Increased Expression of Visfatin in Macrophages of Human Unstable Carotid and Coronary Atherosclerosis Possible Role in Inflammation and Plaque Destabilization

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Background—Although the participation of inflammation in atherogenesis is widely recognized, the identification of the different components has not been clarified. In particular, the role of inflammation in plaque destabilization is not fully understood.

Methods and Results—Our main findings were as follows: (1) In a microarray experiment, we identified visfatin, one of the most recently identified adipokines, as a gene that was markedly enhanced in carotid plaques from symptomatic compared with plaques from asymptomatic individuals. This finding was confirmed when carotid plaques from 7 patients with asymptomatic and 14 patients with symptomatic lesions were examined with real-time reverse transcription polymerase chain reaction. (2) Immunohistochemistry showed that visfatin was localized in areas that were rich in lipid-loaded macrophages. (3) The relationship between visfatin and unstable lesions was also found in patients with coronary artery disease, demonstrating a strong visfatin immunostaining in lipid-rich regions within the material obtained at the site of plaque rupture in patients with acute myocardial infarction. (4) Both oxidized low-density lipoprotein and tumor necrosis factor-α increased visfatin expression in THP-1 monocytes, with a particularly enhancing effect when these stimuli were combined. (5) Visfatin increased matrix metalloproteinase-9 activity in THP-1 monocytes and tumor necrosis factor-α and interleukin-8 levels in peripheral blood mononuclear cells. Both of these effects were abolished when insulin receptor signaling was blocked.

Conclusions—Our findings suggest that visfatin should be regarded as an inflammatory mediator, localized to foam cell macrophages within unstable atherosclerotic lesions, that potentially plays a role in plaque destabilization. (Circulation. 2007;115:972-980.)

Key Words: atherosclerosis ▪ inflammation ▪ leukocytes ▪ plaque ▪ coronary disease

Atherosclerosis is a progressive disease in which lipids, extracellular matrix, and activated vascular smooth muscle cells accumulate in the arterial wall, resulting in growth of an atherosclerotic plaque.1 Recent research has shown that inflammation plays a key role in this process. Hence, immune cells dominate early atherosclerotic plaques, their effector molecules accelerate progression of the lesions, and activation of inflammation can elicit various acute ischemic events such as acute coronary syndromes, transient ischemic attacks, and stroke.2 However, although the participation of inflammatory mediators in the atherosclerotic process has become widely recognized, the identification of the different components, as well as their relative importance, is unclear. In particular, the way in which inflammation may promote the transition from an asymptomatic fibroatheromatous plaque to a vulnerable and symptomatic lesion is not fully understood.

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The analysis of expressed genes in adipose tissue has revealed that adipocytes produce and secrete a variety of
bioactive substances, named adipokines, including growth factors and cytokines. Several studies have suggested a role for these mediators in atherogenesis not only through regulation of lipid and glucose metabolism but also by modulating the inflammatory arm of atherosclerosis. Adipokines have also been found to be expressed within atherosclerotic plaques, suggesting local and endocrine effects of these mediators on atherosclerotic lesions. However, although several studies suggest a role for adipokines in atherogenesis, their role in plaque destabilization has not been clarified.

One of the most recently identified adipokines is visfatin, originally identified as pre–B-cell colony-enhancing factor. Visfatin appears to be preferentially produced by the visceral adipose tissue and seems to have insulin mimetic actions. Interestingly, visfatin has also been shown to be produced by immune cells (eg, neutrophils and macrophages), and animal studies have suggested a role for this adipokine in plaque destabilization by several approaches including both clinical and experimental studies, particularly focusing on its role in inflammation and matrix degradation.

