ORIGINAL ARTICLE

Inflammatory biomarkers CRP, MCP-1, serum amyloid alpha and interleukin-18 in patients with HTN and dyslipidemia: impact of diabetes mellitus on metabolic syndrome and the effect of statin therapy

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The objective of this study was to determine the relationship of HTN (HTN) and the inflammatory markers C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), amyloid alpha (AA) and interleukin-18 (IL-18) in persons with HTN, considering concomitant diabetes mellitus (DM) or metabolic syndrome (MS). This was a multicenter twelve-week, single-step titration, open-label study of individuals with dyslipidemia, assigned according to their initial risk assessment, to atorvastatin starting doses of 10, 20, 40 or 80 mg. In subjects with HTN ($N=677$) versus no HTN ($N=581$), there were significantly ($P<0.02$) higher levels of CRP, IL-18, MCP-1 and AA but not for IL-18 when combined with DM or MS, and AA or CRP when combined with MS. Systolic blood pressure significantly ($P<0.02$) correlated with CRP, MCP-1 and AA but not IL-18. The greatest increase in CRP was with HTN plus DM. Statin therapy produced significant dose-dependent reductions in CRP but not with similar changes in other inflammatory markers. In summary, these data suggest a complex relationship between inflammation and HTN with dyslipidemia. Although HTN is associated with an increase in these inflammatory markers, the associated conditions DM or MS lead to different patterns of increases—MCP-1 being the most consistently increased with HTN, the greatest CRP increase was with HTN and DM, and no relationship was found with IL-18 and HTN in the presence of DM or MS. In addition, there are different responses to statins depending on the nature of the inflammatory marker. *Hypertension Research* (2013) 36, 550–558; doi:10.1038/hr.2012.214; published online 7 February 2013

Keywords: atorvastatin; CRP; interleukin-18; MCP-1; serum amyloid alpha

INTRODUCTION

Inflammation has been proposed to be a component of the process by which conditions such as HTN (HTN), diabetes mellitus and metabolic syndrome produce their complications, but whether inflammation is an independent causal factor is still uncertain.^{1–5} The molecular biology of vascular inflammation has identified a wide array of factors that operate to initiate, develop and/or sustain the process of vascular disease.⁶ Central to the inflammatory process is the family of cytokines or chemokines that are present at the site of inflammation or injury and directly induce vascular injury or attract cells that accelerate the atherosclerotic process.^{7–9} Measurement of circulating concentrations of inflammatory markers that presumably reflect this inflammatory process in the vessel wall have proven to be indicators of atherosclerosis and patient prognosis, although the extent to which some of these inflammatory markers is independent of other known risk factors is currently being defined.

Four circulating inflammatory markers are of special interest—C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), amyloid alpha (AA) and interleukin-18 (IL-18), as these span the range from intensively studied to relatively neglected factors. CRP has attracted the most attention. Elevated levels of CRP have consistently been found to be associated with the increased risk of subsequent cardiovascular events.^{10–12} Data suggest that modification of CRP may be associated with a reduced risk of cardiovascular events.¹³ As CRP is a nonspecific indicator of inflammation, the use of other inflammatory markers may be preferable to develop a construct to predict and limit cardiovascular risk.

MCP-1, also known as CCL2, directs the migration of circulating leukocytes to the sites of inflammation or injury, which include the atherosclerotic plaque.¹⁴ Circulating MCP-1 levels are significantly higher in persons with coronary artery disease or peripheral artery disease compared with individuals without these clinical

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condition.^{15–18} MCP-1 is a marker of adverse cardiovascular events.^{16,17,19} The predictive value of MCP-1 is independent of the plasma levels of CRP.¹⁹ MCP-1 levels reflect the degree of the acute inflammation, endothelial dysfunction²⁰ or atherosclerosis burden in coronary vasculature or peripheral arteries.^{21,22} MCP-1 has been proposed as a more precise marker for coronary inflammation because plasma levels of MCP-1 correlate better than CRP with subsequent development of coronary stenosis after PCI.¹⁸ Furthermore, MCP-1 may mediate the atherogenic effects of other coronary risk factors.¹⁵

Serum amyloid alpha (AA) has been a relatively neglected factor but has been conceptualized to reside at the intersection of inflammation, dyslipidemia, obesity and insulin resistance and may have a pivotal role in regulating the contributions of those processes to atherogenesis.²³ Elevated circulating levels of AA are more common in patients with atherosclerotic coronary artery disease compared with that of controls.²⁴ AA predicts the progression of carotid atherosclerosis.²⁵ Prospective data demonstrate that AA predicts the risk of recurrent coronary events among stable patients with a prior history of myocardial infarction, independently of smoking status and baseline levels of LDL cholesterol, HDL cholesterol and triglycerides.²⁶ In women, AA, after adjustment for traditional coronary artery risk, was independently associated with angiographic coronary artery disease whereas CRP was not.²⁷

IL-18, a pro-inflammatory cytokine, can have a significant role in the induction of vascular damage.⁹ Plasma IL-18 concentrations are elevated in patients with established coronary artery disease and correlate directly with the extent of coronary atherosclerosis independently of C-reactive protein.²⁸ IL-18 levels are even higher in patients with coronary disease (CAD) and diabetes mellitus than those with CAD alone, diabetes mellitus alone or neither condition.⁸ In patients with angina pectoris, higher serum concentrations of IL-18 are associated with an increased risk of fatal cardiovascular events.²⁹ In some but not all population studies, IL-18 remained as a strong predictor of cardiac mortality even after adjustment for other factors such as ejection fraction and C-reactive protein.^{29,30} Some investigators report that statins increase the production of IL-18 from human peripheral blood mononuclear cells³¹ whereas other content that statins reduce serum IL-18 levels.^{32–34}

The ability of HMG-CoA reductase inhibitors (statins) to modify the inflammatory process continues to attract considerable attention. The effect of statins on cytokines ranges from a reduction in circulating levels of CRP^{13,35} to uncertain effects on other cytokines. For MCP-1, some studies show a beneficial effect of statins on MCP-1, whereas others do not.^{36–40} The results are similarly discordant for the effect of statins on AA.^{26,41,42} Most studies showing a benefit of statins on MCP-1 and AA were conducted in patients after an episode of acute coronary syndrome (ACS)^{26,42,43} and may not be relevant for other conditions.

