazine has also been shown to have no influence on leptin release from adipose tissue and skeletal muscle.[17] The above mentioned studies suggest that no one specific DMARD influences serum leptin concentrations. Moreover, a washout period for DMARDs was performed on every patient included in our study 3 weeks before entry—that is, before the time at which blood was collected for leptin determination.

Leptin is known to stimulate T cell mediated immunity. In the case of septic shock, mortality is associated with decreased plasma leptin levels,[18] while genetic leptin deficiencies increase the severity of infections in humans.[5] In addition, severe infections have been reported to occur more often in patients with RA than in the general population,[19] especially in patients receiving anti-TNF drugs.[20] These data suggest that suppression of leptin concentration by chronic inflammation may contribute to the susceptibility of patients with RA to infections.

An additional aim of our study was to investigate whether a short course of anti-TNF treatment can influence leptin concentrations. To date, as far as we know, no study has investigated the effect of in vivo TNFα blockade upon circulating leptin levels. We found that a 2 week course of anti-TNF treatment did not change plasma leptin concentrations, despite decreasing the acute phase reactants. Therefore, a short course of treatment with anti-TNF does not modulate leptin concentrations in patients with RA, and studies investigating the effect of long term treatment on leptin concentration are warranted.

In conclusion, our study shows that circulating leptin concentrations are inversely correlated with the inflammatory status in patients with RA. We suggest that in RA, chronic inflammation down regulates leptin production, which may indirectly contribute to the susceptibility to infections seen in these patients. The precise role of leptin in RA remains uncertain but, possibly, local actions, through

BMC Surgery

RESEARCH ARTICLE Open Access

Sepsis induced changes of adipokines and cytokines - septic patients compared to morbidly obese patients

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Abstract

Background: Hyperglycemia and insulin resistance frequently occur in critically ill and in morbidly obese (MO) patients. Both conditions are associated with altered serum levels of cytokines and adipokines. In addition, obesity related alterations in adipokine expression contribute to insulin resistance in metabolic syndrome. In this study we examined the serum adipocytokine profile in critically ill patients, MO patients, and healthy blood donors.

Methods: 33 patients who fulfilled the clinical criteria for severe sepsis or septic shock (SP) were prospectively enrolled in this study. A multiplex analysis was performed to evaluate plasma levels of adiponectin, resistin, leptin, active PAI-1, MCP-1, IL-1 alpha, IL-6, IL-8, IL-10, and TNF-alpha in 33 critically ill patients, 37 MO patients and 60 healthy blood donors (BD).

Results: In SP, adiponectin was significantly lowered and resistin, active PAI-1, MCP-1, IL-1 alpha, IL-6, IL-8, IL-10, and TNF-alpha were significantly elevated compared to BD. Leptin levels were unchanged. In MO, adiponectin and IL-8 were significantly lowered, leptin, active PAI-1, MCP-1, IL-1 alpha, IL-6, and IL-10 significantly elevated, whereas resistin was unaltered.
In SP, adiponectin correlated negatively with BMI, SAPS II and SOFA scores, while resistin correlated positively with SAPS II and SOFA scores and leptin correlated positively with the BMI. Adiponectin was approximately equally diminished in SP and MO compared to BD. With the exception of active PAI-1, cytokine levels in SP were clearly higher compared to MO.

Conclusion: A comparable adipocytokine profile was determined in critically ill and MO patients. As in MO, SP showed reduced adiponectin levels and elevated MCP-1, active PAI-1, IL-1 alpha, IL-6, and IL-10 levels. Leptin is only elevated in MO, while resistin, IL-8, and TNF-alpha is only elevated in SP. As in MO patients, increased levels of proinflammatory cytokines and altered levels of adipokines may contribute to the development of insulin resistance in critically ill patients.

Background

Sepsis is defined as a systemic inflammatory response syndrome to infection, which, when associated with one or more organ system dysfunctions, is considered as severe sepsis. When sepsis is associated with shock, which is refractory to fluid resuscitation, the patient is considered to be in septic shock [1]. This is associated with an increased production of both pro- and anti-inflammatory cytokines [2]. Cytokines are low-molecular-weight polypeptides or glycoproteins that play an important role in regulating host response to infection, immune responses, inflammation, and trauma.

Some cytokines clearly promote inflammation and are called *proinflammatory cytokines*, whereas other cytokines suppress the activity of proinflammatory cytokines and are called *anti-inflammatory cytokines*.

Tumor necrosis factor (TNF) and interleukin (IL)-1α are proinflammatory cytokines, which can cause fever,

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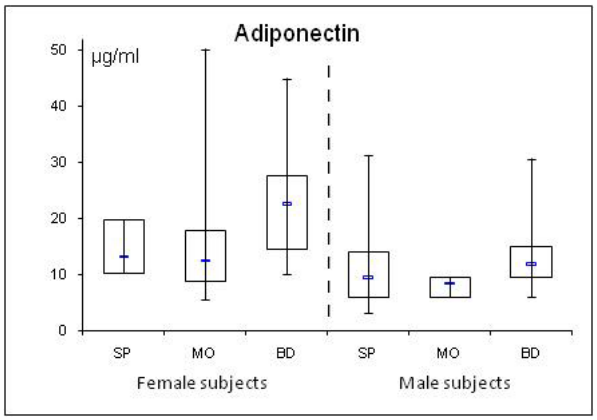

Figure 1 Gender specific serum concentrations of adiponectin in septic (SP) and morbidly obese (MO) patients and healthy controls (BD). The top and bottom of the rectangle represent the 25th and 75th percentile. The line within the rectangle represents the median. The whiskers extend from the 5th percentile to the 95th percentile.

