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# 5

## Metabolic stress–induced inflammation plays a major role in the development of osteoarthritis in mice

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## Abstract

*Objective:* Obesity is associated with systemic inflammation and is a risk factor for osteoarthritis (OA) development. We undertook this study to test the hypothesis that metabolic stress-induced inflammation, and not mechanical overload, is responsible for the development of high-fat diet-induced OA in mice.

*Methods:* Human C-reactive protein (CRP)-transgenic mice received a high-fat diet without or with 0.005% (weight/weight) rosuvastatin or 0.018% (w/w) rosiglitazone, 2 different drugs with antiinflammatory properties. Mice fed chow were included as controls. After 42 weeks, mice were killed and histologic OA grading of the knees was performed. To monitor the overall inflammation state, systemic human CRP levels were determined.

*Results:* Male mice on a high-fat diet had significantly higher OA grades than mice on chow and showed no correlation between OA severity and body weight. In male mice, high-fat diet-induced OA was significantly inhibited by rosuvastatin or rosiglitazone to OA grades observed in control mice. Both treatments resulted in reduced human CRP levels. Furthermore, a positive correlation was found between the relative individual induction of human CRP evoked by a high-fat diet on day 3 and OA grade at end point.

*Conclusion:* High-fat diet-induced OA in mice is due to low-grade inflammation and not to mechanical overload, since no relationship between body weight and OA grade was observed. Moreover, the OA process was inhibited to a great extent by treatment with 2 drugs with antiinflammatory properties. The inflammatory response to a metabolic high-fat challenge may predict individual susceptibility to developing OA later in life. The use of statins or peroxisome proliferator-activated receptor  $\gamma$  agonists (e.g., rosiglitazone) could be a strategy for interfering with the progression of OA.

Osteoarthritis (OA) is a chronic degenerative joint disease with large consequences for the quality of life of patients. It is now generally accepted that OA is not only a disease of articular cartilage, but in fact involves the entire joint, including Hoffa's fat pad, synovium, subchondral bone, menisci, and ligaments. Insight into the different underlying processes leading to the clinical and pathologic outcomes of OA is crucial in the search for new therapies (1, 2).

Obesity is a risk factor for the development of OA and is classically seen as a biomechanical factor, suggesting that the increase of loading forces causes cartilage damage. However, from the association between obesity and OA of non-load bearing joints it is hypothesized that systemic factors induced by obesity contribute considerably to the initiation and progression of OA (3). Obesity is associated with a mild chronic inflammation, and adipokines secreted by adipocytes and macrophages within adipose tissue are suggested to be a metabolic link between obesity and OA (4, 5). However, the relative contribution of these processes in the onset and progression of OA remains unclear.

The association between obesity and the development of OA has been studied in several animal models. Previously, Sokoloff et al showed that high-fat diet-induced obesity caused OA in 2 different mouse strains and in a rat strain. In addition, STR/Ort mice, which are vulnerable to spontaneous obesity, developed OA in a short period of time (6). The incidence of knee OA was found to be twice as high in C57BL/6 mice on a high-fat diet than in the control group fed with a chow diet. Changes associated with the human metabolic syndrome such as abdominal obesity, hyperglycemia, hyperinsulinemia, and hypertension are also present in these animals (7, 8). Although some of these diet-induced OA mouse model studies support a role of mechanical factors in the progression of OA, a correlation between body weight and OA is lacking. The fact that adipose tissue of C57BL/6 mice can become inflamed under conditions of metabolic stress (e.g., high-fat diet feeding) and secrete a broad spectrum of inflammatory mediators (9) suggests that high-fat diet-fed C57BL/6 mice constitute a suitable model to study the role of (metabolic stress-induced) inflammation in the development of OA.

The human C-reactive protein (CRP)-transgenic mouse model enables us to study the role of inflammation in mice. CRP is an acute-phase protein and an established

marker for systemic inflammation in humans, but not in wild-type mice, where its serum levels never rise above 2–3 mg/liter (10). CRP is produced by hepatocytes and adipocytes and is regulated by proinflammatory cytokines. Human CRP–transgenic mice carry the entire human CRP gene, including the coding region and 17 kb of the 5′-flanking promoter region, and the expression of human CRP in these mice resembles its regulation in humans. Unlike its wild-type mouse counterpart, human CRP behaves as a major acute-phase reactant when introduced into the mouse genome (11). Obese individuals are found to have higher CRP levels, indicating a low-grade inflammation state (12, 13). Obese human CRP–transgenic mice on a C57BL/6 background are therefore an interesting model for analyzing the role of inflammation in the development of OA. Modulation of this metabolic stress–induced inflammation provides a tool to gain more insight into the relevant contribution of this process in OA.