### Methods

#### Patients

Carotid plaques from 21 consecutive endarterectomy patients were classified into 2 groups depending on whether or not the patients had experienced ipsilateral stroke, transient ischemic attack, or amaurosis fugax in the 6 months before surgery. Plaques were characterized as symptomatic (n=14) or asymptomatic (n=7) according to the presence or absence of cerebrovascular symptoms, respectively (Table 1). The carotid stenoses were diagnosed and classified by precerebral color Duplex ultrasound and computed tomographic angiography according to consensus criteria. The asymptomatic carotid stenoses were detected during clinical examinations of patients with coronary artery disease (CAD), peripheral artery disease, or stroke/transient ischemic attack >6 months previously. In other experiments, blood samples were collected from patients with stable (n=8) and unstable angina (n=8) (Table 2). Those with unstable disease had experienced ischemic chest pain at rest within the preceding 48 hours (ie, Braunwald class IIIB) but with no evidence of myocardial necrosis by enzymatic criteria. Transient ST-T segment depression and/or T-wave inversion was present in all cases. All patients with stable angina had stable effort angina of >6 months’ duration and a positive exercise test. Coronary angiography was performed by standard techniques within 1 to 2 days after admission, and the diagnosis of CAD was confirmed by at least 1-vessel disease, defined as >50% narrowing of luminal diameter, in all patients. Patients with concomitant inflammatory diseases and liver or kidney disease were excluded from the study. The protocols were approved by the regional ethics committee. Signed informed consent for participation in the study was obtained from all individuals.

#### Carotid Endarterectomy Specimens

Atherosclerotic carotid plaques were retrieved from patients during carotid endarterectomy. Plaques that were used for protein and RNA extraction were rapidly frozen in liquid nitrogen. For Western blots, the tissue powders from the plaques were homogenized in ice-cold...
TABLE 2. Baseline Variables in Patients With CAD

<table>
<thead>
<tr>
<th></th>
<th>Stable Angina Pectoris* (n=8)</th>
<th>Unstable Angina Pectoris† (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59 (52–70)</td>
<td>62 (55–74)</td>
</tr>
<tr>
<td>Male sex, % (n)</td>
<td>100 (0)</td>
<td>63 (5)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26 (25–28)</td>
<td>26 (25–28)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>145 (131–159)</td>
<td>145 (120–148)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80 (77–84)</td>
<td>80 (60–86)</td>
</tr>
<tr>
<td>Antihypertensive treatment, % (n)</td>
<td>88 (7)</td>
<td>100 (8)</td>
</tr>
<tr>
<td>Statin treatment, % (n)</td>
<td>100 (8)</td>
<td>100 (8)</td>
</tr>
<tr>
<td>Platelet count, 10^9/L</td>
<td>270 (250–284)</td>
<td>228 (205–269)</td>
</tr>
<tr>
<td>White blood cell count, 10^9/L</td>
<td>9.0 (7.5–9.5)</td>
<td>8.7 (5.8–9.6)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.1 (3.8–4.5)</td>
<td>5.1 (4.4–5.5)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.2 (1.2–1.2)</td>
<td>1.1 (1.1–1.2)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7 (0.9–2.7)</td>
<td>1.8 (1.6–1.8)</td>
</tr>
<tr>
<td>Current smoking, % (n)</td>
<td>25 (2)</td>
<td>37 (3)</td>
</tr>
</tbody>
</table>

Values are median (25th and 75th percentiles) unless otherwise indicated. HDL indicates high-density lipoprotein.
*Patients used in the PCI study.
†Patients used in the PBMC study.

Blood Sampling Protocol
Peripheral venous blood was drawn into pyrogen-free EDTA tubes that were immediately immersed in melting ice and centrifuged at 2500g for 20 minutes within 20 minutes to obtain platelet-poor plasma. All samples were stored at −80°C and thawed only once.