The primary objective of the CanACTFAST (Canadians Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration) trial was to determine whether a preselected algorithm of atorvastatin dosage based on the patients' cardiovascular risk category and level of LDL-C enables patients to achieve their designated LDL-C and TC/HDL-C ratio targets quickly, with either no titration or just a single titration step.⁴⁴ A prespecified secondary objective of the study was to examine the effect of the HMG-CoA reductase inhibitor atorvastatin on circulating cytokine concentrations comparing CRP, IL-18, MCP-1 and AA by evaluating the blood concentrations and percent change from baseline in specific subgroups—those with HTN, diabetes mellitus or metabolic syndrome.

METHODS

Patient population

The design, recruitment and primary results of the CanACTFAST, a multicenter, twelve-week, single-step titration, open-label study of individuals with dyslipidemia assigned according to their initial risk assessment to a atorvastatin starting doses of 10, 20, 40 and 80 mg, has been reported previously.⁴⁴ The steering committee (SWR, EU, AL, CC and LAL) designed the trial in collaboration with the sponsor. EU and LAL drafted the initial primary and secondary endpoints, which were then modified and approved by the steering committee. Briefly, the entry criteria were: male or female patients, ages 30–79 years old with LDL-cholesterol (LDL-C) level of ≤ 5.7 mmol l⁻¹ and triglyceride level ≤ 6.8 mmol l⁻¹. Subjects were excluded if their LDL > 5.7 mmol l⁻¹ because these individuals might require more vigorous statin treatment than the protocol would permit. Subjects with triglycerides > 6.8 mmol l⁻¹ were excluded because they would require fibrates drugs to prevent pancreatitis. The latter exclusion criterion avoided the potential interaction in some subjects receiving both statins and fibrates while other subjects were only on statins. Patients were required to have an elevated LDL concentration despite adhering to an appropriate diet before entry into the study (except for high-risk group patients where, treatment was started concomitantly) and be eligible for LDL-C-lowering drug therapy at baseline as determined by LDL-C. Subjects who achieved both lipid targets at 6 weeks continued on the same dose of atorvastatin for an additional 6 weeks. Subjects who did not achieve both LDL-C and TC/HDL-C ratio targets had their dose increased according to a single-step titration regimen that was administered for an additional 6-week.

Inclusion and exclusion criteria were based on lipid values and lipid treatment. The only exclusion relevant to inflammatory markers was based on the necessity to remove the influence of an acute infection on inflammatory markers. Subjects with baseline hs-CRP ≤ 10 mg l⁻¹ were included in the per protocol analysis of inflammatory markers.⁴⁵ Although not excluded from the study for the assessment of protocol or drug efficacy on LDL management, laboratory studies on inflammatory markers were not performed or were excluded for all subjects using any of the following concomitant anti-inflammatory oral/parenteral medications for more than 10 days during any treatment period or within 10 days of a laboratory assessment: ASA (at a dose > 325 mg d⁻¹), non-steroidal anti-inflammatory drugs, Cox-2 inhibitors, corticosteroids, 5-ASA preparations (for example, sulfasalazine and mesalazine), cytotoxic agents (cyclophosphamide, methotrexate, hydroxyurea), other immunosuppressants such as azathioprine or infliximab, thiazolidinediones (pioglitazone and rosiglitazone), gold salts and leukotriene receptor antagonists (montelukast and zafirlukast). Only data from that specific visit was removed. Laboratory data on inflammatory markers were similarly excluded from analysis at the time (week 6, week 12) of blood sampling for subject who were suffering from an acute infectious or inflammatory episode or from an acute trauma. The subjects with superficial skin or mucosal infection or injury were not be excluded from the per protocol analysis of inflammatory markers.

Subjects were classified as having HTN if their sitting blood pressure was $> 140/90$ mm Hg on the baseline physical exam or they were taking anti-hypertensive medication. Subjects with severe HTN (that is, sitting systolic/diastolic BP $> 160/100$ mm Hg), whether taking or not taking concurrent antihypertensive medication were excluded from the study because their HTN was not controlled and it was expected that they would be placed on antihypertensive drugs that might alter their lipids.⁴⁶ Diabetes mellitus was defined as either one of the following, (i) symptoms of diabetes plus casual plasma glucose concentration ≥ 11.1 mmol l⁻¹ (200 mg dl⁻¹), (ii) fasting plasma glucose ≥ 7.0 mmol l⁻¹ (126 mg dl⁻¹) or (iii) a plasma glucose value in the 2 h sample (2hPG) of the oral glucose tolerance test (OGTT) ≥ 11.1 mmol l⁻¹. For the purpose of this study, current treatment with insulin or oral hypoglycaemic agents at screening was also sufficient to make the diagnosis of diabetes mellitus. The NCEP ATP III criteria for metabolic syndrome—waist circumference (men > 102 cm and women > 88 cm), triglycerides ≥ 1.7 mmol l⁻¹, HDL < 1 mmol l⁻¹ for men and 1.3 mmol l⁻¹ for women, as well as blood pressure $> 130/85$ mm Hg and fasting glucose between 6.2 and 7 mmol l⁻¹ were used.⁴⁷ Participants were classified as having HTN, diabetes mellitus or metabolic syndrome at baseline. Within the time

frame of the study, it was considered unlikely for subjects to develop diabetes or HTN so that subjects were not examined for the development of one of these conditions within each group.