Statins and peroxisome proliferator–activated receptor  $\gamma$  (PPAR $\gamma$ ) agonists have been shown to have important pleiotropic antiinflammatory effects in a number of studies and are therefore interesting interventions for studying the role of inflammation in OA (14–16). Statins are inhibitors of the rate-determining enzyme in the biosynthesis of cholesterol and are considered the most effective drugs to reduce serum cholesterol levels (17). PPAR $\gamma$  receptors are nuclear hormone receptors and are expressed at high levels in adipose tissue (18). PPAR $\gamma$  agonists bind to and activate these receptors and are used for the treatment of type 2 diabetes mellitus. In recent studies, PPAR $\gamma$  ligands were shown to have a wide spectrum of actions in the treatment of metabolic disorders (19, 20).

Statin or PPAR $\gamma$  agonist therapy for OA has been suggested in the literature (19, 21, 22); however, *in vivo* data demonstrating the use of these drugs are scarce. Treatment with statins or PPAR $\gamma$  agonists in animals with experimentally induced OA resulted in reduced cartilage degradation, probably due to decreased inflammatory cell infiltration and matrix-degrading enzyme expression (23–25). However, their effect in high-fat diet–induced OA has not been investigated.

In this study, we hypothesize that high-fat diet–induced OA in mice is not due to mechanical overload, but is the consequence of metabolic stress–induced inflammation. We used the human CRP–transgenic mouse model to monitor the

overall inflammation state during the high-fat diet. Interventions with statins and PPAR $\gamma$  agonists were applied to suppress metabolic stress-induced inflammation to demonstrate that this process is involved in the pathogenesis of OA under conditions of obesity and the metabolic syndrome.

## Methods

### Animals

Human CRP-transgenic mice (11, 26) on a C57BL/6 background were characterized by polymerase chain reaction and enzyme-linked immunosorbent assay (ELISA) for human CRP expression. Mice were housed in groups under standard conditions on a 12-hour light/dark cycle and had free access to water and food. Experiments were approved by the Institutional Animal Care and Use Committee of TNO and were in compliance with European Community specifications regarding the use of laboratory animals.

### Diets

Human CRP-transgenic 12-week-old male and female mice were fed standard lab chow (V1534; Ssniff Spezialdiäten) (time 0). After 4 weeks (time 4) the diets were changed, and mice were randomly distributed in 3 groups consisting of 9 male and 9 female mice. Group 1 consisted of control mice that remained on a standard chow diet. Mice in group 2 were switched to a high-fat diet (D12451, 23.1% fat; Ssniff Spezialdiäten), and mice in group 3 were fed a high-fat diet containing 0.018% (weight/weight) rosiglitazone (GlaxoSmithKline). Besides these 3 groups, another group (group 4) was added that received chow supplemented with 0.01% (w/w) rosuvastatin (AstraZeneca) (time 0), which was continued in a high-fat diet after 4 weeks but at a lower dosage of 0.005% (w/w) because of increased absorption of rosuvastatin on high-fat diets. Rosuvastatin and rosiglitazone doses were based on their antiinflammatory effects observed in previous studies (27, 28). Chow-fed control mice (group 1), untreated mice on a high-fat diet (group 2), and treated mice on a high-fat diet (groups 3 and 4) remained on their respective diets until the completion of the study at age 54 weeks (time 42).

### **Histologic examination**

At end point (time 42), knee joints of the hind limbs were fixed in a 10% neutral buffered formalin solution (Sigma-Aldrich), decalcified in Kristensen's solution (29), dehydrated, and embedded in paraffin for histologic analysis. Serial coronal 5  $\mu\text{m}$  sections were collected throughout the patella, medial and lateral joint. Sections were stained with hematoxylin, fast green, and Safranin O as well as with hematoxylin–phloxine–safron. Sections were randomly scored in a blinded manner at 3 locations in the joint: the femorotibial joint at the lateral side, the femorotibial joint at the medial side, and the patellofemoral joint. The scoring system included changes in articular cartilage structure, proteoglycan depletion, and chondrocyte morphology. The whole joint was scored following the international guidelines of the Osteoarthritis Research Society International (OARSI) histologic grading system (30). Briefly, this scoring system is based on a combined assessment of severity (from grade 0 = surface and cartilage intact to grade 6 = deformation) and extent (from stage 0 = no OA activity to stage 4 = >50% OA activity) of OA in the articular cartilage (grade by stage).

### **Human CRP levels**

To monitor metabolic stress–induced inflammatory changes and the antiinflammatory effects of rosuvastatin and rosiglitazone using human CRP levels, after 4 hours of food deprivation blood was obtained by tail incisions and collected in EDTA tubes for plasma preparation (Sarstedt) at times 0, 2, 4, 4.5, 5, 11, and 34 weeks. Tubes were centrifuged for 10 minutes at 6,000 revolutions per minute, and plasma was immediately stored at  $-80^{\circ}\text{C}$  until analysis. Human CRP was quantified in the plasma samples by established ELISA (R&D Systems).