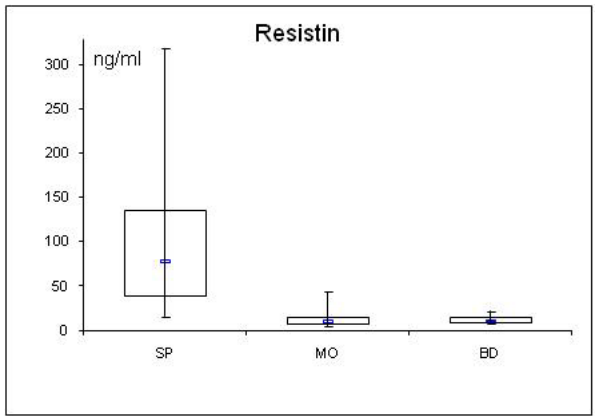

Figure 3 Serum concentrations of resistin in septic (SP) and morbidly obese (MO) patients and healthy controls (BD).

p = 0.085). There was no correlation with patients' age in BD concerning female/male leptin, resistin, active PAI-1, MCP-1, IL-1α, IL-6, IL-8; IL-10, and TNF-α. Values of MO and SP showed no correlation with age.

Seven patients of the sepsis group had preexisting type 2 diabetes. These seven patients showed no difference in adiponectin, resistin, leptin, active PAI-1, MCP-1, IL-1α, IL-6, IL-8, IL-10, and TNF-alpha levels.

Discussion

In this study we determined serum levels of classical cytokines and adipokines in septic and morbidly obese patients and compared them to healthy blood donors. The most important finding of this study is that both, SP and MO have similarly shifted values compared to a control group of healthy BD. Moreover, most cytokine concentrations were greater in sepsis patients than in the morbidly obese patients.

Adipokines

Our results revealed that the only known adipose tissue derived factor with major insulin-sensitizing and anti-inflammatory properties, adiponectin, is equally diminished in SP and MO. Adiponectin is the most abundant protein produced by adipose tissue and its blood concentration is much higher than the concentration of other known hormones. Normal serum adiponectin levels are higher in women compared to men. Adiponectin attenuates inflammatory actions on several levels. It suppresses function of mature macrophages and inhibits growth of macrophage precursors [23]. Further, adiponectin attenuates the production of TNF-α and IL-6 production in macrophages and induces that of IL-10 [24]. Thus, the lower levels of adiponectin in the

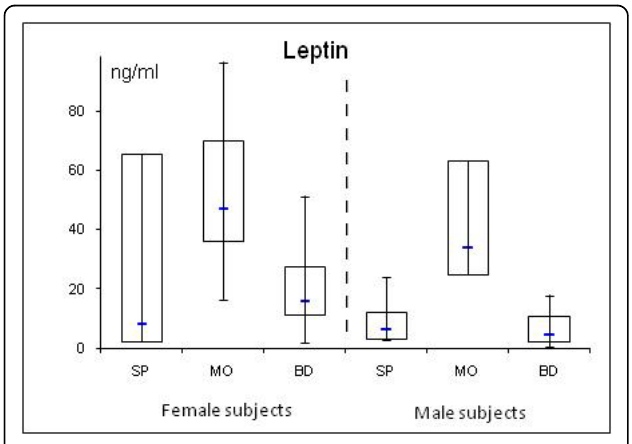

Figure 2 Gender specific serum concentrations of leptin in septic (SP) and morbidly obese (MO) patients and healthy controls (BD).

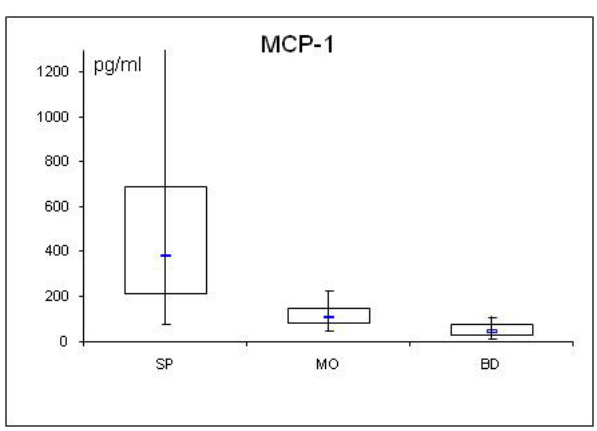

Figure 4 Serum concentrations of MCP-1 in septic (SP) and morbidly obese (MO) patients and healthy controls (BD).

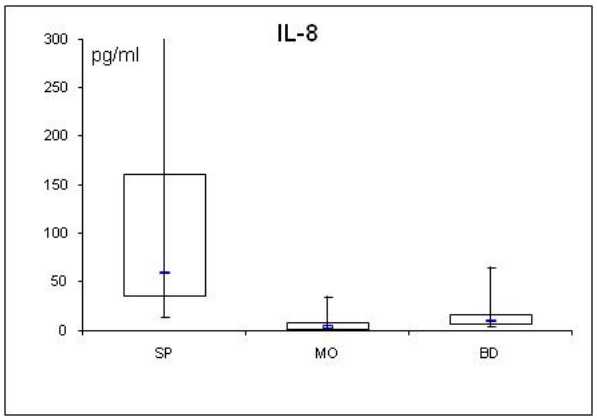

Figure 9 Serum concentrations of IL-8 in septic (SP) and morbidly obese (MO) patients and healthy controls (BD).

different casuistics

Since pro-inflammatory cytokines were higher in SP than in MO, this could have led to the higher resistin levels in SP than in MO in our study. Otherwise, the higher resistin levels in SP compared to MO may have contributed to the higher pro-inflammatory cytokines in SP compared to MO. In our SP and MO patients, as well as in prior studies of septic patients [35,36], resistin did not correlate to obesity measured by BMI which suggests that in circumstances of critical illness the release of resistin by macrophages plays a superior role compared with the secretion from adipocytes. This could possibly also explain why there was no difference in resistin levels of patients with preexisting diabetes mellitus.

