Adiponectin is Not Associated With Blood Pressure in Normotensives and Untreated Hypertensives With Normal Kidney Function

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Abstract: The role of adiponectin in hypertension is still a matter of debate. Obtained conflicting results could be mostly explained with diversity of subjects included in different studies. Our aim was to analyze association of adiponectin with blood pressure (BP) in a group of normotensive and untreated hypertensive subjects.

Participants (N = 257) were selected from a random sample of 2487 subjects enrolled in an observational cross-sectional study. Subjects with diabetes and chronic kidney diseases were excluded. BP was measured using Omron M6 device followingESH/ESC guidelines. Adiponectin concentration was determined by ELISA.

There were no differences in adiponectin values (mg/L) between hypertensives and normotensives (median 9.75; iqr: 7.44–17.88 vs 11.35; iqr: 7.43–12.63; P = 0.17). On univariate linear regression adiponectin was not associated with systolic or diastolic BP (P > 0.05). Furthermore, multivariate analysis did not show significant contribution of log-transformed adiponectin either to systolic (β = −0.040; P = 0.43) or diastolic BP (β = 0.066; P = 0.33).

In our group of normotensives and untreated hypertensives with normal kidney function adiponectin was not associated with BP even after adjustment for other risk factors. Our results and conclusions should not be extrapolated to subjects with other characteristics.

INTRODUCTION

Hypertension and obesity are growing to epidemic proportions primarily due to unhealthy lifestyle and inefficient primary prevention. Contrary to the majority of adipokines, adiponectin was reported to be lower in obese individuals and weight-loss regimes significantly increase its concentration.1 It was reported that hypoadiponectinemia has a role in the pathogenesis of atherosclerosis, type-2 diabetes and hypertension.2 Adamczak et al found that adiponectin plasma concentration was significantly lower in patients with essential hypertension compared to normotensives with similar body mass index (BMI).3 Iwashima et al showed that hypoadiponectinemia is a risk factor for hypertension independent of insulin resistance and diabetes.4 An inverse correlation was found between adiponectin concentrations and risk of developing hypertension later in life.5 Kim et al reported that 1 μg/mL increase in adiponectin levels was associated with 6% reduction in risk of hypertension.6 On the contrary, Murakami et al in a study encompassing 3 populations found no significant difference in adiponectin concentration between normotensives and insulin sensitive hypertensive patients.7 No correlation was observed between adiponectin plasma concentrations and blood pressure (BP) in children.8,9 In addition, Copenhagen City Heart Study failed to show a predictive value of adiponectin for BP values in adults which is in line with British Women’s Heart and Health Study where no relationship between adiponectin and BP was found although high molecular weight multimer of adiponectin was analyzed.10,11,12

Due to conflicting published results obtained in populations with different levels of cardiovascular risk and/or chronic kidney disease (CKD), our aim was to analyze the relationship of adiponectin with BP in a selected group of normotensives and untreated, newly diagnosed hypertensives with estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m².

SUBJECTS AND METHODS

In this cross sectional observational study, we enrolled 257 adults (160 females, 97 males) selected from a random sample of 2487 people who were recruited in a survey conducted between 2008 and 2010 in rural continental Croatian area. Study interviewers went door-to-door asking if the adult resident (older than 18 years of age) would be willing to participate
in the study. All households in the target villages were approached. If no one was home, interviewers returned over the next several days and attempted to make contact with the resident. Individuals agreeing to participate in the study completed an extensive survey administered by the study personnel and also provided spot urine and fasting blood sample. Among villages participation rate ranged from 60.8% to 88.7%. Inclusion criteria in this study were: written informed consent, normotension or untreated hypertension and eGFR > 60 mL/min/1.73 m². Exclusion criteria were: unsigned written consent, treated hypertensives, pregnancy, diabetes, terminally ill patients and subjects with severe disabilities, people with one or more amputated limbs and those suffering from dementia and psychiatric illnesses. To diminish and exclude the effect of kidney impairment on adiponectin values patients with eGFR < 60 mL/min/1.73 m² were excluded. Study was approved by Institutional Review Boards of University of Zagreb School of Medicine and Croatian Public Health Institute.

BP was measured according to ESH/ECS guidelines. Patients rested in a sitting position for 10 minutes in a quiet room at 22°C before BP measurements were recorded. They were asked to avoid caffeine-containing drinks and to refrain from smoking and exercise for the 12 hours preceding the measurements. BP and heart rate were measured using the Omron M6 Comfort device (Omron Corporation, Kyoto, Japan) and 2 cuff sizes (standard and large) on 2 visits (one at participants’ home and the other in office). At each visit BP was measured 3 times with 3 minutes period between measurements and average of second and third measurement was used further. Final BP value which was used for analyses was calculated as a mean of average values obtained from office and home BP measurements. Hypertension was defined as BP ≥ 140/90 mm Hg while BP values lower than 140/90 mm Hg were considered normotensive. All hypertensive patients enrolled in this study were newly diagnosed hypertensives who were never treated. BMI was calculated as: weight [kilograms] divided by square of height [meters]. Waist circumference was measured by elastic tape in 3 separate occasions and arithmetic mean of these measurements was used. Waist circumference larger than 102 cm for men and 88 cm for women was used as a measurement of central obesity. In our group, out of 257 subjects, 97 were ex or current smokers (25 and 72, respectively) and 8 subjects had missing smoking information, which gives us a prevalence of ex or current smoking of 39%. Smoking was quantified as pack-years (number of pack-years = (number of cigarettes smoked per day × number of years smoked)/20).