High-Density Oligonucleotide Microarrays
Total RNA was isolated from frozen carotid tissue with the use of MagNA Pure Kit III (Roche Applied Science, Indianapolis, Ind), quantified spectrophotometrically, and stored at −80°C. The Human Genome U133A 2.0 Array encoding 14 500 genes was purchased from Affymetrix (Santa Clara, Calif), and hybridization was performed according to the manufacturer’s 2-cycle target labeling protocol. Briefly, cDNA was prepared from 100 ng total RNA, and cRNA was obtained from in vitro transcription of the cDNA. Then the cycle was repeated, making cDNA from 600 ng cRNA. Thereafter, biotin-labeled cRNA was generated from in vitro transcription of cDNA and fragmented before hybridization to the array. For data analyses, GeneChip Operation Software (1.3) and ArrayAssist (3.4) were used. Microarray suite calculation of the signal intensities was performed, and genes with very low intensity were not included in further analyses. Hypothesis testing with Benjamini-Hochberg correction was performed between the 2 groups, resulting in no significant expression of genes/probes (P<0.05), possibly because of the low number of samples in each group and the high biological variation between individuals. Therefore, a list of genes with a probability value <0.05, without multiple correction, was obtained. To eliminate the effects of the calculation method chosen, we also used the same approach after robust multichip analysis calculation. To further strengthen our results, we compared each sample in one group with every sample in the other group (microarray suite calculation). Lists of genes were made after these filtering criteria: genes with A (absent) or M (marginal) in both samples were
removal, genes with NC (no change) were removed, and genes with a signal log ratio <2-fold were also removed.

**Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction**

Total RNA was isolated from frozen THP-1 monocytes and carotid tissue by the MagNA Pure LC instrument (Roche Applied Science), with the use of MagNA Pure LC RNA isolation Kit II and III, respectively, and stored in RNA storage solution (Ambion, Houston, Tex) at −80°C until further analysis. Primers for matrix mettalloproteinase (MMP)-9 (forward primer: 5'-GCTCACCTTCACTCGCCGTGTA-3' and reverse primer: 5'-TCCGTGTCGGCGACAGA-3'), visfatin (forward primer: 5'-CTTCTGTAACATTAGATGTCTGGAA-3' and reverse primer: 5'-GCTCCTATGCGACAGTCTCTT-3'), 18S (forward primer: 5'-CGCTACACCATCAAGGAA-3' and reverse primer: 5'-GCTGGAATTACCCGGCGCT-3'), and β-actin (forward primer: 5'-AAGCACCAGGGCGCTGAT-3' and reverse primer: 5'-TCGTCCTCAGTTGGTGACGA-3') were designed with the use of Primer Express software version 1.10 (Applied Biosystems, Foster City, Calif). Quantification of mRNA was performed with the use of the ABI Prism 7000 (Applied Biosystems, Foster City, Calif). Quantification of mRNA was performed with the use of the ABI Prism 7000 (Applied Biosystems). SyBr Green assay was performed with the qPCR Master Mix for SYBR Green I (Eurogentec, Seraing, Belgium). Gene expression of the housekeeping gene β-actin or 18S was used for normalization.

**Immunohistochemistry**

Paraformaldehyde-fixed sections of material obtained during PCI and acetone-fixed sections of asymptomatic and symptomatic carotid plaques were stained with the use of purified polyclonal rabbit anti-human visfatin IgG (Phoenix Pharmaceuticals, Belmont, Calif) and mouse anti-human monocytes/macrophages (calprotectin) IgG (MCA874G, Serotec Ltd, Oxford, UK). The primary antibodies were followed by biotinylated anti-rabbit or anti-mouse IgG (Vector Laboratories, Burlingame, Calif). The immunoreactivities were further amplified with avidin-biotin-peroxidase complexes (Vectastain Elite kit, Vector Laboratories). Diaminobenzidine was used as the chromogen in a commercial metal-enhanced system (Pierce Chemical, Rockford, Ill). The sections were counterstained with hematoxylin. Omission of the primary antibody served as a negative control.

**Western Blotting**

Western blotting was performed as previously described,12 separating equal amounts of protein from each sample by SDS-PAGE (10%) before transferring it onto polyvinyl difluoride membranes (NEN; Life Science, Boston, Mass). The membranes were incubated with rabbit antibody against visfatin (Phoenix Pharmaceuticals), stripped, and reprobed with mouse anti-β-tubulin (Sigma) and rabbit anti-MCM3 (DNA polymerase-α holoenzyme-associated protein P1; Abcam, Cambridge, UK) to ensure equal loading, followed by incubation with species-specific horseradish peroxidase–coupled secondary antibodies (Cell Signaling, Beverly, Mass). The immune complex was visualized with the use of the Supersignal West Pico Western blot detection system (Pierce) and exposure to Hyperfilm ECL (Amersham Biosciences, Buckinghamshire, UK) and detected with the use of the Kodak 440 CF imaging station (Boston, Mass). The software Total Laboratory v.1.10 (Phorexic, Newcastle, UK) was used for quantification.

