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Original Article

24-Hour ambulatory blood pressure associates inversely with prostaglandin $F_{2\alpha}$, interleukin-6 and F_2-isoprostane formation in a Swedish population of older men

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Abstract: Vasoconstrictive prostaglandins (PGs), such as $PGF_{2\alpha}$, F_2-isoprostanes, and systemic inflammation may be involved in the physiological regulation of blood pressure (BP) and the pathophysiology leading to hypertension. However, studies evaluating these parameters and BP in human populations are sparse. We analysed the cross-sectional associations between 24-hour ambulatory BP and urinary 15-keto-dihydro-$PGF_{2\alpha}$ (indicator of PG-mediated vasoconstriction and inflammation), plasma interleukin-6 (IL-6), C-reactive protein (CRP), serum amyloid A (SAA) and urinary F_2-isoprostanes (indicator of vasoconstriction and oxidative stress) in 619 men in a Swedish older population (Uppsala Longitudinal Study of Adult Men, age 78 years). Both systolic and diastolic 24-hour BP correlated inversely with concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ ($P<0.01$) and F_2-isoprostanes ($P<0.01$) independent on other cardiovascular risk factors. Additionally, diastolic 24-hour BP inversely correlated with plasma IL-6 ($P<0.05$) and 24-hour pulse pressure showed a positive linear correlation with IL-6, CRP and SAA. In conclusion, high BP is associated with decreased formation of vasoconstrictive $PGF_{2\alpha}$ and F_2-isoprostanes in this population of older men. These findings, although unlike our original hypothesis, might have an important physiological function which needs to be further evaluated.

Keywords: Blood pressure, pulse pressure, eicosanoids, prostaglandin $F_{2\alpha}$, F_2-isoprostanes, interleukin-6, inflammation, human, population

Introduction

Blood pressure (BP) homeostasis is crucial for an adequate cardiovascular balance and elevated BP is a major risk factor for cardiovascular diseases in most populations. BP regulation involves complex cascades of physiological systems including the sympathoadrenal and renin-angiotensin-aldosterone systems where prostaglandins (PGs) may also contribute to blood pressure homeostasis [1-3]. PGs of the 2-series (such as PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2) are formed from free arachidonic acid catalysed by cyclooxygenases (COX) in a variety of cells and possess potent biological activity *in situ*. $PGF_{2\alpha}$ is a major prostaglandin with potent vasoconstricting and pro-inflammatory properties [4, 5], and has been suggested to contribute to BP homeostasis by hypertensive and reno-vasoconstricting mechanisms [3]. $PGF_{2\alpha}$ dose-dependently elevates BP in mice via activation of the FP receptor which is primarily expressed in the kidney [3]. However, the role of $PGF_{2\alpha}$ for BP regulation in humans is not yet known. $PGF_{2\alpha}$ formation *in vivo* can be reliably estimated by measurement of 15-keto-dihydro-$PGF_{2\alpha}$ a major stable metabolite of $PGF_{2\alpha}$ [5-7].

Systemic inflammation may contribute to the progression of atherosclerosis subsequently leading to the development of cardiovascular diseases [8, 9]. Besides the PGs, cytokines (interleukin-6 [IL-6]), and acute-phase proteins (C-reactive protein [CRP], serum amyloid A pro-

tein [SAA]) are putative biomarkers of systemic inflammation [10-12]. Elevated blood pressure is a major risk factor for atherogenesis, and thus inflammation may be one of the potential links relating hypertension to atherogenesis. The association between systemic inflammation and BP is uncertain and has not been much studied in older populations.

F_2-isoprostanes are potent vasoconstricting prostanoids formed from esterified arachidonic acid catalysed by free radicals (not catalysed by cyclooxygenases as the primary PGs) in the state of oxidative stress or lipid peroxidation. 8-Iso-$PGF_{2\alpha}$, a major F_2-isoprostane, is currently regarded as one of the most reliable indicators of *in vivo* oxidative stress and increased formation of isoprostanes have been associated with many cardiovascular risk factors [13-17]. However, studies have shown conflicting results concerning hypertension and isoprostanes, thus the association between F_2-isoprostanes and BP still remains uncertain [18-21].

This study aimed at investigating the associations between 24-hour ambulatory BP and concentrations of 15-keto-dihydro-$PGF_{2\alpha}$, IL-6, CRP, SAA and F_2-isoprostanes in an older population.