Leptin was the only measured adipokine in the present study that was marginally reduced in SP compared to BD. The MO group had significantly elevated levels. In the present study, normal leptin serum levels were higher in women compared to men. In the obese, increased fat storage will lead to enhanced leptin levels [37]. Leptin can affect glucose metabolism and increases insulin sensitivity. Obese humans are often insulin- and leptin-resistant [38]. The role of leptin in sepsis and septic shock is controversial. Earlier reports suggested that high lepin levels are associated with increased survival in sepsis and septic shock [39,40], several other reports -such as our study- fail to show a correlation between leptin and sepsis [41]. Our minor changes of leptin serum levels in SP are in line with a reported temporal decrease in leptin after operative stress followed by a subsequent rise slightly above the initial levels [42].

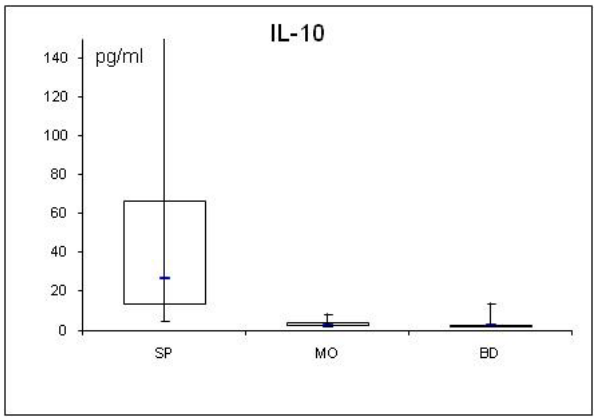

Figure 10 Serum concentrations of IL-10 in septic (SP) and morbidly obese (MO) patients and healthy controls (BD).

Cytokines

Several studies suggest that TNF-α and IL-6 are both involved in obesity-related insulin resistance and that TNF-α is one of the most important mediators of inflammation [43]. TNF-α is not secreted by adipocytes but by infiltrating macrophages in adipose tissue, whereas adipose tissue is a significant source of IL-6 [44]. Expression and secretion of TNF-α increases with obesity and correlates positively with body mass index [45]. TNF-α and IL-6 are known to promote lipolysis and the secretion of free fatty acids, which contributes to an increase in hepatic glucose production and insulin resistance [46]. On a cellular level, TNF-α is a potent inhibitor of the insulin-stimulated tyrosine phosphorylations on the beta-chain of the insulin receptor and insulin receptor substrate-1, suggesting that TNF-α may play a crucial role in the systemic insulin resistance of non insulin dependent diabetes mellitus [47]. In analogy, the elevated IL-6 levels in our septic and morbidly obese patients may contribute to insulin resistance. In contrast to the morbidly obese patients, in addition, the elevated TNF-α levels in our septic patients might play a role in insulin resistance.

Human fat cells are also known to produce proinflammatory IL-8 which was increased in insulin-resistant subjects [48]. Surprisingly, we found reduced levels of IL-8 in MO compared to BD. Serum levels of MO patients (median: 3.7 pg/ml) match reported levels in the obese. Serum level of BD seems (median: 9.8 pg/ml) markedly elevated compared to reported levels of approximately 3.2 pg/ml [49]. Insofar, reported IL-8 levels should be interpreted with care. Nevertheless, the higher IL-8 levels in septic patients compared to the morbidly obese patients underline the infection induced origin of IL-8 in our study.

The presented study has several limitations. In the healthy control group, there was no investigation of hidden or not yet clinically manifest side diagnoses. Consequently, there is no information about type 2 diabetes or other chronic inflammatory diseases without clinical manifestation. Moreover, the control group was

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Adiponectin is a Mediator of the Association of Adiposity with Radiographic Damage in Rheumatoid Arthritis

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Abstract

Objectives—Recent reports have suggested that increasing adiposity may protect against radiographic damage in rheumatoid arthritis. We explored the role of serum adipokines (adiponectin, resistin, leptin) in mediating this association.

Methods—RA patients underwent total-body dual-energy absorptiometry for measurement of total and regional body fat and lean mass, abdominal computed tomography for measurement of visceral fat area, and radiographs of the hands and feet scored according to the Sharp-van der Heijde method. Serum levels of adipokines were measured and cross-sectional associations with radiographic damage were explored, adjusting for pertinent confounders. The associations of measures of adiposity with radiographic damage were explored with the introduction of adipokines into multivariable modeling as potential mediators.

Results—Among the 197 patients studied, adiponectin demonstrated a strong association with radiographic damage, with the log Sharp score increasing 0.40 units for each log unit increase in adiponectin (p=0.001) after adjusting for pertinent predictors of radiographic damage. Adiponectin independently accounted for 6.1% of the explainable variability in Sharp score, a proportion comparable to rheumatoid factor and greater than HLA-DRB1 shared epitope alleles or C-reactive protein. Resistin and leptin were not associated with radiographic damage in adjusted models. An inverse association between visceral fat area and radiographic damage was attenuated when adiponectin was modeled as a mediator. The association of adiponectin with radiographic damage was stronger in patients with longer disease duration.

Conclusions—Adiponectin may represent a mechanistic link between low adiposity and increased radiographic damage in RA. Adiponectin modulation may represent a novel strategy for attenuating articular damage.

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Study Design: Giles, Bathon
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in fat and muscle tissue, antagonizing atherogenesis and improving insulin sensitivity (13), its activity in the joint may be pro-inflammatory, as suggested by several recent *in vitro* studies. Tang et al (17) demonstrated an increase in IL-6 production in cultured synovial fibroblasts from RA and osteoarthritis patients when stimulated with increasing concentrations of adiponectin. Upregulation of IL-6 was induced by adiponectin stimulated signaling through the NF-κB pathway. In another study, Luo et al (29) demonstrated that stimulation with adiponectin induced RANKL and inhibited osteoprotegerin in cultured human osteoblasts, leading to increased osteoclast formation. Taken together, these studies provide circumstantial evidence that adiponectin may have pro-inflammatory activity in the joint, inducing the terminal mechanisms involved in erosive joint damage. Indeed, the effect may not be specific to RA, as adiponectin levels were higher in patients with erosive vs. non-erosive osteoarthritis of the hands in a recently report (30).