To characterize the inflammatory responsiveness of the mice and to verify the antiinflammatory effects of rosuvastatin and rosiglitazone, all groups on high-fat diets were injected intraperitoneally (IP) with 100  $\mu\text{l}$  interleukin-1 $\beta$  (IL-1 $\beta$ ; PeproTech) at a mild dose ( $\sim$ 200 ng) 2 weeks before (time 2) and 8 weeks after (time 12) diet switch. The concentration used in this study is negligible compared with that used in previous models in which IL-1 was injected intraarticularly into the mouse knee joint (31). Plasma samples were collected prior to and 18 hours after this challenge and used to quantify the human CRP level.

### Cytokine measurements

At end point, blood was collected by heart puncture and centrifuged for 5 minutes at 10,000 rpm. Serum samples were immediately stored at  $-80^{\circ}\text{C}$ . Levels of the cytokines IL-6, leptin, resistin, and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) were measured in the serum samples using a multiplex bead immunoassay (Millipore) for mice with the Luminex 100 instrument. All samples were analyzed as recommended by the manufacturer.

### Insulin levels

To determine the effects of high-fat diet and treatment on insulin, levels were measured at times 5 and 11 in plasma samples using an ELISA (Mercodia).

### Statistical analysis

Statistical analysis was performed using the nonparametric Kruskal-Wallis and Mann-Whitney U tests. Pearson's correlation coefficient was used to assess the relationship between OA grade and human CRP levels. P values less than 0.05 were considered significant. Unless stated otherwise, data are shown as the mean  $\pm$  SD.

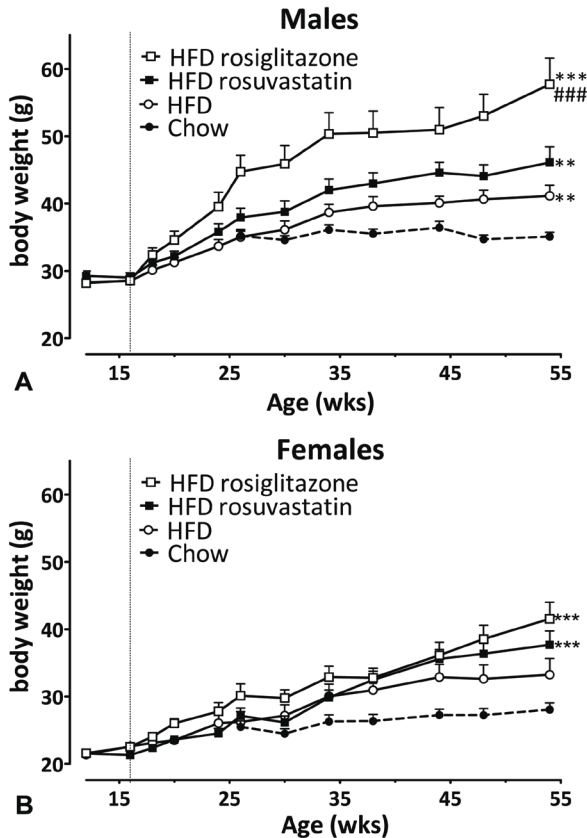
## Results

### Body weight and body fat

Male mice in all the high-fat diet groups (treated and untreated) had significantly higher mean  $\pm$  SEM body weights at the end of the study compared to the chow-fed control group. Male mice treated with rosiglitazone had the highest mean  $\pm$  SEM body weight due to an increased fat accumulation, and this weight was significantly higher than that in the untreated high-fat diet group (untreated high-fat diet group  $41.4 \pm 1.6$  grams,  $P < 0.01$  versus chow-fed control group [ $35.1 \pm 0.6$  grams]; high-fat diet with rosuvastatin group  $46.1 \pm 2.3$  grams,  $P < 0.01$  versus chow-fed control group; high-fat diet with rosiglitazone group  $57.7 \pm 3.9$  grams,  $P < 0.001$  versus chow-fed control group and untreated high-fat diet group) (Figure 1A). Female mice on a high-fat diet also had increased body weights compared to the chow-fed control group (untreated high-fat diet group  $33.2 \pm 2.4$  grams,  $P$  not significant versus chow-



fed control group [ $28.0 \pm 1.0$  grams]; high-fat diet with rosuvastatin group  $37.7 \pm 2.0$  grams,  $P < 0.001$  versus chow-fed control group; high-fat diet with rosiglitazone group  $41.6 \pm 2.5$  grams,  $P < 0.001$  versus chow-fed control group) (Figure 1B).