**Cell Culture Experiments**

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, Md) was cultured in RPMI-1640 (Sigma, St Louis, Mo) with 10% fetal calf serum (Sigma), penicillin-streptomycin, and 2 mmol/L L-glutamine (Sigma) in 6-well trays (106 cells/mL; Costar, Cambridge, Mass). Before the experimental studies, the cells were washed once in RPMI-1640 and further incubated in serum-free medium (RPMI 1640 with 2 mmol/L L-glutamine supplemented without fetal calf serum) with and without different concentrations of recombinant human (rh) visfatin (Phoenix Pharmaceuticals, Calif), oxidized low-density lipoprotein (oxLDL) (20 μg/mL), tumor necrosis factor-α (TNF-α) (5 ng/mL; R&D Systems, Minneapolis, Minn), or a combination thereof. In a separate set of experiments, peripheral blood mononuclear cells (PBMCs) were obtained from heparinized blood by Isopaque-Ficoll (Lymphoprep; Nycomed, Oslo, Norway) gradient centrifugation and incubated in flat-bottomed 96-well trays (2×105/mL; Costar) in medium alone (RPMI 1640 with 2 mmol/L L-glutamine supplemented with 5% fetal calf serum [Sigma]) or stimulated with rh-visfatin as described above. LDL was isolated from human endotoxin-free heparin plasma and oxidatively modified by Cu2+ ions (10 μmol/L).13 In some experiments, hydroxy-2-naphthalenylmethylphosphonic acid tris-acetoxy-methyl ester (HNMPA-[AM]), hereafter named HNMPA; 100 μmol/L; Biomol Research Laboratories, Plymouth Meeting, Pa), a tyrosine kinase
intra-assay and interassay coefficients of variation were no assays were 2.8 ng/mL, 15 pg/mL, and 15 pg/mL, receptively. The sensitivity values for visfatin, TNF-α, and interleukin (IL)-8 in PBMC supernatants were measured by DuoSet (R&D Systems). Gels were scanned by a Kodak 440 CF imaging station looking for large differences (ie, >2-fold increase or >50% decrease), and we did not perform any sample size calculation. Statistical Analyses Data are presented as median and interquartile range. We were looking for large differences (ie, >2-fold increase or >50% decrease), and we did not perform any sample size calculation. However, on the basis of previous experiments in our laboratory, a sample size of 7 to 15 individuals in each group will be sufficient to detect large and biologically relevant differences in the gene expression of inflammatory genes. The Mann-Whitney U test was used for comparison of 2 independent groups, and the Wilcoxon signed rank test was used in the paired situation. The Kruskal-Wallis test (Figures 4 and 5), Friedman test (Figure 3A), and 2-way ANOVA (Figure 6A and 6B) were used a priori where appropriate; the results of these tests are stated in the figure legends. The Fisher exact test was used for comparison of proportions. Probability values (2-sided) were considered significant when <0.05. All authors have read and agree to the manuscript as written. All authors had full access to and take full responsibility for the integrity of the data.

Results

Expression of Visfatin in Symptomatic Carotid Plaques
To screen for genes that were regulated differently in symptomatic compared with asymptomatic carotid plaques, we used oligonucleotide microarrays encoding 14 500 human genes to analyze the gene expression profiles in specimens from atherosclerotic carotid plaques isolated from 4 patients with symptomatic and 4 patients with asymptomatic lesions. The patients were selected randomly from the total study population. The analysis identified 136 genes to be regulated differently in symptomatic compared with asymptomatic plaques (>2-fold increase or >50% decrease; Table I in the online-only Data Supplement), and visfatin was identified as one of these genes, being markedly upregulated in all symptomatic patients (2.1- to 3.2-fold increase). This increased expression of visfatin in symptomatic plaques was confirmed by real-time reverse transcription polymerase chain reaction when plaques from the total study population were analyzed (Figure 1A) and on the protein levels as assessed by Western blot assays when carotid plaques from 6 asymptomatic and 10 symptomatic patients were analyzed (Figure 1B and 1C).