Three prior studies have identified an association between increasing BMI (a surrogate for adiposity) and lower rates of radiographic progression in RA patients (5–7). This association is seemingly paradoxical, as adipose tissue is a potent source of cytokines (31) and was associated with increased systemic inflammation in RA patients (32). Thus, one might hypothesize that increasing adiposity would result in higher, rather than lower, rates of radiographic progression. However, a key feature of adiponectin physiology is that circulating levels diminish as adiposity increases, with highest levels noted in individuals with the lowest fat mass (33). In this light, considering that adiponectin may have detrimental effects on the joint, adiponectin becomes an excellent candidate to mediate the inverse relationship between increasing adiposity and radiographic damage observed in prior RA studies. Two of the studies identified a protective effect of obesity only in patients seropositive for RF (6) or anti-CCP antibodies (7), a heterogeneity not detected in our study. Differences in study design (prospective and enrolling only early RA patients in the prior studies and cross-sectional with early and established patients in our study) may account for the discrepancy in findings.

Adiponectin expression is suppressed to the greatest extent in visceral fat (fat located within the visceral abdominal cavity); under a pathophysiologic mechanism that is not well defined (34). As expected, we observed in our RA patients that adiponectin levels were highest in the patients with the lowest visceral fat area. These patients also demonstrated higher radiographic damage scores, even after accounting for multiple pertinent confounders. When adiponectin and visceral fat were co-modeled, the association of increasing visceral fat area with radiographic damage was subsumed in part by adiponectin, suggesting that adiponectin functions as an intermediate in the pathway between visceral fat and radiographic damage (35). These mediating effects of adiponectin provide further clues to demystifying the apparent paradoxical association between increasing BMI and protection from radiographic progression noted in RA (6,7). We did not, however, observe significant associations between other measures of adiposity (e.g. BMI, total fat by DXA) and radiographic damage. However, adiponectin tends to be more strongly correlated with visceral fat than BMI or DXA measures of fat. Longitudinal assessments of radiographic damage are underway to explore the ability of measures of adiposity and adipokines to predict radiographic change scores over time.

The origin of the adiponectin implicated in the joint damage observed in our study remains unclear. Adipocytes are abundant in the joint (36) and may express adiponectin that acts locally in a paracrine fashion. Another possibility is that circulating adiponectin produced by remote adipocytes acts in the joint as an endocrine hormone. A prior study in RA patients showed that serum adiponectin concentration was higher than synovial fluid concentration (16), with significant correlation noted in adiponectin levels between the two sources. This finding suggests that the source of articular adiponectin may be peripheral adipose rather

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ORIGINAL ARTICLE

Resistin gene variation is associated with systemic inflammation but not plasma adipokine levels, metabolic syndrome or coronary atherosclerosis in nondiabetic Caucasians

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Summary

Objective Resistin causes insulin resistance and diabetes in mice whereas in humans it is linked to inflammation and atherosclerosis. Few human genetic studies of resistin in inflammation and atherosclerosis have been performed. We hypothesized that the –420C>G putative gain-of-function resistin variant would be associated with inflammatory markers and atherosclerosis but not with metabolic syndrome or adipokines in humans.

Design and methods We examined the association of three resistin polymorphisms, –852A>G, –420C>G and +157C>T, and related haplotypes with plasma resistin, cytokines, C-reactive protein (CRP), adipokines, plasma lipoproteins, metabolic syndrome and coronary artery calcification (CAC) in nondiabetic Caucasians ($n = 851$).

Results Resistin levels were higher, dose-dependently, with the –420G allele (CC 5·9 ± 2·7 ng/ml, GC 6·5 ± 4·0 ng/ml and GG 7·2 ± 4·8 ng/ml, trend $P = 0·04$) after age and gender adjustment [fold higher for GC + GG *vs.* CC; 1·07 (1·00–1·15), $P < 0·05$)]. The –852A>G single nucleotide polymorphism (SNP) was associated with higher soluble tumour necrosis factor-receptor 2 (sol-TNFR2) levels in fully adjusted models [1·06 (95% CI 1·01–1·11), $P = 0·01$)]. The estimated resistin haplotype (GGT) was associated with sol-TNFR2 ($P = 0·04$) and the AGT haplotype was related to CRP ($P = 0·04$) in the fully adjusted models. Resistin SNPs and haplotypes were not associated with body mass index (BMI), fasting glucose, insulin resistance, metabolic syndrome, adipokines or CAC scores.

Conclusions Despite modest associations with plasma resistin and inflammatory biomarkers, resistin 5′ variants were not associated with metabolic parameters or coronary calcification. This suggests that resistin is an inflammatory cytokine in humans but has little influence on adiposity, metabolic syndrome or atherosclerosis.

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Introduction

Resistin was originally described as an adipose-derived protein in rodents that links obesity to insulin resistance.[1,2] In mice, resistin expression in adipose tissue is increased in both diet-induced and genetic models of obesity and is related to measures of insulin resistance.[3–5] In humans, however, the relationship between resistin and insulin resistance is disputed, with some studies suggesting a positive association[6,7] and others finding no relationship.[8–10] This may relate to species differences in gene homology and tissue expression. Unlike rodents, where resistin is adipocyte derived, resistin expression is myeloid restricted in humans[11,12] and has an unclear relationship with adiposity, insulin resistance and metabolic syndrome, in lean nondiabetic subjects.[13] Consistent with a myeloid origin in humans, recent studies suggest a positive relationship with chronic inflammatory states including rheumatoid arthritis,[14] chronic kidney disease[15] and atherosclerotic cardiovascular disease (CVD), although the strength and significance of this latter association remains uncertain.[16]

Up to two-thirds of plasma resistin variation may be attributable to heritable influences.[17] Several studies, with conflicting results, have examined the relationship of resistin gene variation with insulin resistance, obesity and glucose homeostasis.[18–21] Among these, a putative gain-of-function promoter single nucleotide polymorphism (SNP),

An Inflammatory Cascade Leading to Hyperresistinemia in Humans

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Author Contributions: ML, MPR, NI, DJR, and MAL designed the study. ML, MPR, and SCM performed experiments. ML, MPR, NI, and MAL analyzed the data. MPR and NI enrolled patients. ML, MPR, NI, DJR, and MAL contributed to writing the paper. MAL obtained funding for the study and provided the research environment where the studies were conducted.