Laboratory methods

CRP-high sensitive was assayed on the Roche/Hitachi 911 analyzer using Tina-quant C-reactive protein high sensitive immunoturbidometric assay (Roche Diagnostics, Mannheim, Germany) using latex microparticles coated with mouse monoclonal antibodies to CRP by a central commercial laboratory. IL-18 was measured using enzyme-linked immunosorbent assay (ELISA) with a quantitative sandwich immunoassay method (MBL Inc., Mississauga, ON, Canada, distributed by R&D Systems Inc., Minneapolis, MN, USA) by a central commercial laboratory. Serum human AA was assessed using an ELISA with the quantitative sandwich immunoassay technique (Biosource Inc., Grand Island, NY, USA) by a central commercial laboratory. Human MCP-1 was measured by an ELISA using a commercially available assay using an antibody specific for MCP-1 that was coated on microtitre wells for the quantitative detection of human MCP-1 in human serum (R&D systems) by a central commercial laboratory.

Statistical analysis

All analyses were performed on an intention-to-treat basis. All randomized subjects who were assigned a starting dose of active study drug, had taken at least one dose of study medication and had at least one subsequent assessment, were included in the analysis. The inflammatory markers: hsCRP, IL18, MCP and AA were not normally distributed and therefore non-parametric approaches—Wilcoxon rank sum test or Kruskal–Wallis were used to calculate the *P*-value for between-group comparisons.

Percentage changes in inflammatory markers from baseline to different timepoints (weeks 6 and 12) and the respective confidence intervals were calculated from the paired differences in the log-transformed baseline and post-baseline values and then back transformation via exponentiation of the mean of log-transformed data, and expressed as $(\text{geometric mean} - 1) \times 100$. The last analysis carried forward analysis (LOCF) approach was also used to analyze all available data closest to the final visit, which takes into account excluding missing or excluded samples for reasons outlined above. Spearman's rank (with untransformed data) or Pearson's (with log-transformed data) correlation coefficient was used to assess the correlations between changes in inflammatory markers and changes in blood pressure. Significance level was preselected at 0.05.

RESULTS

The study population ($N=1258$) consisted of men (62.5%) and women with an average age of 58.4 ± 10.4 years, of whom 54.5% had HTN, 27.2% had diabetes mellitus type 2 and 40% fulfilled the criteria for metabolic syndrome. In the study population, 21.3% were receiving ACE inhibitors, 21.5% were receiving a diuretic with or without another agent, 15% received beta blockers, 13% received calcium channel blockers, 10.3% received angiotensin receptor antagonist and 1.1% received alpha blocker or centrally acting antihypertensive agent. The wide spectrum of antihypertensive drug usage precluded meaningful subgroup analysis within each antihypertensive drug class. For subjects with diabetes mellitus, 66.7% were on oral hypoglycaemic agents and 10.9% were on insulin. These antihypertensive or diabetes mellitus drug therapies, to our knowledge, were not changed during the course of the study. The majority of individuals had not previously been on statin agents before commencement of the study and are referred to as statin-free individuals. At baseline, compared with individuals without HTN, individuals with HTN had significantly higher levels of CRP (mean values, 3.18 vs. 2.46 mg l^{-1} ; $P<0.0001$), IL-18 (267.3 vs. 258.3 pg ml^{-1} ; $P=0.022$), MCP-1 (289.2 vs. 262.9 pg ml^{-1} ; $P<0.0001$) and AA (25.7 vs. $20.7 \text{ } \mu\text{g ml}^{-1}$; $P=0.0001$) (Table 1). Persons with diabetes mellitus had significantly higher levels of IL-18 (288.7 vs. 253.2 pg ml^{-1} ; $P<0.0001$) compared with those without

diabetes mellitus, but there were no significant differences for the other inflammatory markers. The presence of HTN with diabetes mellitus was associated with higher levels of all inflammatory markers compared with individuals with only diabetes mellitus, and the increase was statistically significant for CRP (3.63 vs. 2.51 mg l^{-1} ; $P=0.0051$), MCP-1 (294.7 vs. 242.8 pg ml^{-1} ; $P=0.0002$) and AA (28.5 vs. $18.7 \text{ } \mu\text{g ml}^{-1}$; $P=0.0091$). Metabolic syndrome was associated with significantly increased levels of CRP (3.50 vs. 2.48 mg l^{-1} ; $P<0.0001$), IL-18 (280.3 vs. 253.6 pg ml^{-1} ; $P<0.0001$) and AA (mean 23.6 vs. $23.7 \text{ } \mu\text{g ml}^{-1}$; median 16.5 vs. $14.5 \text{ } \mu\text{g ml}^{-1}$; $P=0.0065$) but not MCP-1. Table 1 reports the mean, median and the *P*-values, obtained from the non-parametric model with the rank of the value, rather than the value itself. The presence of HTN with metabolic syndrome significantly increased the levels of MCP-1 compared with those with MS without HTN (288.7 vs. 255.6 pg ml^{-1} ; $P=0.0025$), but there were no significant further increases in the levels of CRP, IL-18 or AA.

At baseline, there were relationships between inflammatory markers and blood pressure or waist circumference. There were significant correlations between systolic but not diastolic blood pressure for CRP ($r=0.108$; $P=0.0001$), MCP-1 ($r=0.084$; $P=0.0033$) and AA ($r=0.084$; $P=0.003$), but not IL-18 ($r=0.018$). There was a significant correlation between waist circumference and CRP ($r=0.294$; $P<0.0001$), IL-18 ($r=0.160$; $P<0.0001$) and AA ($r=0.098$; $P=0.0006$), but not MCP-1 ($r=-0.004$). The relationships were stronger in persons who had not previously been on statin therapy at baseline.