Materials and methods

Study population and sample collection

This study is based on the participants from the third reinvestigation of the population-based ULSAM cohort (Uppsala Longitudinal Study of Adult Men), which was performed when the participants were 78 years old (mean age 77.5±0.8 years). The ULSAM cohort originated in 1970, when all men born between 1920 to 1924 and residing in Uppsala county were invited to participate in a health survey (2841 men, participation rate 82%) [22]. In the present reinvestigation, the 1398 men still alive were invited, and 839 agreed to participate. Of the 839 men, 619 collected urine for eicosanoid analyses and were eligible for 24-hour BP measurements, thus constitute the present study population. The Ethics Committee at Uppsala University approved to the study, and all participants gave their informed consent.

Twenty-four hour urine was collected for analysis of eicosanoids. Blood samples were drawn in heparinized tubes in the morning after an overnight fast and serum was separated. Both urine and serum were stored at -70°C until analysis.

BP measurements

Twenty-four hour ambulatory BP was recorded using the Accutracker 2 equipment (Suntech Medical Instruments, Raleigh NC, USA). The device was attached to the subjects' non-dominant arm by a skilled lab technician. Systolic (SBP) and diastolic (DBP) BPs were measured every 20 minutes during the whole 24 hour period. Data editing have been desribed previously [23]. Short fixed-clocktime intervals were used, defining daytime as 10 a.m. to 8 p.m. and night-time as midnight to 6 a.m. We used 135/85 mmHg as the threshold for normal daytime ambulatory BP. Pulse pressure (PP) was calculated as the difference between SBP and DBP.

Measurements of urinary 15-keto-dihydro-$PGF_{2\alpha}$, serum IL-6, CRP and SAA

Urinary 15-keto-dihydro-$PGF_{2\alpha}$ was analysed by a radioimmunoassay (RIA) developed as previously described by Basu [7]. The intra-assay coefficient of variation (CV) was 12-14%. Concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ were corrected for urinary creatinine (IL Test creatinine 181672-00, Monarch 2000 analyser, Instrumental Laboratories, Lexington, MA, USA). High sensitivity CRP and SAA were analysed in serum by latex enhanced reagent (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring). The intraassay CV of the CRP method was 1.4% at both 1.23 mg/L and 5.49 mg/L and the intraassay CV of the SAA method was 5.9 % at 12.8 mg/L and 3.2 % at 81.7 mg/L. Serum IL-6 was analyzed by an ELISA kit (IL-6 HS, R&D Systems, Minneapolis, MN). The total CV of the method was 7% and interassay CV was 5%.

Measurements of urinary F_2-isoprostanes

Free 8-iso-$PGF_{2\alpha}$ in urine was analysed by a RIA as previously described by Basu [24]. The intra-assay CV was 12-15%. Concentrations of 8-iso-$PGF_{2\alpha}$ were corrected for urinary creatinine.

Statistics

Variables with skewed distribution, according to Shapiro-Wilks test ($W<0.95$), were log-

transformed to reach normal distribution. Associations between 24-hour and day-time BP and eicosanoids, cytokines and acute phase proteins, respectively, were tested in linear regression models. Body mass index, diabetes (plasma glucose ≥ 7.0 mmol/L or anti-diabetic medication), smoking status (obtained by questionnaires), history of cardiovascular diseases (according to Swedish Hospital Discharge Registry), low-dose aspirin treatment (75 mg to 160 mg salicylic acid daily) and antihypertensive treatment were considered as potential confounders in the models. P-values <0.05 were regarded as statistically significant. Calculations were performed with Stata IC8.2 and IC11 (Stata Corporation, College Station, TX).

Results

Characteristics of study population

24-Hour ambulatory SBP and DBP, daytime ambulatory SBP and DBP, pulse pressure, urinary 15-keto-dihydro-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$ and serum IL-6, CRP and SAA are presented in **Table 1** and **2**. Seven percent of the subjects were smokers and 14% had diabetes mellitus. Mean BMI was 26.2 ± 3.4 kg/m^2 and 28% had a previous history of clinical cardiovascular disease (including myocardial infarction, ischemic stroke and angina pectoris). Twenty-nine percent were treated with low-dose aspirin and 292 men out of 614 (48%) had antihypertensive treatment (7 had alpha blockers, 104 diuretics, 153 beta blockers, 94 calcium antagonists and 96 ACE-inhibitors or angiotensin receptor blockers). Seventy-nine percent of the study population could be classified as hypertensive, defined as day-time ambulatory blood pressure >135/85 mmHg or antihypertensive treatment. This hypertensive group did not significantly differ in urinary concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ or F_2-isoprostanes compare to the normotensive group (data not shown).