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Abbreviations: ANOVA, analysis of variance; IL, interleukin; LPS, lipopolysaccharide; MAPK, MAP-kinase; SEM, standard error of the mean; sTNFR2, soluble tumor necrosis factor receptor 2; TNFα, tumor necrosis factor α

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ABSTRACT

Background

Obesity, the most common cause of insulin resistance, is increasingly recognized as a low-grade inflammatory state. Adipocyte-derived resistin is a circulating protein implicated in insulin resistance in rodents, but the role of human resistin is uncertain because it is produced largely by macrophages.

Methods and Findings

The effect of endotoxin and cytokines on resistin gene and protein expression was studied in human primary blood monocytes differentiated into macrophages and in healthy human participants.

Inflammatory endotoxin induced resistin in primary human macrophages via a cascade involving the secretion of inflammatory cytokines that circulate at increased levels in individuals with obesity. Induction of resistin was attenuated by drugs with dual insulin-sensitizing and anti-inflammatory properties that converge on NF-κB. In human study participants, experimental endotoxemia, which produces an insulin-resistant state, causes a dramatic rise in circulating resistin levels. Moreover, in patients with type 2 diabetes, serum resistin levels are correlated with levels of soluble tumor necrosis factor α receptor, an inflammatory marker linked to obesity, insulin resistance, and atherosclerosis.

Conclusions

Inflammation is a hyperresistinemic state in humans, and cytokine induction of resistin may contribute to insulin resistance in endotoxemia, obesity, and other inflammatory states.

Introduction

Dietary and lifestyle changes during the last century have entailed an unprecedented epidemic of obesity and associated metabolic diseases, including type 2 diabetes and atherosclerosis [1]. Many individuals suffer simultaneously from more than one of these conditions, and epidemiological studies in humans, as well as studies in animal models, suggest that obesity-related insulin resistance is a common pathogenic feature [2]. Indeed, insulin resistance is the keystone of the "metabolic syndrome," a major cardiovascular risk factor even in the absence of demonstrable glucose intolerance or diabetes [3]. Obesity and insulin resistance are strongly associated with systemic markers of inflammation, and, indeed, inflammation may contribute to insulin resistance [4]. Similarities and overlap between obesity and inflammatory states are emerging. Inflammatory cytokines such as tumor necrosis factor α (TNF α) and interleukin (IL)-6 are produced by adipocytes as well as by monocytes and macrophages, and they circulate at increased levels in individuals with obesity [5,6]. Moreover, bone-marrow-derived macrophages home in on adipose tissue in individuals with obesity [7,8], and adipocytes and macrophages may even be interconvertible [9]. Furthermore, inflammation is increasingly recognized as a major component and predictor of atherosclerotic vascular disease, a major clinical consequence of insulin resistance [10]. Hence, the interrelationships between obesity, insulin resistance, and atherosclerosis are of great scientific and clinical interest.

We originally identified and characterized resistin as a circulating mouse adipocyte gene

product that is regulated by antidiabetic drugs [11]. In rodents, resistin is derived exclusively from adipocytes [11,12], circulates at increased levels in obese animals [11], and causes dysregulated hepatic glucose production, leading to insulin resistance [13,14]. A syntenic gene exists in humans, but is expressed at higher levels in monocytes and macrophages than in adipocytes [15,16], raising questions about the relationship between resistin and human metabolic disease. Recently, several studies have suggested that metabolic abnormalities are associated with polymorphisms in the human resistin gene [17,18]. Furthermore, several studies, though not all, have reported increased serum resistin levels in patients with obesity, insulin resistance, and/or type 2 diabetes [19,20,21,22,23,24,25,26]. However, the mechanism and importance of increased resistin levels in human metabolic disease are not known.

Here we show that the endotoxin lipopolysaccharide (LPS), a potent inflammatory stimulant, dramatically increases resistin production by inducing secretion of inflammatory cytokines such as TNFα. This increase in resistin production is blocked by both aspirin and rosiglitazone, drugs that have dual anti-inflammatory and insulin-sensitizing actions and have been shown to antagonize NF-κB. Indeed, activation of NF-κB is sufficient to induce resistin expression, and loss of NF-κB function abolishes LPS induction of resistin. Resistin serum levels are increased dramatically by endotoxemia in humans, and correlate with a marker of inflammation in patients with type 2 diabetes. Thus, systemic inflammation leads to increased resistin production and circulating levels in humans. The increased level of resistin in humans with obesity is likely an indirect result of elevated levels of inflammatory cytokines characteristic of states of increased adiposity. Hence, obesity and acute inflammation are both hyperresistinemic states associated with insulin resistance.