The baseline values for inflammatory markers were assessed according to the subject's baseline risk, which was based on Framingham 10-year risk assessment (Table 2A). There was a significant increase in IL-18 (low: 255.2 vs. moderate: 269.6 vs. high: 271.7 vs. very high: 281.7 pg ml^{-1} ; $P=0.0076$) and MCP-1 (low: 270.9 vs. mod: 282.7 vs. high 289.1 vs. very high: 287.5 ; $P=0.0079$) according to the risk level at baseline. Levels of AA were significantly lower with higher risk categories at baseline (low: 26.5 vs. moderate: 20.9 vs. high: 17.7 vs. very high: $18.5 \text{ } \mu\text{g ml}^{-1}$; $P=0.0322$). The relationships were minimally stronger for those who were statin-free at baseline that comprises the majority of the population (Table 2B).

Subjects showed a significant reduction in CRP at 6 and 12 weeks of treatment ranging from -23 to -27% (Table 3). We assessed significance from a confidence interval approach, which is if the confidence interval does not enclose the value reflecting 'no effect', that is 0% change; this represents a % change that is statistically significant. All persons improved when the 95% confidence intervals was not containing the 'no effect' value of 0% change. Those with HTN showed a slightly greater, but not statistically significant, reduction in CRP that was more evident at 12 compared with 6 weeks (Table 3). The effect was slightly greater in the statin-free group.

There was a significant reduction in IL-18 at 6 and 12 weeks. The magnitude of the percent change was much smaller than the change in CRP. There was no difference between persons with HTN compared with those without HTN with respect to the change in IL-18. Individuals with HTN showed a significant reduction in MCP-1 at 6 weeks, (-4 ; 95% CI: -7 to -2%) whereas those without HTN did not show a similar change. At 12 weeks, there was no change in MCP-1 in the group without HTN. Serum AA levels were significantly reduced at 6 weeks for those with or without HTN. There was less of a difference from baseline at 12 weeks for all subjects.

Subjects with HTN and diabetes mellitus showed a much greater reduction in CRP than persons with diabetes and no HTN (Table 4). This was particularly evident at 12 weeks of treatment

Table 1 Baseline data on inflammatory markers in the subject population at baseline according to whether or not they had HTN (HTN), diabetes mellitus (diabetes) or the metabolic syndrome (MS)

<i>hsCRP</i> (mg l ⁻¹)			<i>IL-18</i> (pg ml ⁻¹)		<i>MCP-1</i> (pg ml ⁻¹)		<i>AA</i> (μg ml ⁻¹)	
<i>HTN</i>	<i>HTN</i>	<i>No HTN</i>	<i>HTN</i>	<i>No HTN</i>	<i>HTN</i>	<i>No HTN</i>	<i>HTN</i>	<i>No HTN</i>
<i>N</i>	677	567	673	560	671	558	671	560
Mean	3.18	2.46	267.3	258.3	289.2	262.9	25.7	20.7
Median	1.76	1.23	242.6	227.6	256.0	225.0	16.5	13.7
s.d.	5.44	4.46	134.9	146.6	152.6	184.8	46.3	30.1
<i>P</i> -value	<0.0001		0.022		<0.0001		0.0001	
<i>Diabetes</i>	<i>Diabetes 2</i>	<i>No diabetes 2</i>	<i>Diabetes 2</i>	<i>No diabetes 2</i>	<i>Diabetes 2</i>	<i>No diabetes 2</i>	<i>Diabetes 2</i>	<i>No diabetes 2</i>
<i>N</i>	351	893	348	885	348	881	348	883
Mean	3.24	2.71	288.7	253.2	276.5	277.6	25.1	22.8
Median	1.58	1.40	260.3	227.0	241.5	237.0	16.4	15.0
s.d.	6.33	4.40	170.1	125.5	133.8	180.4	52.7	33.4
<i>P</i> -value	0.1110		<0.0001		0.5637		0.2666	
<i>MS</i>	<i>MS</i>	<i>No MS</i>	<i>MS</i>	<i>No MS</i>	<i>MS</i>	<i>No MS</i>	<i>MS</i>	<i>No MS</i>
<i>N</i>	472	706	464	703	464	700	463	702
Mean	3.50	2.48	280.3	253.6	278.5	278.5	23.6	23.7
Median	1.94	1.22	255.1	221.9	248.0	237.0	16.5	14.5
s.d.	5.45	4.89	134.8	146.6	127.5	193.5	34.9	44.2
<i>P</i> -value	<0.0001		<0.0001		0.1793		0.0065	
<i>Diabetes ± HTN</i>	<i>Diabetes + HTN</i>	<i>Diabetes + No HTN</i>	<i>Diabetes + HTN</i>	<i>Diabetes + No HTN</i>	<i>Diabetes + HTN</i>	<i>Diabetes + No HTN</i>	<i>Diabetes + HTN</i>	<i>Diabetes + No HTN</i>
<i>N</i>	228	123	226	122	226	122	226	122
Mean	3.63	2.51	292.8	281.1	294.7	242.8	28.5	18.7
Median	1.85	1.35	260.3	260.1	263.0	219.0	17.1	13.2
s.d.	7.35	3.69	167.4	175.6	141.5	111.2	63.7	19.1
<i>P</i> -value	0.0051		0.2566		0.0002		0.0091	
<i>MS ± HTN</i>	<i>MS + HTN</i>	<i>MS + No HTN</i>	<i>MS + HTN</i>	<i>MS + No HTN</i>	<i>MS + HTN</i>	<i>MS + No HTN</i>	<i>MS + HTN</i>	<i>MS + No HTN</i>
<i>N</i>	326	146	321	143	321	143	320	143
Mean	3.42	3.69	283.7	272.5	288.7	255.6	24.0	22.7
Median	2.03	1.80	257.1	248.3	259.0	222.0	16.9	14.2
s.d.	5.15	6.10	129.4	146.2	127.9	123.9	35.6	33.3
<i>P</i> -value	0.2023		0.1579		0.0025		0.1757	

Note: As *hsCRP*, *IL18*, *MCP*, and amyloid data do not normally distribute, the Wilcoxon rank sum test was used to calculate the *P*-value. This non-parametric approach does not assume normal distribution from the data.