24-Hour ambulatory BP and urinary 15-keto-dihydro-$PGF_{2\alpha}$

Both 24-hour and daytime SBP and DBP were linearly significantly inversely associated with urinary concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ as shown in **Table 1**. The associations remained significant after adjustment for established cardiovascular risk factors. Urinary concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ were also negatively associated with DBP (but not SBP) quartiles, P = 0.02 for linear trend across quartiles. The highest DBP quartile (range 80-104 mmHg) had significantly lower urinary 15-keto-dihydro-$PGF_{2\alpha}$ concentrations than the lowest DBP quartile (range 51-67 mmHg), P = <0.01. 24-hour PP showed a trend towards an inverse association with concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ however this was not significant (**Table 1**).

24-Hour ambulatory BP and serum IL-6, CRP, SAA

Serum IL-6 was significantly negatively associated with DBP and with day-time DBP in the studied population whereas serum CRP and SAA did not show any linear association with ambulatory DBP (**Table 1**). Serum IL-6, CRP and SAA were all significantly positively associated with 24-hour PP even after adjustment for potential confounders.

24-Hour ambulatory BP and urinary F_2-isoprostanes

Both 24-hour and day-time SBP and DBP were linearly inversely correlated with urinary F_2-isoprostanes as shown in **Table 2**. The associations remained significant after adjustment for established cardiovascular risk factors. Urinary concentrations of F_2-isoprostanes were negatively associated with SBP across quartiles, P = 0.04 for linear trend, and DBP across quartiles, P = 0.003 for linear trend. The highest SBP quartile (range 143 - 203 mmHg) had significantly lower urinary F_2-isoprostanes concentrations than the lowest SBP quartile (range 88-124 mmHg), P = 0.02. The highest DBP quartile (range 80 - 104 mmHg) had significantly lower urinary F_2-isoprostanes concentrations than the lowest DBP quartile (range 51-67 mmHg), P = 0.02. 24-Hour PP was significantly inversely related to urinary F_2-isoprostanes (**Table 2**).

Discussion

This is the first study to show measurements of *in vivo* formation of $PGF_{2\alpha}$ in relation to BP in a human population and the data indicate that 24 -hour ambulatory SBP and DBP correlate inversely with $PGF_{2\alpha}$ formation. The explanation for these unexpected inverse associations is unclear and can unfortunately not be fully answered in this study. However, we speculate that the role of $PGF_{2\alpha}$ in BP regulation may be

Table 1. Linear associations between 24-h blood pressure and urinary 15-keto-dihydro-$PGF_{2\alpha}$ and serum IL-6, CRP and SAA in the study population.

		Inflammatory biomarkers, median [interquartile interval]											
		15-keto-dihydro-$PGF_{2\alpha}$ 280 [213-374] (pmol/mmol Cr)			IL-6 2.8 [2.1-4.7] (ng/L)			CRP 1.8 [1.0-4.3] (mg/L)			SAA 4.0 [2.4-7.0] (mg/L)		
		r	P	P (adj.)	r	P	P (adj.)	r	P	P (adj.)	r	P	P (adj.)
Blood pressure (mmHg) mean ± SD (CV%)	24-hour SBP 134 ± 15 (11)	-0.11	<0.01	<0.01	-0.03	0.50	0.64	0.09	<0.05	0.16	0.09	<0.05	0.08
	Day-time SBP 138 ± 16 (12)	-0.10	<0.05	<0.05	-0.05	0.90	0.89	0.07	0.07	0.29	0.08	<0.05	0.14
	24-hour DBP 73 ± 8.1 (11)	-0.10	<0.05	<0.01	-0.08	<0.05	<0.05	-0.03	0.43	0.16	-0.01	0.90	0.55
	Day-time DBP 76 ± 8.6 (11)	-0.10	<0.05	<0.01	-0.11	<0.01	<0.05	-0.05	0.22	0.09	-0.03	0.47	0.24
	24-hour PP 61 ± 12 (20)	-0.07	0.08	0.10	0.09	<0.05	<0.05	0.14	<0.001	<0.01	0.13	<0.01	<0.01

Cr, urinary creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; r, correlation coefficient; P, unadjusted p-value; P (adj.), linear regression model adjusting for antihypertensive treatment, history of cardiovascular diseases, smoking, diabetes, BMI and low-dose aspirin treatment; PP, pulse pressure

Table 2. Linear associations between 24-h blood pressure and urinary F_2-isoprostanes in the study population.