Methods

Differentiation of Primary Human Macrophages

Peripheral blood mononuclear cells were isolated from whole blood of healthy donors following apheresis and elutriation. Greater than 90% of these monocytes expressed CD14 and HLA-DR. Cells were plated in 24-well plates at a density of 10^6 cells per well, allowed to adhere for 4 h, then washed with Dulbecco's Modified Eagles Medium and further cultured in 10% FBS in Dulbecco's Modified Eagles Medium supplemented with 5 ng/ml GM-CSF (Sigma, St. Louis, Missouri, United States) to promote macrophage differentiation. All experiments were performed after overnight equilibration with macrophage serum-free medium (GIBCO, San Diego, California, United States; Invitrogen, Carlsbad, California, United States) supplemented with 5 ng/ml GM-CSF. Cells were treated with LPS (Sigma), aspirin (Sigma), SN50, and/or control peptide (Biomol, Plymouth Meeting, Pennsylvania, United States), MG132, PD98059, SB20358 (Calbiochem, San Diego, California, United States), and TNFα (R&D Systems, Minneapolis, Minnesota, United States). Neutralizing antibodies to TNFα, IL-6, and anti-IL-1β, as well as control IgG, were obtained from R&D Systems. Adenovirus expressing activated IKK in pAD easy with GFP and control vector was a generous gift from Steven Shoelson.

RNA Isolation and Quantification

RNA was isolated using RNeasy Mini Kit (Qiagen, Valencia, California, United States), then subjected to DNase digestion followed by reverse transcription (Invitrogen). mRNA transcripts were quantified by the dual-labeled fluorogenic probe method for real-time PCR, using a Prism 7900 thermal cycler and sequence detector (Applied Biosystems, Foster City, California, United States). Real-time PCR was performed using Taqman Universal Polymerase Master Mix (Applied Biosystems). The primers and probes used in the real-time PCR were the following: Sense-Resistin, 5′-AGCCATCAATGATAGGATCCA-3′; Antisense-Resistin, 5′-TCCAGGCCAATGCTGCTTAT-3′; Resistin Probe, 5′-Fam-AGGTCGCCGGCTCCCTAATATTTAGGG-TAMRA-3′; Sense human 36B4 sense, 5′- TCGTGGAAGTGACATCGTCTTT-3′; Antisense 36B4, 5′-CTGTCTTCCCTGGGCATCA-3′; and 36B4 Probe, 5′-FAM-TGGCAATCCCTGACGCACCG-TAMRA-3′.

Primer and probe for TNFα were obtained from Applied Biosystems. The cycle number at which the transcripts of the gene of interest were detectable (CT) was normalized to the cycle number of 36B4 detection, referred to as deltaCT. The fold change in expression of the gene of interest in the compound-treated group relative to that in the vehicle-treated group was expressed as $2^{-\text{deltadeltaCT}}$, in which deltadeltaCT equals the deltaCT of the compound-treated group minus the deltaCT of the chosen control group, which was normalized to 1.

ELISA

Resistin concentrations, in cell media and human plasma, were assessed with a commercially available ELISA (Linco Research, St. Charles, Missouri, United States) and normalized to cell protein. The average correlation coefficient for standards using a four-parameter fit was 0.99. Intra-assay and inter-assay coefficients of variance were 4.7% and 9.1%, respectively. Direct comparison of standard curves generated by the Linco kit with those yielded by another commercially available resistin ELISA (Biovendor Laboratory Medicine, Brno, Czech Republic) yielded high correlation (rho = 0.99, $p < 0.001$), except that the Biovendor values were approximately 30% lower than those determined with the Linco assay. This appeared to be related to the standards used for calibration. Discrepant absolute values among different assays, including the Biovendor assay, were recently described by others [22]. Resistin levels in 40 plasma samples were measured using both Linco and Biovendor ELISA kits, with moderate correlation (rho = 0.66). Levels of soluble TNFα receptor 2 (sTNFR2) were measured using a commercially available immunoassay (R&D Systems). Intra-assay and inter-assay coefficients of variance were 5.1% and 9.8%, respectively.

Human Endotoxemia Study

Healthy volunteers (n = 6, three male and three female), aged 18–45 y with BMI between 20 and 30 and on no medications, were studied. The University of Pennsylvania Institutional Review Board approved the study protocol, and all participants gave written informed consent. Following screening and exclusion of individuals with any clinical or laboratory abnormalities, participants were admitted to the General Clinical Research Center at the University of

EXTENDED REPORT

Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum

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Background: Resistin is a newly identified adipocytokine which has demonstrated links between obesity and insulin resistance in rodents. In humans, proinflammatory properties of resistin are superior to its insulin resistance-inducing effects.
Objectives: To assess resistin expression in synovial tissues, serum and synovial fluid from patients with rheumatoid arthritis, osteoarthritis and spondylarthropathies (SpA), and to study its relationship with inflammatory status and rheumatoid arthritis disease activity.
Methods: Resistin expression and localisation in synovial tissue was determined by immunohistochemistry and confocal microscopy. Serum and synovial fluid resistin, leptin, interleukin (IL)1β, IL6, IL8, tumour necrosis factor α, and monocyte chemoattractant protein-1 levels were measured. The clinical activity of patients with rheumatoid arthritis was assessed according to the 28 joint count Disease Activity Score (DAS28).
Results: Resistin was detected in the synovium in both rheumatoid arthritis and osteoarthritis. Staining in the sublining layer was more intensive in patients with rheumatoid arthritis compared with those with osteoarthritis. In rheumatoid arthritis, macrophages (CD68), B lymphocytes (CD20) and plasma cells (CD138) but not T lymphocytes (CD3) showed colocalisation with resistin. Synovial fluid resistin was higher in patients with rheumatoid arthritis than in those with SpA or osteoarthritis (both $p<0.001$). In patients with rheumatoid arthritis and SpA, serum resistin levels were higher than those with osteoarthritis ($p<0.01$). Increased serum resistin in patients with rheumatoid arthritis correlated with both CRP ($r=0.53$, $p<0.02$), and DAS28 ($r=0.44$, $p<0.05$), but not with selected (adipo) cytokines.
Conclusion: The upregulated resistin at local sites of inflammation and the link between serum resistin, inflammation and disease activity suggest a role for resistin in the pathogenesis of rheumatoid arthritis.