(−30% (−38 to −21%) vs. −12% (−25 to 3%)). A similar trend was evident for *IL-18* but the differences were not as great. There were no meaningful changes in *MCP-1* in the subgroup with diabetes mellitus. Individuals with diabetes mellitus and HTN showed a slight reduction in *AA* at 12 weeks, which was in sharp contrast to the increase in *AA* observed in those with diabetes mellitus, but without HTN (−4% (−12 to 6%) vs. 10% (−3 to 25%)).

In contrast to diabetes mellitus, individuals with metabolic syndrome had similar reductions in *CRP* and *IL-18* regardless of whether or not they had HTN (Table 5). *MCP-1* did not change or went up slightly in the group with metabolic syndrome whereas it decreased slightly in the subgroup with HTN. There was no change in *AA* in the group with metabolic syndrome.

There was a significant reduction in the blood pressure of 1.7 [CI −2.4 to −0.9] mm Hg for systolic and 0.6 [CI −0.12 to −1.11] mm Hg for diastolic from the baseline to the 12-week measurement. Thus, we sought to determine whether change in blood pressure might be associated with change in inflammatory markers. There was no correlation between change in blood pressure and most of the inflammatory markers, although the relationship with *MCP-1* was possible ($r=0.057$; $P=0.046$) (Table 6).

DISCUSSION

The present study contributes novel data to the understanding of the relationship between HTN and inflammation in persons with

associated diabetes mellitus or metabolic syndrome. In persons with dyslipidemia, HTN is associated with an increase in the inflammatory markers *CRP*, *IL-18*, *MCP-1* and *AA*. The presence of diabetes mellitus in persons with HTN and dyslipidemia further accentuates the levels of *CRP*, *MCP-1* and *AA*. The presence of HTN in persons with obesity defined as metabolic syndrome increases *MCP-1* above levels in persons without HTN. The presence of HTN did not increase the already elevated levels of *IL-18* found in persons with diabetes mellitus nor was it associated with greater values of *CRP*, *IL-18* or *AA* in metabolic syndrome. These data suggest different and potentially unique relationships between different inflammatory processes in HTN compared with that in diabetes mellitus and metabolic syndrome.

Low-grade inflammation has received increasing attention as a factor mediating the development of HTN. Increased levels of cytokines, reflecting the inflammatory state, may increase smooth muscle cell proliferation producing hypertensive vascular changes, induce kidney damage or affect key CNS regulatory centers leading to sympathetic nervous system activation. Our finding that *CRP* is higher in persons with HTN and dyslipidemia is substantiated by our finding of a significant correlation between blood pressure and *CRP*. *CRP*, an important marker of inflammation, has been subjected to extensive investigation. There is, however, limited previous investigation examining the impact in a hypertensive population compared with a nonhypertensive one with dyslipidemia. Previous data support

Table 2A Baseline values for all subjects according to risk group at baseline

	hsCRP (mg l ⁻¹)	IL-18 (pg ml ⁻¹)	MCP-1 (pg ml ⁻¹)	AA (μg ml ⁻¹)
<i>Low risk</i>				
N	714	710	707	708
Mean	3.08	255.2	270.9	26.5
Median	1.36	228.0	230.0	16.0
s.d.	6.07	136.5	189.7	50.2
<i>Moderate risk</i>				
N	219	217	217	217
Mean	2.58	269.6	282.7	20.9
Median	1.49	246.9	245.0	15.9
s.d.	3.45	129.1	134.8	20.0
<i>High risk</i>				
N	137	134	134	134
Mean	2.35	271.7	289.1	17.7
Median	1.61	237.5	254.0	13.4
s.d.	2.57	176.6	144.9	14.0
<i>Very high risk</i>				
N	174	172	171	172
Mean	2.69	281.7	287.5	18.5
Median	1.61	256.6	263.0	13.4
s.d.	2.97	136.8	124.6	15.5
P-value	0.3678	0.0076	0.0079	0.0322

The low-, moderate-, high- and very high-risk groups were calculated on the basis of their 10-year probability of coronary artery disease/or the presence of cardiovascular disease (which was assigned to the very high risk category). The P-value was calculated by the Kruskal-Wallis test to assess whether the distributions were difference between different risk groups.

a link between HTN and CRP, although the role of C-reactive protein as a causal factor in promoting HTN and its complications remains to be elucidated.⁴⁸ Our finding of a stronger relationship between CRP and systolic rather than with diastolic blood pressure is consistent with the concept that CRP is linked to arterial stiffness.^{49,50} This is also consistent with the proposition that cytokines increase smooth muscle cell proliferation producing hypertensive vascular changes. We found that atorvastatin induced a greater reduction in CRP than any of the other inflammatory markers studied. Statin-induced reduction in CRP occurred in hypertensive individuals to a slightly greater extent than in individuals without HTN.