**Figure 1.** Body weights in each group during the study of male (A) and female (B) human C-reactive protein–transgenic mice. Male mice had a higher average body weight compared to female mice. Values are the mean  $\pm$  SEM. \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$  versus chow-fed control group. ### =  $p < 0.001$  versus untreated high-fat diet (HFD) group.

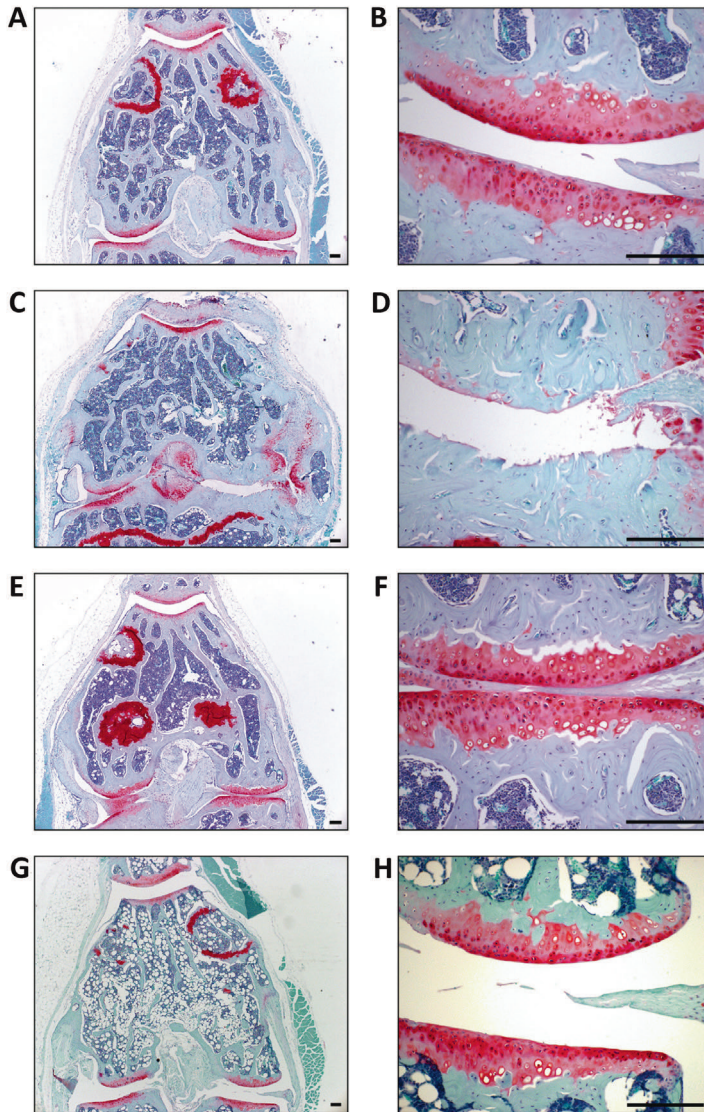
Gonadal, visceral, and subcutaneous fat content of each mouse was summed together to obtain the total fat content and was subsequently expressed as a percentage of the body mass of each mouse at end point. Control female mice on chow had a relatively higher mean  $\pm$  SD percentage of fat than did male mice on chow ( $5.0 \pm 4.1\%$  versus

3.4 ± 1.0%;  $P < 0.05$ ). High-fat diet groups (treated and untreated) had significantly higher percentages of fat than did the chow-fed control group, both for male mice (untreated high-fat diet group 7.1 ± 1.4%, high-fat diet with rosuvastatin group 8.5 ± 3.1%, high-fat diet with rosiglitazone group 7.7 ± 1.4%; all  $P < 0.001$  versus chow-fed control group) and for female mice (untreated high-fat diet group 9.2 ± 3.0%,  $P < 0.001$  versus chow-fed control group; high-fat diet with rosuvastatin group 12.4 ± 2.6%,  $P < 0.001$  versus chow-fed control group; high-fat diet with rosiglitazone group 6.5 ± 1.5%,  $P < 0.05$  versus chow-fed control group).

### **Effects of high-fat diet and antiinflammatory interventions on OA development**

On macroscopic evaluation, male mice from the untreated high-fat diet group had red, swollen knee joints with malformed morphology, which was not present in male mice from the chow-fed control group. High-fat diet groups treated with rosuvastatin or rosiglitazone did not have such macroscopic alterations. No macroscopic abnormal joint morphologies were observed in any female mice in the chow-fed control group or in the untreated or treated high-fat diet groups.