Resistin, also known as adipocyte secreted factor or found in inflammatory zone 3, is a recently described adipocytokine that is a member of low molecular weight cystein-rich secreted peptides.[1] Resistin has been previously suggested to link obesity to the insulin resistance and type II diabetes mellitus in animal models.[2] It is expressed and secreted by adipose tissue, but has also been identified in several other tissues in rodents.[3] In contrast with animal models, immunocompetent cells rather than adipocytes seem to be the major source of resistin in humans.[4] Although several studies reported increased circulating resistin levels in human obesity and type II diabetes mellitus, these findings were not observed consistently[5–7]; moreover, serum resistin levels correlated better with the degree of subclinical inflammation than insulin resistance itself.

Rheumatoid arthritis represents the most common form of chronic inflammatory joint disease leading to cartilage and bone destruction. The inflammatory process causes diffuse thickening and hyperplasia of the rheumatoid arthritis synovium. It is infiltrated with numerous inflammatory cells that produce several proinflammatory cytokines including interleukin (IL)1, IL6 and tumour necrosis factor (TNF)α. The blockade of these factors has already led to the development of highly efficient biological treatments of rheumatoid arthritis.[8] Resistin gene expression in peripheral blood mononuclear cells (PBMCs) was shown to be upregulated, particularly on stimulation with the aforementioned proinflammatory cytokines IL1, IL6 and TNFα.[9] Moreover, it has been shown recently, that plasma resistin levels correlate significantly with inflammatory markers such as C reactive protein (CRP), IL6 and TNF receptor 2.[10] These findings may provide a novel link between elevated resistin levels and associated inflammatory processes. Furthermore, elevated levels of resistin have been shown in the synovial fluid from patients with rheumatoid arthritis and also correlated strongly with inflammatory markers such as erythrocyte sedimentation rate (ESR) and CRP.[11] In addition, resistin has also been found to be upregulated on TNFα stimulation and has been postulated as an important molecule triggering NF-κB activation and cytokine production in human peripheral blood mononuclear cells.[12] Moreover, administration of recombinant mouse resistin into the knee joints of healthy mice induced leucocyte infiltration and hyperplasia of the synovia.[12] These data support the hypothesis of resistin being an important member of the cytokine family with potent regulatory functions that might be involved in the pathogenesis of inflammatory diseases such as rheumatoid arthritis.

This study was designed to determine the difference in expression of resistin between rheumatoid arthritis and control osteoarthritis synovial tissue samples. Furthermore, serum and synovial fluid resistin levels in patients with different arthritides such as rheumatoid arthritis, spondylarthropathy (SpA) and osteoarthritis were analysed, and the hypothesis that resistin could be linked to the inflammation and/or disease activity of rheumatoid arthritis was evaluated.

PATIENTS AND METHODS

Patient characteristics

In all, 20 patients with active rheumatoid arthritis who met the 1987 revised criteria of the American College of

Abbreviations: DAS28, Disease Activity Score 28; DMARDs, disease-modifying antirheumatic drugs; SpA, spondylarthropathy; TNF, tumour necrosis factor

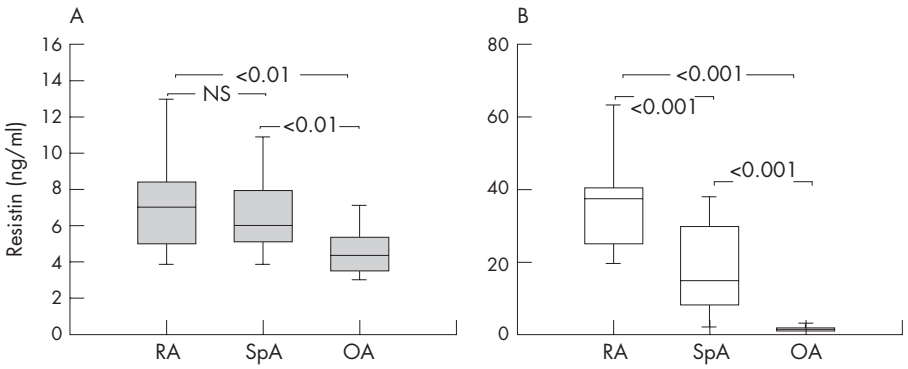

Figure 3 Serum (A) and synovial fluid (B) levels of resistin in patients with rheumatoid arthritis (RA), spondylarthropathy (SpA) and osteoarthritis (OA). The horizontal bar within the box represents the median; the boxes represent a range of ±25% around the median. Vertical bars indicate 95% CI.

of the disease. It has been demonstrated previously that circulating levels of other adipocytokines such as leptin and adiponectin in patients with rheumatoid arthritis were not affected by treatment with biologicals.[19]

Resistin was expressed in numerous cell types within the synovial tissue in our study, including macrophages, which have been identified as a source of resistin by Jung *et al*.[17] Here we demonstrate that other cell types such as plasma cells, B lymphocytes and synovial fibroblasts can also produce resistin. Moreover, in an ex vivo setting, we were able to show much higher resistin levels in eluates from rheumatoid arthritis in contrast with osteoarthritis synovial tissue samples when incubated with PBS. These findings support the idea that resistin may represent a novel secreted signalling molecule, that could be involved in the activation of the above-mentioned cell types during chronic inflammatory processes such as rheumatoid arthritis. We have previously shown that other inflammatory conditions such as hepatitis C or B also significantly increase circulating resistin levels.[20]