		F_2-isoprostanes 185 [142-241] pmol/mmol Cr		
		r	P	P (adj.)
Blood pressure (mmHg) mean ± SD (CV%)	24-hour SBP 134 ± 15 (11)	-0.14	<0.001	<0.001
	Day-time SBP 138 ± 16 (12)	-0.13	<0.01	<0.01
	24-hour DBP 73 ± 8.1 (11)	-0.13	<0.01	<0.01
	Day-time DBP 76 ± 8.6 (11)	-0.11	<0.01	<0.01
	24-hour PP 61 ± 12 (20)	-0.09	0.03	<0.05

Cr, urinary creatinine; SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); r, correlation coefficient; P, unadjusted p-value; P (adj.), linear regression model adjusting for antihypertensive treatment, history of cardiovascular diseases, smoking, diabetes, BMI and low-dose aspirin treatment; PP, pulse pressure.

complex due to the well known dual properties of $PGF_{2\alpha}$. $PGF_{2\alpha}$ mediates inflammation but is also a potent local vasoconstrictor [25, 26], thus the measured $PGF_{2\alpha}$ may indicate both inflammation and vasoconstriction to different extent depending on the study settings. Thus far, most studies evaluating $PGF_{2\alpha}$ in clinical settings collectively highlight mainly the pro-inflammatory properties of $PGF_{2\alpha}$. Patients with cardiovascular risk factors such as type 1 and type 2 diabetes mellitus [27, 28], obesity [29], present atherosclerosis [30], selenium deficiency [31], low dietary vitamin intake [32] and smokers [33] have shown increased $PGF_{2\alpha}$ formation in clinical studies. These studies together suggest that $PGF_{2\alpha}$ with its pro-inflammatory properties may indicate a pathogenetic link between traditional cardiovascular risk factors and atherosclerosis progression. However the results from this present study of older men shows that high BP, which usually is cardiovascular risk factor in the population, is not associated with increased $PGF_{2\alpha}$ formation and thus do not comply with the inflammation linkage theory in this pathology.

We speculate that the inverse relationships seen between BP and $PGF_{2\alpha}$ may be related to the potent vasoconstricting properties of $PGF_{2\alpha}$ *in vivo* [25, 26], and these properties may be part of the normal physiology of BP homeostasis rather than representing a pathological state in this setting. The family of PGs possibly contributes to BP homeostasis by a delicate balance between antihypertensive and vasodilatating prostanoids (PGE_2 and PGI_2) or by hypertensive and vasoconstricting prostanoids ($PGF_{2\alpha}$) [3]. However the exact roles of the prostanoids in blood pressure regulation in humans are still not known [2]. The presented data in this study are cross-sectional and therefore no firm conclusions about cause and effect can be drawn. Thus, lower $PGF_{2\alpha}$ concentrations may be seen as a compensatory consequence to high BP. It is possible that an elevation in BP physiologically leads to less production of $PGF_{2\alpha}$ and perhaps a higher production of other PGs with vasodilatating properties locally (PGE_2 or PGI_2) to balance and possibly counteract the elevated BP. The majority of the participants of this study population had well regulated BP levels considering their high age. Studies in mice have suggested that angiotensin II which mediate BP elevation induce the vasoconstrictor $PGF_{2\alpha}$ but also the counter-regulatory, vasodilatator PGE_2 (via activation of AT_2 and AT_1 receptors, respectively) in the kidney and that these prostanoids interact locally to maintain normal BP homeostasis [34]. Thus, a lower BP level may via activation of the renin-angiotensin system (RAAS) and induction of angiotensin II induce renal vasoconstrictive $PGF_{2\alpha}$ in attempt to counteract the low BP and maintain a healthy BP level. This may be a speculative reason why both SBP and DBP correlate inversely with systemic $PGF_{2\alpha}$ formation.