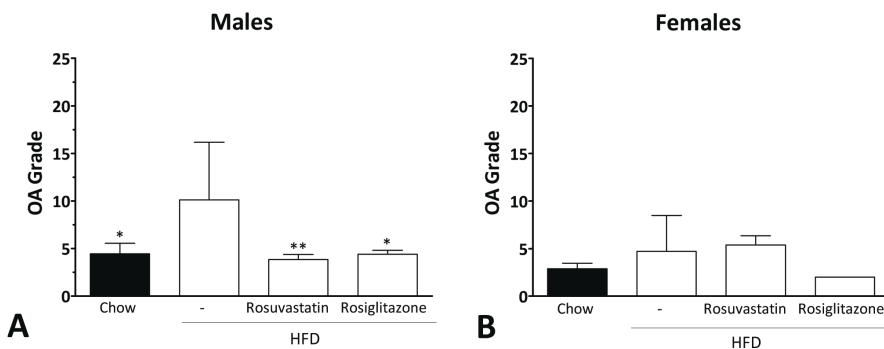
To determine microscopic differences, hematoxylin–, fast green–, and Safranin O–stained slides as well as hematoxylin–phloxine–safron–stained slides of all knee joints were scored on different components. All joints had at least some minor OA changes such as slight fibrillation, hypocellularity, and Safranin O loss (Figure 2). Using the OARSI grading system, male and female mice on chow had comparable low mean ± SD OA grades (4.4 ± 2.9 versus 2.9 ± 1.6, respectively;  $P = 0.3$ ). Male mice receiving a high-fat diet instead of chow had significantly higher OA grades at end point. Severely affected mice had cartilage loss, bone deformation, and osteophyte formation (Figures 2C and D), while the joints of treated mice were comparable to those of their control counterparts (Figures 2E–H).



**Figure 2.** Representative Safranin O-, fast green-, and hematoxylin-stained coronal sections of the whole knee joint (A, C, E, and G) with a detailed picture of the medial femoral condyle and tibial plateau (B, D, F, and H) of human C-reactive protein-transgenic male mice in the chow-fed control group (A and B), the untreated high-fat diet group (C and D), the high-fat diet with rosuvastatin group (E and F), and the high-fat diet with rosiglitazone group (G and H). Bars = 2 mm. Original magnification  $\times 4$  in A, C, E, and G;  $\times 20$  in B, D, F, and H.

Modulation of metabolic stress-induced inflammation with rosuvastatin or rosiglitazone in male mice on high-fat diets resulted in completely reduced mean  $\pm$  SD OA grades (high-fat diet with rosuvastatin group  $3.8 \pm 1.3$ ,  $P < 0.01$  versus untreated high-fat diet group [ $10.1 \pm 6.0$ ]; high-fat diet with rosiglitazone group  $4.4 \pm 0.9$ ,  $P < 0.05$  versus untreated high-fat diet group) (Figure 3A). Remarkably, OA grades observed in the treatment groups were comparable to those in mice fed with chow. In contrast to male mice, untreated female mice receiving high-fat diets hardly developed OA, as judged from their OA grades, which were comparable to those of their chow-fed controls. Treatment with rosuvastatin and rosiglitazone in female mice yielded mean  $\pm$  SD OA grades similar to those of the untreated high-fat diet group (untreated high-fat diet group  $4.7 \pm 3.7$ , high-fat diet with rosuvastatin group  $5.4 \pm 2.7$ , high-fat diet with rosiglitazone group  $2.0 \pm 0.0$ ) (Figure 3B). Analysis of the individual components (lateral and medial femorotibial joint, patellofemoral joint) for cartilage structure, chondrocyte morphology, and Safranin O staining summed together showed results similar to those of the OA score (OARSI grade by stage) for the whole joint.

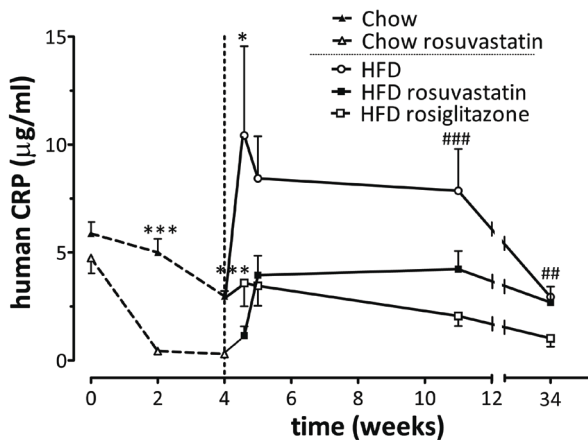
To investigate a possible influence of mechanical forces, body weight and body fat were correlated with OA development. OA grades of male mice in the untreated high-fat diet group were not significantly correlated with body weight or body fat, ruling out mechanical stress and fat mass as important factors for OA in this model.



**Figure 3.** Histologic osteoarthritis (OA) grade of the knee joints of male (A) and female (B) human C-reactive protein-transgenic mice. Rosuvastatin and rosiglitazone decreased the OA grade in male mice significantly. There were no significant differences in female mice ( $p = 0.13$  by Kruskal-Wallis test). Values are the mean  $\pm$  SD. \* =  $p < 0.05$ ; \*\* =  $p < 0.01$  versus untreated high-fat diet (HFD) group.