IL-18, either directly or through oxidative stress pathways and matrix metalloproteins, can alter endothelial function, and/or induce vascular smooth muscle cell migration and/or proliferation leading to the vascular changes of HTN.⁹ We found a significant increase in IL-18 in hypertensive compared with that in non-hypertensive individuals. IL-18 levels were also greater in persons with diabetes mellitus or metabolic syndrome compared with individuals without either of those two conditions. Abdominal obesity, as reflected in waist circumference, correlated more strongly with IL-18 than HTN. Furthermore, once metabolic syndrome was considered, HTN did not further increase IL-18 levels, suggesting that IL-18 was increased mainly in persons with HTN and obesity. IL-18 levels were increased in hypertensive individuals in several population-based trials such as the MONICA/KORA Augsburg study,⁵¹ an Australian community-based population screening study²⁰ and the Dallas Heart Study.⁵² In contrast, another population-based study in Western Europe (France and Ireland) reported no significant difference in IL-18

Table 2B Baseline values for the statin free group according to their risk group at baseline

	hsCRP (mg l ⁻¹)	IL-18 (pg ml ⁻¹)	MCP-1 (pg ml ⁻¹)	AA (μg ml ⁻¹)
<i>Low risk</i>				
N	714	710	707	708
Mean	3.08	255.2	270.9	26.5
Median	1.36	228.0	230.0	16.0
s.d.	6.07	136.5	189.7	50.2
<i>Moderate risk</i>				
N	101	101	101	101
Mean	3.33	285.0	263.0	24.6
Median	1.68	253.5	217.0	17.2
s.d.	4.63	135.9	133.7	25.2
<i>High risk</i>				
N	114	112	112	112
Mean	2.52	279.7	287.2	18.1
Median	1.70	244.7	243.0	13.5
s.d.	2.69	188.1	147.6	14.4
<i>Very high risk</i>				
N	158	158	157	158
Mean	2.60	281.8	287.1	18.3
Median	1.58	254.7	264.0	13.2
s.d.	2.85	139.3	125.0	15.9
P-value	0.2342	0.0026	0.0178	0.0165

The low-, moderate-, high- and very high-risk groups were calculated on the basis of their 10-year probability of coronary artery disease/or the presence of cardiovascular disease (which was assigned to the very high risk category). The P-value was calculated by the Kruskal-Wallis test to assess whether the distributions were difference between different risk groups.

levels in those individuals with HTN compared with those without HTN.⁵³ The entry criteria of individuals with acute coronary syndromes and the exclusively male population⁵³ may have influenced these results. In our study, diabetes and obesity were associated with a greater increase in IL-18 levels than HTN. We found that atorvastatin lowered IL-18 in the hypertensive group, especially in those with HTN and diabetes mellitus. In a small (52 person) study, fluvastatin was reported to reduce IL-18 levels.³² Experimental studies, however have reported that fluvastatin and pravastatin increase IL-18 production in human monocytes while atorvastatin suppressed IL-18-mediated AP-1 and NF-kappaB activation, MMP9 expression, and inhibited IL-18-mediated aortic smooth muscle cells in culture.⁵⁴

MCP-1 was elevated in the hypertensive group, but not in those with diabetes mellitus or metabolic syndrome. This finding suggests a unique relationship between HTN and MCP-1 that is not present in diabetes mellitus or metabolic syndrome. This suggestion is further supported by the significant correlation between MCP-1 and systolic blood pressure but not waist circumference. Hypercholesterolemia itself does not increase MCP-1.⁵⁵ MCP-1 is synthesized and released by vascular, cardiac and renal cells in response to hemodynamic stress, oxidative stress and hormonal stimuli that increase blood pressure such as angiotensin II and endothelin-1.⁵⁶ MCP-1 has been proposed to be a key mediator of angiotensin II-induced target organ damage in hypertensive heart disease.⁵⁶ We found that MCP-1 correlated significantly with the calculated level of cardiovascular risk. There was, however, only a small reduction in MCP-1 with statin therapy. It was only significantly reduced at 6 weeks but not at the end of

Table 3 Percent change in inflammatory markers compared with baseline in subjects with and without HTN at baseline

	Statin-free				All persons			
	–HTN week 6	HTN week 6	–HTN week 12	HTN week 12	–HTN week 6	HTN week 6	–HTN week 12	HTN week 12
hsCRP	N = 493 –25% [–30%, –19%]	N = 536 –26% [–31%, –21%]	N = 506 –26% [–32%, –19%]	N = 560 –30% [–35%, –24%]	N = 541 –23% [–28%, –17%]	N = 640 –24% [–29%, –19%]	N = 555 –23% [–29%, –17%]	N = 668 –27% [–32%, –22%]
IL-18	N = 488 –5% [–7%, –4%]	N = 538 –6% [–8%, –5%]	N = 500 –6% [–7%, –4%]	N = 555 –5% [–6%, –3%]	N = 535 –5% [–6%, –3%]	N = 638 –6% [–7%, –4%]	N = 548 –5% [–7%, –3%]	N = 659 –4% [–5%, –2%]
MCP-1	N = 484 –1% [–4%, 2%]	N = 534 –5% [–8%, –1%]	N = 497 2% [–1%, 5%]	N = 552 0% [–3%, 3%]	N = 531 –2% [–4%, 1%]	N = 634 –4% [–7%, –2%]	N = 545 1% [–2%, 4%]	N = 656 0% [–3%, 3%]
AA	N = 488 –7% [–12%, –2%]	N = 537 –7% [–12%, –2%]	N = 498 0% [–7%, 6%]	N = 554 –5% [–11%, 0%]	N = 535 –5% [–10%, 0%]	N = 637 –5% [–10%, –1%]	N = 546 0% [–6%, 6%]	N = 658 –3% [–8%, 2%]

The numbers in [] are the confidence intervals.

–HTN: Without HTN.

HTN: With HTN.