Cellular Localization of Visfatin Protein in Atherosclerotic Carotid Plaques
To determine the cellular localization of visfatin, immunohistochemical analysis was performed on carotid plaques from 2 patients with symptomatic disease and 2 patients with asymptomatic disease (Figure 2A). Staining of serial sections of these atherosclerotic lesions with anti-visfatin IgG showed strong immunostaining in plaques from symptomatic patients that were localized to the lipid-rich core of the plaque with numerous CD68-positive macrophages (Figure 2B).
positive macrophages outside this region of the lesion did not display anti-visfatin immunoreactivity. Visfatin immunoreactivity was also seen in lipid-rich regions in lesions from patients with asymptomatic disease (Figure 2C). However, in these patients, both the atherosclerotic plaque and the lipid-rich visfatin-positive core were substantially smaller than in lesions from patients with symptomatic disease.

Visfatin Expression During Plaque Ruptures in CAD
To further elucidate a potential role of visfatin during plaque destabilizations, we tested 2 clinical models of plaque rupture in another group of patients with atherosclerotic disorder, ie, CAD patients. First, we examined plasma levels of visfatin in patients with stable angina (n=8) undergoing PCI. This procedure, representing a mechanically induced plaque rupture, induced a significant increase in plasma levels of visfatin within 4 hours that returned to baseline levels after 24 hours (Figure 3A). Second, we examined the expression of visfatin in material obtained from the site of plaque rupture in 4 patients with ST-elevation myocardial infarction undergoing primary PCI. Immunohistochemical staining of plaque material from these patients showed results similar to those found in the atherosclerotic carotid lesions, with visfatin immunoreactivity in lipid-rich regions with strong CD68-positive immunostaining (Figure 3B to 3D). In addition, visfatin immunoreactivity was found in CD68-positive macrophages surrounding these regions.

Regulation of Visfatin Expression in the Human Monocytic Cell Line THP-1
The exposure of monocytes to oxLDL appears to be a key event in both atherogenesis and plaque destabilization. Notably, in accordance with the strong immunostaining in lipid-loaded macrophages in carotid plaques, we found that oxLDL markedly increased the gene expression of visfatin in THP-1 monocytes, reaching a maximum after 6 hours (Figure 4A).

Moreover, TNF-α, a prototypical inflammatory cytokine with enhanced expression in advanced atherosclerotic lesions, also increased the expression of this adipokine with a particularly prominent effect when the cells were costimulated with oxLDL (Figure 4B).

Effects of Visfatin on MMP Activity in the Human Monocytic Cell Line THP-1
The vulnerability of the plaque is to a large degree defined by the integrity of the intercellular matrix of the vessel wall, which is compromised by actions of MMPs. To map any pathogenic consequences of the increased visfatin level

Figure 4. OxLDL and TNF-α induce visfatin gene expression in THP-1 monocytes. The human monocytic cell line THP-1 was stimulated with oxLDL (20 μg/ml) and TNF-α (6 ng/ml), a combination of these, or vehicle (controls). A, Visfatin gene expression at different time points after oxLDL stimulation. B, Effect of oxLDL and TNF-α on visfatin mRNA levels after incubation for 6 and 24 hours. mRNA levels were quantified with real-time reverse transcription polymerase chain reaction, and data are presented relative to the gene expression of β-actin (median and 25th to 75th percentiles of 4 separate experiments). Kruskal-Wallis test; *P=0.002 and #P=0.005 for 6 and 24 hours, respectively; *P=0.029 vs controls (vehicle).