### Effects of high-fat diet and antiinflammatory interventions on human CRP, cytokine, and insulin levels

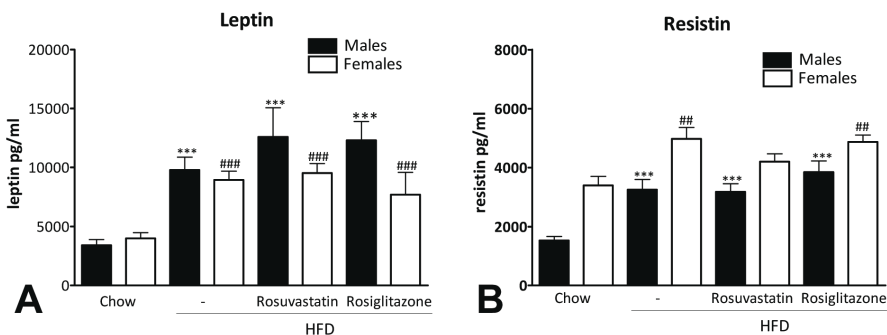
Human CRP levels were measured during the experiment to monitor the overall inflammation state during high-fat diets as well as the effects of intervention with rosuvastatin and rosiglitazone. Before diet switch, all male mice on chow had similar baseline human CRP levels. In all cases, the switch to high-fat diets resulted in an increase in human CRP levels. Although not significant at every analyzed time point, rosiglitazone- and rosuvastatin-treated mice had lower human CRP levels than those in untreated mice on a high-fat diet. At a later phase of the study (time 34), human CRP levels decreased back to baseline levels (Figure 4). All female mice had an ~1,000-fold lower level of human CRP compared to male mice, and there was no effect of rosuvastatin or rosiglitazone (data not shown).



**Figure 4.** Human C-reactive protein (CRP) levels in male mouse plasma at several time points during the study. Values are the mean  $\pm$  SD. Dashed lines represent groups before diet switch. Overall, rosiglitazone- and rosuvastatin-treated mice showed lower human CRP levels compared to untreated mice on a high-fat diet (HFD) after diet switch (vertical and horizontal dotted lines), although the difference was not significant at every time point. At times 2 and 4 weeks, untreated mice in the chow-fed control group showed significantly higher human CRP levels compared to rosuvastatin-treated mice in the chow-fed control group. \*\*\* =  $p < 0.001$  versus rosuvastatin-treated mice in the chow-fed control group. At time 4.5 weeks, untreated mice on a high-fat diet showed significantly higher human CRP levels compared to rosuvastatin-treated mice on a high-fat diet. \* =  $p < 0.05$  versus rosuvastatin-treated mice on a high-fat diet. At times 11 and 34 weeks, untreated mice on a high-fat diet showed significantly higher human CRP levels compared to rosiglitazone-treated mice. ## =  $p < 0.01$ ; ### =  $p < 0.001$  versus rosiglitazone-treated mice. Human CRP levels in female mice were 1,000-fold lower (data not shown).

To assess obesity-related cytokines, a multiplex assay was performed on serum samples collected from male and female mice at end point. Levels of IL-6 and TNF $\alpha$  were below the limit of detection. Leptin and resistin levels were high and increased in high-fat diet groups (treated and untreated) compared to those in the respective chow-fed control groups (Figures 5A and B). Treatment with rosuvastatin or rosiglitazone did not significantly alter leptin or resistin levels compared to those in the untreated high-fat diet group. No apparent differences between male and female mice were observed for leptin and resistin levels. No significant correlations were found in any of the groups between leptin or resistin levels and OA grade at end point (data not shown).

Insulin levels were measured to detect the effects of high-fat diet and intervention with rosuvastatin or rosiglitazone on the development of insulin resistance in this mouse model. At time 5, 1 week after diet switch, mean  $\pm$  SD insulin levels were significantly suppressed in rosiglitazone-treated male mice on a high-fat diet compared to those in untreated male mice on a high-fat diet ( $0.5 \pm 0.2$   $\mu$ g/liter versus  $0.9 \pm 0.5$   $\mu$ g/liter;  $P < 0.004$ ). Treatment with rosuvastatin did not alter insulin levels ( $1.1 \pm 0.6$   $\mu$ g/liter) compared to those in untreated mice on a high-fat diet. The same effects were still observed at time 11. Insulin levels in female mice were very low, and there were no significant differences between groups.