Table 4 Percent change in inflammatory markers compared with baseline in subjects with diabetes mellitus with or without HTN at baseline

	Statin-free				All persons			
	DM – HTN week 6	DM + HTN week 6	DM – HTN week 12	DM + HTN week 12	DM – HTN week 6	DM + HTN week 6	DM – HTN week 12	DM + HTN week 12
hsCRP	N = 106 –21% [–30%, –10%]	N = 160 –25% [–34%, –14%]	N = 110 –13% [–26%, 2%]	N = 168 –37% [–45%, –27%]	N = 117 –21% [–30%, –11%]	N = 216 –22% [–30%, –13%]	N = 121 –12% [–25%, 3%]	N = 225 –30% [–38%, –21%]
IL-18	N = 105 –4% [–7%, 0%]	N = 160 –6% [–10%, –3%]	N = 109 –1% [–4%, 2%]	N = 168 –5% [–8%, –3%]	N = 116 –4% [–7%, 0%]	N = 214 –5% [–8%, –2%]	N = 120 –1% [–4%, 3%]	N = 223 –3% [–6%, –1%]
MCP-1	N = 105 1% [–6%, 8%]	N = 159 –2% [–7%, 4%]	N = 109 2% [–5%, 10%]	N = 169 1% [–4%, 6%]	N = 116 0% [–7%, 6%]	N = 213 –4% [–8%, 1%]	N = 120 1% [–5%, 9%]	N = 224 1% [–3%, 6%]
AA	N = 105 –3% [–12%, 8%]	N = 161 –7% [–17%, 3%]	N = 109 11% [–3%, 27%]	N = 169 –7% [–16%, 4%]	N = 116 –1% [–10%, 9%]	N = 215 –5% [–13%, 4%]	N = 120 10% [–3%, 25%]	N = 224 –4% [–12%, 6%]

The numbers in [] are the confidence intervals.

DM – HTN: Subjects with diabetes mellitus but without HTN.

DM + HTN: Subjects with diabetes mellitus and HTN.

study, suggesting that the effects of statins may be only transient. The decline in MCP-1 was significant only for persons with HTN or for persons with HTN and metabolic syndrome. These findings offer a potential explanation for the discordant findings between other studies examining the effect of statins on MCP-1.^{36–40} The nature of the hypertensive population including the proportion of controlled hypertensive individuals^{38,57} may influence the effect of statin treatment. The magnitude of the reduction in MCP-1 in response to statins therapy was small and transient. It was only evident after 6 weeks of therapy suggesting that other factors regulate MCP-1 levels and the response to statins. Our clinical data would not support the experimental data that CRP increases the production of MCP-1 (Pasceri *et al.*⁵⁸) because we observed a much greater change in CRP than MCP-1 and the effect of atorvastatin on CRP remained present at 12 weeks, which was not the case for MCP-1.

We found an increase in serum amyloid alpha in hypertensive individuals. Serum AA is associated with the complications of HTN specifically left ventricular hypertrophy or cerebrovascular disease.^{59,60} In addition, serum AA predicts the occurrence of a cerebrovascular event in patients who have had a cardiac event.⁶¹ We found that serum AA correlated with systolic and not diastolic BP. This is supported by the finding in a multivariate analysis that systolic but not diastolic blood pressure was independently associated with serum AA.⁶² The effect of statins on AA is controversial and is likely depending on the clinical setting. Atorvastatin and pravastatin significantly reduced AA, but in the setting of acute coronary syndrome—a state of heightened inflammatory responses.^{26,42} In contrast, simvastatin did not significantly alter serum AA in two multicenter, randomized, double-blind, placebo-controlled studies designed to examine the lipid-altering efficacy a non-acute setting⁴¹

Table 5 Percent change in inflammatory markers compared with baseline in subjects with metabolic syndrome with or without HTN at baseline

	<i>MS-HTN</i> week 6	<i>MS+ HTN</i> week 6	<i>MS-HTN</i> week 12	<i>MS+ HTN</i> week 12	<i>MS-HTN</i> week 6	<i>MS+ HTN</i> week 6	<i>MS-HTN</i> week 12	<i>MS+ HTN</i> week 12
hsCRP	<i>N</i> = 124 –25% [–33%, –16%]	<i>N</i> = 248 –25% [–31%, –18%]	<i>N</i> = 130 –32% [–41%, –21%]	<i>N</i> = 262 –27% [–33%, –19%]	<i>N</i> = 135 –23% [–31%, –14%]	<i>N</i> = 305 –23% [–30%, –17%]	<i>N</i> = 141 –29% [–39%, –18%]	<i>N</i> = 321 –25% [–32%, –18%]
IL-18	<i>N</i> = 121 –5% [–8%, –2%]	<i>N</i> = 245 –8% [–11%, –6%]	<i>N</i> = 130 –7% [–10%, –4%]	<i>N</i> = 258 –5% [–7%, –3%]	<i>N</i> = 131 –4% [–7%, –1%]	<i>N</i> = 300 –7% [–9%, –5%]	<i>N</i> = 140 –6% [–9%, –3%]	<i>N</i> = 315 –4% [–7%, –2%]
MCP-1	<i>N</i> = 121 4% [–3%, 10%]	<i>N</i> = 243 –7% [–12%, –2%]	<i>N</i> = 130 5% [–2%, 13%]	<i>N</i> = 258 0% [–4%, 5%]	<i>N</i> = 131 4% [–2%, 10%]	<i>N</i> = 298 –7% [–11%, –3%]	<i>N</i> = 140 5% [–2%, 12%]	<i>N</i> = 315 0% [–4%, 4%]
AA	<i>N</i> = 121 0% [–8%, 9%]	<i>N</i> = 245 –3% [–9%, 3%]	<i>N</i> = 129 3% [–8%, 14%]	<i>N</i> = 258 –2% [–9%, 6%]	<i>N</i> = 131 1% [–7%, 10%]	<i>N</i> = 300 –1% [–7%, 5%]	<i>N</i> = 139 2% [–8%, 13%]	<i>N</i> = 315 1% [–6%, 9%]

The numbers in [] are the confidence intervals.