Similarly, this study showed an inverse linear association between 24-hour ambulatory DBP and the inflammatory biomarker IL-6. High BP is a major cardiovascular risk factor in most populations, and thus it could be expected that high

BP rather would associate with an enhanced systemic inflammatory pattern. Studies of IL-6 and BP in other age groups than elderly including middle-aged women [35] and men [36, 37] generally show positive associations and support the role of inflammation as a pathogenetic link between hypertension and cardiovascular diseases. CRP, an acute phase protein induced in the liver by IL-6 regulation, have also shown positive associations between BP and hypertension cross-sectionally [38-40], and CRP may predict hypertension in middle-aged men and women [41-45]. We could however not find significant associations between CRP and BP in this present study of older men. The reasons for the inverse associations seen between BP and IL-6 in this study are not known but we speculate that they may be related to the very high age of the study participants (77-78 years). Studies of hypertension in the very elderly (>75-80 years) are few and there is an ongoing debate whether low BP always relates to a longer life in very old populations [46-48]. Some studies have reported a J or U-curve relationship between BP and cardiovascular events [49] and between SBP, PP and mortality in an elderly population (>69 years), respectively [47]. Moreover, low SBP and DBP are associated with higher mortality risk in very old (>75 years) patients with type 2 diabetics [48]. In this Swedish cohort of men, PP was a better prognostic indicator of future cardiovascular events than merely SBP or DBP alone [50], thus low DBP would represent a cardiovascular risk factor in this study population. This fits well with the findings in this study that IL-6, CRP and SAA in serum correlated positively with 24-hour PP. Thus, an increased PP seemed to be associated with increased systemic inflammatory activity and speculatively higher atherosclerotic load in the arteries.

This is the first study to show inverse linear associations between F_2-isoprostane formation and both systolic and diastolic ambulatory BP. Even this result is contrary to what we originally expected. It has been postulated that oxidative stress, i. e. when the extent of free radicals produced severely exceeds the capacity of the antioxidative defence, may contribute to the pathogenesis of essential hypertension and atherogenesis [8, 9]. Based on this subtle theory a positive correlation between blood pressure and the gold standard indicator of oxidative stress F_2-isoprostanes could have been expected. However, previous studies evaluating urinary and plasma F_2-isoprostanes in relation to office BP [18] and 24-hour BP [19, 21] could not find any significant associations. The exception is that severe hypertensives have increased concentrations of plasma F_2-isoprostanes [20].

The F_2-isoprostane 8-iso-$PGF_{2\alpha}$, which is quantified in this study, has like $PGF_{2\alpha}$ dual properties. 8-Iso-$PGF_{2\alpha}$ is not only an indicator of oxidative stress but also a potent renal vasoconstrictor [51, 52] and its effect partly mediates by the thromboxane A_2 receptor. Further, F_2-isoprostanes are induced by angiotensin II infusion [53] and may contribute to the vasoconstriction and hypertensive effects of angiotensin II. In analogy with the discussion above concerning physiological regulation of normal BP and $PGF_{2\alpha}$ formation, a lower BP level may, in order to assure an adequate circulating blood flow, via activation of the renin-angiotensin-aldosterone system and induction of angiotensin II induce renal vasoconstrictive 8-iso-$PGF_{2\alpha}$ to counteract the low BP. Thus, the inverse linear correlation seen between both SBP and DBP and systemic 8-iso-$PGF_{2\alpha}$ concentrations may speculatively be related to this proposed feedback regulation.

Antihypertensive treatment (ACE inhibitors or angiotensin II receptor blockers) has been associated with decreased formation of F_2-isoprostanes, and thus proposed to have antioxidative properties [21]. Low-dose aspirin treatment has a direct therapeutic cyclooxygenase-inhibitor effect on PG-synthesis and is related to decreased $PGF_{2\alpha}$ formation [54]. Thus, due to the heterogeneity and considerably high frequency of antihypertensive and low-dose aspirin treatment among the study participants it can not be excluded that the inverse associations between BP and $PGF_{2\alpha}$ formation and F2-isoprostanes to some extent may be confounded by medication. However, the associations remained significant after adjustment for antihypertensive and low-dose aspirin treatment, thus medication does not seem to be a major confounder of the associations seen in this setting.

The strengths of this study are the community-based design and the very high age of the study participants which is an important and rarely studied age group. The interpretation of the results is limited because of the relatively weak

correlation coefficients observed. This may be related to the heterogeneity of the study population with respect to several diseases or medication and the results may be confounded by other factors than those we have adjusted for. Further, the problems of generalizing these results to women, other age-groups and other ethnic groups have to be acknowledged.

In conclusion, this study is first to report inverse associations between ambulatory BP and concentrations of $PGF_{2\alpha}$, F_2-isoprostanes and IL-6, respectively. Although these results oppose our original hypothesis the findings might have an important physiological function but yet to further evaluate in detail.

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