**Figure 5.** Serum cytokine concentrations of leptin (A) or resistin (B) at end point (time 42 weeks) in chow-fed control mice, untreated mice on a high-fat diet (HFD), and mice on a high-fat diet treated with rosuvastatin or rosiglitazone. Values are the mean  $\pm$  SD. \*\*\* =  $P < 0.001$  versus chow-fed control male mice. ## =  $P < 0.01$ ; ### =  $P < 0.001$  versus chow-fed control female mice.

### Initial response to metabolic high-fat stress correlates with OA at end point

The initial response to metabolic high-fat stress (during the first days of high-fat diet feeding) was determined by calculating the difference in plasma levels of human CRP before and after diet switch. We correlated individual changes in human CRP level with individual OA grades at end point (Table 1). The initial response to high-fat diet feeding (3 days on a high-fat diet; time 4.5 weeks) was significantly correlated with OA. This relationship was lost for human CRP levels determined at later time points, indicating that the inflammatory response evoked by a metabolic stressor predicts susceptibility to developing OA at the level of an individual subject. This notion was further substantiated by demonstrating that no correlation was found between OA and change in human CRP level when human CRP was evoked by a nonmetabolic inducer of inflammation (200 ng IL-1 injected IP) (data not shown).

**Table 1.** Correlation between relative individual induction of huCRP levels (compared to t=4 weeks; diet switch) and individual end point OA grades.

Mouse	t#	$\Delta$ huCRP level $\mu\text{g/ml}$ weeks (t)			OA grade	
		4.5	5	11	34	42
1		8.8	3.9	17.6	1.2	9
2		16.3	16.2	13.5	4.1	15
3		37.5	5.5	4.9	1.1	20
4		-1.2	4.3	1.4	-0.8	4
5		9.0	11.2	3.7	-1.1	18
6		4.1	10.1	1.1	0.3	6
7		-0.6	0.8	3.3	-0.4	6
8		-0.6	2.5	2.1	0.6	9
9		-0.1	0.9	2.5	0.6	4
<b>Mean <math>\pm</math> SEM</b>		8.1 $\pm$ 4.2	6.1 $\pm$ 1.8	5.6 $\pm$ 2.0	0.6 $\pm$ 0.5	10.1 $\pm$ 2.0
<b>correlation</b>						
$\Delta$ CRP-OA grade		<b>0.83**</b>	0.54	0.28	0.30	

Values are tested with a 2-tailed Pearson correlation test. Only correlation directly after diet switch (t=4.5) was statistically significant \*\* $p < 0.01$

## Discussion

Obesity is associated with low-grade systemic inflammation and is a risk factor for OA development. Whether this mild chronic inflammation or other factors such as mechanical stress are responsible for the development of OA is under debate.

This study provides evidence for a role of metabolic stress–induced systemic inflammation in the development of OA. High-fat diet feeding in human CRP–transgenic mice led to severe OA development compared to chow-fed control mice. We found no correlation between OA and individual body weight or fat mass, which excludes mechanical stress as a major trigger of OA in the present experimental setting. Instead, we demonstrated that antiinflammatory intervention with either rosuvastatin or rosiglitazone suppressed OA development to a great extent. This strongly suggests that metabolic stress–induced inflammation plays a major role in the onset and progression of high-fat diet–induced OA in mice.

In the clinic, statins are used as cholesterol-lowering drugs, and PPAR $\gamma$  agonists are administered as treatments for type 2 diabetes mellitus (17, 20). These different types of drugs only have antiinflammatory properties in common. Therefore, it is most likely that these drugs exert their inhibitory effect on high-fat diet–induced OA through suppression of low-grade systemic inflammation associated with obesity and metabolic stress. Indeed, both treatments resulted in decreased levels of human CRP, which served as a marker of inflammation in this study. This is in line with previously reported data demonstrating that statins reduce basal and IL-1 $\beta$ –induced human CRP expression independent of their cholesterol-lowering effect (12). This implies that systemic inflammatory factors play an important role in the development of high-fat diet–induced OA.

A primary role for inflammation in general in the pathogenesis of OA is also supported by the finding that a statin and a PPAR $\gamma$  agonist suppressed mechanically induced OA in animal models by interfering with local inflammatory processes. Intraarticular administration of mevastatin reduced inflammatory cell infiltration and matrix-degrading enzyme expression in a rabbit model of experimental OA (23). Furthermore, Boileau et al showed an inhibitory effect of pioglitazone (a PPAR $\gamma$  agonist) on the activation of signaling pathways of inflammation in a dog model of experimental OA (24). In addition to models of mechanically induced OA, Yudoh and Karasawa recently demonstrated significantly reduced cartilage degeneration due to statin treatment in STR/Ort mice, a strain that spontaneously develops OA (32). In vitro data suggest that statins and PPAR $\gamma$  agonists have several direct effects on chondrocytes or synoviocytes. For example, statins inhibited matrix metalloproteinase



3 (MMP-3) expression in stimulated human OA chondrocytes and also inhibited apoptosis of synoviocytes. Furthermore, PPAR $\gamma$  agonists suppressed the expression of inducible nitric oxide synthase and MMP-13 in human chondrocytes as well as the expression of MMP-1 in human synovial fibroblasts (21, 22, 33, 34). Stimulation with IL-1 in these in vitro experiments suggests that induction of inflammatory pathways is important for these outcomes and explains the effect of statins and PPAR $\gamma$  agonists on these OA-related processes through their antiinflammatory action.