MS–HTN: Subjects with metabolic syndrome, but without HTN.

MS+ HTN: Subjects with metabolic syndrome and HTN.

Table 6 Correlation between change in blood pressure and change in inflammatory markers from baseline to 12 weeks for all subjects and those with HTN, diabetes mellitus or the metabolic syndrome

	<i>Log (CRP/CRP_base)</i>	<i>Log (IL18/IL18_base)</i>	<i>Log (MCP/MCP_base)</i>	<i>Log (SSA/SSA_base)</i>
ALL				
Change in DBP	–0.013 0.02% n.s.	–0.017 0.03% n.s.	0.057 0.32% <i>P</i> = 0.046	–0.022 0.05% n.s.
Change in SBP	0.008 0.006% n.s.	–0.0188 0.04% n.s.	0.032 0.10% n.s.	0.030 0.09% n.s.
HTN				
Change in DBP	–0.006 0.004% n.s.	–0.033 0.11% n.s.	0.046 0.21% n.s.	–0.036 0.13% n.s.
Change in SBP	–0.0007 0.00005% n.s.	–0.049 0.24 n.s.	0.008 0.006% n.s.	0.005 0.003% n.s.
DM				
Change in DBP	–0.008 0.006% n.s.	–0.019 0.036% n.s.	0.022 0.05% n.s.	0.020 0.04% n.s.
Change in SBP	0.060 0.36% n.s.	0.011 0.01% n.s.	0.011 0.01% n.s.	0.063 0.40% n.s.
MS				
Change in DBP	–0.054 0.29% n.s.	–0.029 0.08% n.s.	0.093 0.86% <i>P</i> = 0.046	–0.075 0.56% n.s.
Change in SBP	–0.024 0.06% n.s.	–0.010 0.01% n.s.	0.092 0.85% <i>P</i> = 0.047	0.004 0.002% n.s.

We found that changes in serum AA in response to atorvastatin was of a smaller magnitude than the change in CRP but greater than the change in MCP-1. Statin therapy, however, was associated with a more persistent reduction in serum AA in the hypertensive individuals compared with non-hypertensive individuals. The difference was largest for individuals who have HTN and diabetes mellitus.

Metabolic syndrome is a composite construct that has been the subject of considerable debate. Elevated blood pressure is one of the components for many of the diagnostic criteria of metabolic syndrome. Our analysis separated out those individuals with HTN from those without HTN. There were no significant differences in the CRP or IL-18 response to statins although individuals with HTN had a slightly smaller response. There were no consistent changes in MCP-1 or AA; although for MCP-1, the difference between those with HTN and those without HTN favored a greater decline for those with HTN.

Comparisons across the different inflammatory markers showed that HTN was associated with a consistent and meaningful increase in MCP-1 in all individuals as well as those with HTN and either diabetes mellitus or metabolic syndrome. Our data suggest that investigative efforts should be focused on these three factors especially MCP-1. MCP-1 is an important chemokine that regulates migration and infiltration of monocytes/macrophages and may serve as a key mediator of hypertensive-induced vascular disease.⁵⁶

Statins have been shown to reduce blood pressure⁶³ and to favorably affect vascular stiffness.⁶⁴ We did not intend to study the effect of statins on blood pressure and did not have a placebo group to assess this possibility. It is noteworthy that there was a significant reduction in blood pressure from baseline in the study. We found a significant but weak relationship between change in blood pressure and change in MCP-1 that occurred in individuals with metabolic syndrome. These data provide some support for focusing attention on MCP-1 in HTN.

There are several important considerations relevant to the limitations of the study. Firstly, the study measured circulating levels of four inflammatory markers. The tissue concentrations of these inflammatory markers or the relationship of these biomarkers to the presence of vascular disease was not ascertained. Secondly, the study was not designed as a placebo control trial to compare the effects of statin to placebo, which is relevant to changes over time in blood pressure, lipids and inflammatory markers. Instead, the study permits comparisons between inflammatory markers and between different cardiovascular risk factors HTN, diabetes mellitus, metabolic syndrome and their combination. Thirdly, causal relationships are difficult to infer from association analysis. Fourth, the designation—'metabolic syndrome' remain controversial. Our data however, should be viewed within the construct of factors that define metabolic syndrome.

In summary, this study in a relatively large number of individuals presents three kinds of data in persons with HTN and dyslipidemia—(i) levels of four inflammatory markers, (ii) their differences when there is concomitant diabetes mellitus or metabolic syndrome and (iii) changes in the inflammatory markers in each of disease combinations in response to statin therapy. In persons with dyslipidemia, HTN is associated with increased levels of CRP, IL-18, MCP-1 and serum AA, but the concomitant presence of diabetes mellitus or metabolic syndrome is associated with different kinds of activation of these inflammatory markers. In diabetes mellitus, HTN increases MCP-1, CRP and AA. In metabolic syndrome, HTN increases mainly MCP-1. There were no relationships with IL-18 and HTN associated with DM or MS. MCP-1 is the biomarker that is most consistently increased with HTN and the greatest CRP increase with HTN is in the

presence of DM. The ability of statins to reduce CRP and IL-18 is similar or slightly greater in hypertensive individuals. The smaller impact of statin therapy on AA and MCP-1 compared with CRP suggests that the action of statins is different depending on the mechanism of production for each cytokines. These data suggest a complex relationship between inflammation and HTN in persons with dyslipidemia, with different pattern of activation and responses to statins that has implications for pathogenesis of vascular disease and its treatment in HTN according to the concomitant presence of other conditions.

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