Figure 5. Visfatin stimulates the matrix-degrading capacity of THP-1 monocytes. The human monocytic cell line THP-1 was stimulated with different concentrations of rh-visfatin or vehicle (controls). A, Effect of different concentrations of rh-visfatin on the mRNA level (median and 25th to 75th percentiles of 5 separate experiments) of MMP-9 after culturing for 6 and 24 hours. Kruskal-Wallis test; P=0.006 and P<0.001 for 6 and 24 hours, respectively. B, MMP-9 activity in conditioned media after 24 hours of incubation with different concentrations of rh-visfatin or control (vehicle) as assessed by zymography. Top, Representative zymogram; bottom, median and 25th to 75th percentiles of 5 separate experiments. Kruskal-Wallis test; *P=0.004. In parallel sets of experiments, the insulin receptor signaling blocker HNMPA (100 μmol/L, 24-hour incubation) was used in combination with rh-visfatin (800 ng/ml), showing that HNMPA totally abolished the effect of rh-visfatin on MMP-9 mRNA level (C; Kruskal-Wallis test; P=0.004). Data are presented as median and 25th to 75th percentiles of 5 separate experiments. *P=0.03 vs controls; #P=0.029 vs rh-visfatin alone.
within the unstable lesions, we examined the ability of rh-visfatin to induce MMP-9 expression in THP-1 cells. As shown in Figure 5A, rh-visfatin markedly increased MMP-9 mRNA levels (~14-fold increase) in a dose-dependent manner, with the most marked effects after culturing for 24 hours. Importantly, such a visfatin-mediated increase was also seen when MMP-9 activity was analyzed by zymography in cell supernatants (Figure 5B). The endotoxin level in the rh-visfatin preparation was ~70 pg/mL in all experiments. Adding polymyxin B (10 μg/mL), a cationic polypeptide that inhibits lipopolysaccharide activity, to the cell culture 30 minutes before rh-visfatin activation had no effect on the MMP-9 levels (data not shown). This suggests strongly that the demonstrated effects of rh-visfatin were not related to endotoxin contamination of the culture medium or the rh-visfatin preparation. Visfatin has been reported to be bound to producer of visfatin.15 Some reports also suggest a positive correlation between plasma levels of visfatin and visceral adiposity.6 On the basis of our findings in the present study, showing potent inflammatory effects of visfatin, it is tempting to hypothesize that this adipokine also could contribute to the inflammatory state and increased risk for cardiovascular events characterizing patients with high abdominal fat/obesity.16

### Discussion

Visfatin has been reported previously to be produced in adipose tissue, bone marrow, skeletal muscle, and liver.15 In this report, to the best of our knowledge, we demonstrate for the first time that visfatin is strongly expressed within lipid-loaded macrophages in atherosclerotic lesions, with increased expression particularly in plaques from symptomatic patients. On the functional level, visfatin was found to be a potent inducer of MMP-9 and inflammatory cytokines in THP-1 monocytes and PBMCs, respectively. Visfatin has previously been indirectly linked to atherogenesis through its effect on glucose homeostasis.6 Our findings in the present study suggest that this adipokine could be related to atherogenesis and plaque destabilization in a more direct way. Moreover, visceral white adipose tissue seems to be a major producer of visfatin.15 Some reports also suggest a positive correlation between plasma levels of visfatin and visceral adiposity.6 On the basis of our findings in the present study, showing potent inflammatory effects of visfatin, it is tempting to hypothesize that this adipokine also could contribute to the inflammatory state and increased risk for cardiovascular events characterizing patients with high abdominal fat/obesity.16

It was reported recently that macrophages from visceral white adipose tissue expressed higher levels of visfatin than did mature adipocytes.17 In the present study we extend this finding by showing that visfatin is strongly expressed within symptomatic atherosclerotic carotid plaques and is localized to areas with lipid-loaded macrophages. The relationship between visfatin and unstable lesions was further supported by our findings of strong visfatin immunostaining in lipid-rich regions within plaque material obtained at the site of plaque rupture in patients with ST-elevation myocardial infarction undergoing PCI as well as by the demonstration of a marked increase in plasma visfatin levels during PCI in stable angina patients, representing a mechanically induced plaque rupture.
Previously, stimuli such as dexamethasone and peroxisome proliferator-activated receptor agonists have been found to increase visfatin expression in adipocytes. In the present study we found that oxLDL is a potent stimulus for visfatin expression in THP-1 monocytes, which is in agreement with localization of visfatin in regions with foam cell macrophages. Although TNF-α seems to decrease visfatin expression in adipocytes, this and other inflammatory cytokines have been reported to increase visfatin expression in neutrophils, suggesting that visfatin is regulated differently in different cell types. In the present study we found that a TNF-α-mediated upregulation of visfatin is also operating in THP-1 monocytes, with a particularly strong induction when TNF-α is acting in concert with oxLDL. An unstable atherosclerotic lesion is characterized by increased levels of oxLDL and inflammatory cytokines like TNF-α. On the basis of our findings in THP-1 monocytes, such a milieu could be a potent stimulus for visfatin expression, potentially explaining its enhanced expression in unstable atherosclerotic lesions.