The adipokines leptin and resistin are important systemic factors that are associated with obesity and suggested to be involved in the development of OA (35). Griffin et al showed in leptin-deficient (*ob/ob*) and leptin receptor-deficient (*db/db*) mice that body fat adiposity in the absence of leptin signaling is insufficient to induce systemic inflammation and knee OA (36). Consistent with this, we recently showed that quality of adipose tissue (and not its mass) is linked to systemic inflammation status (37). This confirms that proinflammatory factors induced by fat gain are more important in our model than mechanical overload due to an increase in body mass, although previously an association was suggested between body mass and fat and the loss of cartilage matrix proteoglycans in C57BL/6 mice on a high-fat diet (38). The proposed proinflammatory effect of leptin would match very well with increased serum leptin levels in the high-fat diet model and the involvement of inflammation in high-fat diet-induced OA. However, conclusive evidence for this is lacking, since we found no correlation between severity of OA and circulating leptin levels at end point. Furthermore, rosuvastatin and rosiglitazone treatment did not reduce leptin levels, indicating that if leptin is involved, the inhibitory effect of these treatments is not by acting directly on leptin, but probably by influencing downstream inflammatory processes regulated by leptin.

Obesity is also associated with the development of insulin resistance (39). All mice developed higher insulin levels after switching to a high-fat diet except for those treated with rosiglitazone. The antidiabetic property of rosiglitazone is thereby confirmed in this model, but the insulin-lowering capacity is most likely not the cause of the suppressive effect seen on OA development. Since rosuvastatin-treated mice display insulin levels comparable to those in untreated mice, insulin resistance as part of the metabolic syndrome cannot be used to clarify the inhibitory effect of these drugs on OA development in this model.

Besides variation in body weight, the human CRP levels, which were used to monitor inflammation status, also varied between mice of the same group. The variation seen in our study is typical for human CRP–transgenic mice, and similar variation has been reported previously (40, 41). In several human studies, human CRP level is correlated with various features of OA, such as extent and severity of knee OA, knee pain or markers of local synovial inflammation, and knee OA progression. However, human CRP level is not associated with incidence of knee or hip OA when possible confounding factors such as body mass index are taken into account, suggesting that human CRP is not a functional protein in the onset of OA (42, 43).

In our study, we found a correlation between OA grade at end point and change in human CRP level 3 days after diet switch, which suggests that the responsiveness to a metabolic challenge can be used as a marker to estimate the individual susceptibility to developing OA later in life. Consistent with this, we showed that the acute response to high-fat diet–induced stress results in a transient inflammatory response that normalizes as soon as metabolically active organs have adapted their glucose and lipid metabolism (44). Indeed, after several weeks, human CRP levels decreased to baseline levels, and the correlation with OA grade was lost. Since only 9 male mice were in this group, an increased number should be investigated to confirm these results. Our finding of no correlation between human CRP level and OA when an inflammatory response was evoked by IL-1 suggests that metabolic stress–induced inflammation encompasses more inflammatory signaling routes than only those triggered by IL-1.

It is known that in different inbred strains, male mice have a higher susceptibility to developing OA than do female mice (45–47). Concordant with this, in our study only male mice developed substantial and severe OA characteristics after 38 weeks on a high-fat diet. Also, it is well established that female mice on the C57BL/6 background are less sensitive to developing features of the metabolic syndrome (obesity, adiposity, metabolic inflammation, and the like) than are their male counterparts (48). Highlighting this is the fact that insulin levels, as a measure of diabetes, were very low in female mice compared to male mice on a high-fat diet. This indicates that metabolic stress is more important than age-dependent processes on the development of OA in this model.

The present study demonstrates that low-grade inflammation associated with obesity plays an important role in the development of high-fat diet–induced OA, since rosuvastatin and rosiglitazone suppress development of OA features via their antiinflammatory mode of action. Mechanical overload seems to be less important in the development of OA in this high-fat diet–induced disease model, since no correlation with body weight or body fat mass was observed. Individual susceptibility to a metabolic challenge may predict OA development at end point and needs to be further investigated. These findings suggest that the use of statins and PPAR $\gamma$  agonists could be a strategy for interfering with the progression of the disease, at least in those patients with metabolic disorders. Identification of the inflammatory mediators involved may provide leads to new treatment regimens in OA.

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