Resistin was originally discovered as a potential link between obesity and insulin resistance in rodents, and its role, especially in the development of liver insulin resistance has been clearly documented by later experimental studies.[21] On the contrary, its exact pathophysiological role in humans is still a matter of debate. Our data, together with previously published papers, suggest that in humans resistin is more directly related to inflammation, whereas its relationship with insulin resistance has been documented only in some but not in all studies. For example, it was shown that, in vitro, resistin expression can be upregulated upon proinflammatory stimuli.[9 12] Moreover, several inflammatory markers correlated well with plasma resistin levels in patients with metabolic disorders.[10] In our study, serum resistin postitively correlated with ESR and CRP, but not with other pro-inflammatory cytokines such as IL6, IL8, TNFα or MCP-1. While synovial fluid resistin levels in patients with rheumatoid arthritis were markedly higher than its serum counterparts, the opposite was true for another adipocytokine leptin. Leptin concentrations were higher in systemic circulation than locally in synovial fluid, and it was related neither to resistin levels nor to other proinflammatory markers in body fluids from patients with rheumatoid arthritis. In agreement with Schäffler *et al*,[11] we found significantly higher levels of resistin in the synovial fluid in the case of rheumatoid arthritis than in the case of osteoarthritis. Interestingly, we also detected higher serum resistin levels in patients with rheumatoid arthritis in contrast with control patients with osteoarthritis. This finding is in disagreement with recently published data[12 22] showing no significant difference in blood resistin levels between patients with rheumatoid arthritis and healthy controls. The explanation for higher resistin levels in patients with rheumatoid arthritis from our study group may lie in the fact that our group consisted of patients with rheumatoid arthritis with more severe disease course as measured by acute-phase reactants and disease activity score. This is further supported by our finding that serum resistin levels correlated not only with inflammatory status (ESR, CRP) but also with the clinical disease activity (DAS28) in patients with rheumatoid arthritis. On the other hand, we did not see a significant relationship between increased synovial fluid resistin and inflammatory markers as shown previously.[11 12] Furthermore, we did not confirm speculations that serum resistin levels would be reflected by those in synovial fluid in inflammatory arthritides.[23] Taken together, it can be concluded that the joint compartment represents a major site of resistin production in patients with rheumatoid arthritis. Resistin levels in synovial fluid might reflect both the intensity of the inflammatory infiltrates within synovial tissue and the number of inflammatory cells within the synovial fluid. Since rheumatoid arthritis represents a condition with polyarticular involvement, we suggest that serum resistin levels could be more relevant to

Table 2 Serum and synovial fluid levels of selected adipose- and inflammatory-derived markers, and their correlations with resistin in patients with rheumatoid arthritis

	Serum (n = 20)	P1	Synovial fluid (n = 20)	P2
IL1β (pg/ml)	NA	NA	NA	NA
IL6 (pg/ml)	24.9 (31.3)	0.56	8190.8 (4257.8)	0.83
IL8 (pg/ml)	7.9 (7.3)	0.84	2413.5 (1528.7)	0.24
Leptin (ng/ml)	15.2 (19.4)	0.89	11.5 (13.2)	0.62
TNFα (pg/ml)	6.8 (23.5)	0.70	10.8 (15.2)	0.13
MCP-1 (pg/ml)	275.1 (177.4)	0.45	426.1 (769.7)	0.60
CRP (mg/l)	66.1 (40.8)	<0.02	NE	NE
ESR (mm/1st h)	35 (24)	0.03	NE	NE

CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; MCP, monocyte chemoattractant protein; NE, not evaluated; NA, not applicable; P1, correlation between resistin and selected markers in serum; P2, correlation between resistin and selected markers in synovial fluid; TNF, tumour necrosis factor.

systemic inflammation and/or disease activity, whereas synovial fluid resistin reflects the particular inflammatory process of the affected joint.

Adipose tissue in males expresses higher levels of resistin than in females as shown in animal models.[3] However, we did not find any influence of sex or age with regard to the resistin levels in human body fluids, which is in agreement with the previous study by Schäffler *et al*.[24] Although we observed no influence of BMI on resistin levels, it can support the idea that resistin may be the link to inflammatory processes rather than to obesity or insulin resistance in humans. Moreover, numerous hormonal factors including glucocorticoids can regulate resistin levels. As glucocorticoids can increase resistin production,[25] we could speculate that the increased resistin level in patients with rheumatoid arthritis is the result of glucocorticoid treatment. To further assess the possible influence of glucocorticoids on resistin level, we compared the subgroups with and without glucocorticoid treatment and found no significant differences between these two groups in terms of resistin levels. We thus suggest that mechanism(s) other than stimulation by exogenous glucocorticoids is (are) responsible for the increase of resistin levels in patients with rheumatoid arthritis. With regard to the recently published data by Bokarewa *et al*,[12] the role of proinflammatory cytokines and a positive feedback loop (resistin itself) can be hypothesised as a cause for the upregulation of resistin under inflammatory conditions such as rheumatoid arthritis. Furthermore, exogenous resistin induced NF-κB activation, resulting in a strong upregulation of proinflammatory cytokines such as TNFα or IL6, and, when injected into healthy murine joints, induced synovial pannus formation and cartilage destruction.[12]

It has to be noted that the present study has several limitations. Firstly, it was designed as a cross-sectional study with a relatively low number of enrolled patients, and hence the role of DMARDs on the resistin levels in follow-up could not be determined due to this cross-sectional character. Secondly, the synovial tissue samples were obtained at the time of both arthroscopy and open joint surgery in our study. As recent work showed increased cell infiltration, expression of proinflammatory cytokines, matrix-degrading enzymes and growth factors in synovium obtained by arthroscopy in contrast with end stage destructive rheumatoid arthritis synovium obtained by total joint replacement,[26] the possible influence of the tissue harvesting procedure on resistin expression has also to be taken into account.

In summary, we have shown markedly increased production of resistin at local sites of inflammation such as synovial tissue and synovial fluid in patients with rheumatoid arthritis. This local overproduction of resistin was also reflected by increased circulating resistin levels in patients with rheumatoid arthritis compared with those with osteoarthritis. Resistin was produced not only by activated macrophages but also by synovial fibroblasts and several other inflammatory cell types. The link between increased serum resistin, inflammation and disease activity of rheumatoid arthritis suggests a role of resistin as a novel proinflammatory mediator and supports the idea that, except for adiponectin,[27] resistin may also play a role in chronic joint inflammatory diseases.

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