Several reports have been published on the metabolic effects on visfatin, including its insulin-mimicking actions. Thus, studies addressing the molecular mechanisms have revealed that visfatin activates the intracellular signaling cascade for insulin, but, interestingly, visfatin activates the insulin receptor in a manner distinct from that of insulin. However, the effects of visfatin are not restricted to the modification of glucose metabolism. Some previous studies have suggested a role for this adipokine in inflammation and immune responses. In fact, visfatin was originally isolated from a cDNA library derived from activated peripheral blood lymphocytes as a factor that synergizes with IL-7 to promote the differentiation of B-cell precursors. More recently, visfatin has been reported to promote the survival of neutrophils during experimental inflammation and clinical sepsis, to stimulate the expression of inflammatory cytokines in epithelial cells, and to be involved in the thrombin-induced endothelial cell dysfunction during various forms of acute lung injury. In the present study we show that visfatin is a potent inducer of MMP-9 activity in THP-1 monocytes and that it promotes a marked release of inflammatory cytokines (ie, IL-8 and TNF-α) in PBMCs, with a particularly enhancing effect on IL-8 in cells from unstable angina patients. As previously reported for the metabolic effects of visfatin, these inflammatory actions were abolished by blocking insulin receptor signaling. MMP and inflammatory cytokines and their mutual interactions play an important role in atherogenesis and plaque destabilization. The marked induction of these mediators by visfatin in cells with relevance to atherosclerotic lesions suggest that visfatin, showing enhanced expression in symptomatic plaques, could be an important mediator in these processes. Moreover, the combined ability of visfatin to induce TNF-α as well as to respond with increased expression on TNF-α stimulation suggests that the interaction between TNF-α and visfatin could represent a pathogenic loop operating in foam cell macrophages within unstable atherosclerotic lesions, further promoting plaque destabilization.

Several reports have been published on the metabolic effects of visfatin in particular in relation to its possible role in diabetes and metabolic syndrome. Although relatively few patients were examined, our findings in the present study suggest that visfatin also should be regarded as an inflammatory mediator, localized to foam cell macrophages within unstable atherosclerotic lesions, that potentially plays a role in plaque destabilization.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

The analysis of expressed genes in adipose tissue has revealed that adipocytes produce and secrete a variety of bioactive substances, named adipokines, including growth factors and cytokines. Several studies have suggested a role for these mediators in atherogenesis not only through regulation of lipid and glucose metabolism but also by modulating the inflammatory arm of atherosclerosis. Visfatin, one of the most recently identified adipokines, has previously been reported to be produced in adipose tissue, bone marrow, skeletal muscle, and liver. Herein we demonstrate that visfatin is strongly expressed within lipid-loaded macrophages in carotid atherosclerotic lesions, with particularly increased expression in carotid plaques from symptomatic patients. On the functional level, visfatin was found to be a potent inducer of matrix degradation and inflammation in monocytes and mononuclear cells from peripheral blood, respectively. Several reports have been published on the metabolic effects of visfatin, in particular in relation to its possible role in diabetes and metabolic syndrome. Our findings in the present study suggest that visfatin also should be regarded as an inflammatory mediator, localized to foam cell macrophages within unstable atherosclerotic lesions, potentially playing a role in plaque destabilization. Our findings further underscore the link between lipid accumulation and inflammation in atherogenesis.
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