Subjects provided fasting blood samples and first morning spot urine samples. Serum creatinine was determined on Olympus AU 2700 using continuous photometric method with alkaline pikrate (Olympus, Tokyo, Japan). α1-microglobulin and urine albumin were determined on Siemens Dade Behring BN II Nephelometer (Siemens, Germany), and were corrected to urine creatinine (ACR and α1CR, respectively). Glomerular filtration was calculated using abbreviated MDRD equation: eGFR = 186.3 × [Serum Creatinine]⁻¹.⁰₁⁵ × AGR⁻⁰.²⁰³ × (0.742 for females). HOMA index was used to express insulin resistance (HOMA-IR = [Insulin (μIU/mL) × [Glucose][mmol/L]]/22.5). In this study, insulin resistance was defined as HOMA-IR > 3. Total adiponectin and leptin concentrations were determined using enzyme immunoassay method (ELISA) (Behring GmbH, Germany), high sensitivity C-reactive protein (hsCRP) using an immunoturbidimetric method on latex particles, insulin concentrations by immunochemical detection with electroluminescence and glucose using photometry with hexokinase or glucose oxidase (Olympus System Reagent Kit na Olympus AU2700, Tokyo, Japan).

Statistical analysis was performed in SPSS v.21 (IBM Corp., Armonk, NY). Numerical variables were tested for normality of distribution using D’Agostino-Pearson test. Categorical variables were compared with chi square test. Normally distributed variables were presented as arithmetic mean ± standard deviation and Student t test was used to compare the 2 groups. Analysis of covariance (ANCOVA) was used to test for differences across group after adjustment for multiple covariates and factors. Multiple regression analysis was used to determine the impact of several independent variables on one dependent variable. For correlation analysis we used Pearson’s and Spearman’s test. In all tests a 2 sided P-value <0.05 indicated statistical significance.

Power calculation was carried out and the equation using Cohen’s $d = 0.45$ ($\alpha = 0.05$, $\beta = 0.80$) estimated minimum needed sample sized for two-tailed hypothesis of 79 per group (158 total).

**RESULTS**

There was no difference in gender distribution between hypertensives and normotensives ($P > 0.05$). Hypertensives were older, had higher BMI and greater proportion of visceral obesity (all $P < 0.001$) (Table 1). Higher values of total cholesterol, LDL cholesterol, triglycerides, fasting blood glucose, insulin values and HOMA index were determined in hypertensives (all $P < 0.01$). They also had significantly lower eGFR and higher ACR values (both $P < 0.001$) (Table 2). Hypertensives also had higher values of hsCRP and leptin (both $P < 0.05$) but there was no significant difference in adiponectin values (9.75 [iqr: 7.43–12.63] mg/L vs 11.35 [iqr: 7.44–17.88] mg/L; $P = 0.17$). Additionally, we failed to find a difference in adiponectin even after adjustment for factors known to affect adiponectin concentrations (age, sex, BMI, waist circumference, HOMA-IR, eGFR, total cholesterol and hs-CRP) (mean (standard error), SE): 10.97 (1.10) vs 10.72 (1.15), $P = 0.95$.

As shown in Table 2, adiponectin values were higher in women than in men in both hypertensives and normotensives but difference did not reach statistical significance ($P > 0.05$). We failed to find difference in adiponectin values between insulin resistant and insulin sensitive subjects in both groups.

We have carried out a sensitivity analysis using Receiver Operated Curves analysis (ROC) and failed to observe difference between HOMA-IR > 3 (AUC = 0.568, $P = 0.15$), HOMA-IR > 2.5 (AUC = 0.553, $P = 0.29$) and HOMA-IR > 2 (AUC = 0.554, $P = 0.30$). We tested if a lower cut-off value of HOMA-IR might show differences in adiponectin concentrations between IR and non-IR subjects, but again there was no difference either with cut off HOMA-IR > 2.5 (10.10 (iqr: 6.70–13.68) mg/L vs 11.20 (iqr: 7.85–16.65) mg/L; $P = 0.29$) and HOMA-IR > 2 (10.40 (iqr: 7.08–13.90) mg/L vs 11.35 (iqr: 7.44–17.88) mg/L; $P = 0.31$). Based on this analyses it could be concluded that our results are comparable with results of other studies where different cut off values for HOMA-IR were used.

On correlation analysis in normotensive as well as in hypertensive group adiponectin did not correlate with systolic BP ($r = -0.103$, $P = 0.39$; $r = -0.201$, $P = 0.27$, respectively) or diastolic BP ($r = 0.073$, $P = 0.54$; $r = 0.020$, $P = 0.91$, respectively).

For regression analysis adiponectin was log-transformed because of significant positive skew in distribution of
| TABLE 1. Anthropometric and Clinical Parameters in the Whole Group Stratified According to Blood Pressure, and in Normotensives and Hypertensives Stratified According to Gender |
| --- |
| **Number of subjects (M/F)** | Male | Female | P-Value |
| **Normotensive** | **Hypertensive** | 58/106 | 39/54 | 0.30 |
| **Weight (kg)** | 74 (17) | 81 (17) | 0.31 |
| **BMI (kg/m²)** | 25.9 (5.3) | 25.9 (5.3) | 0.109 |
| **BMI ≥35 (%)** | 52 (4%) | 77 (39%) | <0.001 |
| **Waist circumference (cm)** | 97 (44) | 97 (44) | <0.001 |
| **Diastolic blood pressure (mm Hg)** | 74 (8) | 74 (8) | <0.001 |
| **Systolic blood pressure (mm Hg)** | 106 (15) | 106 (15) | <0.001 |

**DISCUSSION**

According to vast majority of reports, adiponectin, has beneficial properties, and it was reported that it improves insulin sensitivity, has anti-inflammatory role and is related to lower BP values. A recently published meta-analysis by Kim...
TABLE 2. Laboratory Parameters in the Whole Group Stratified According to Blood Pressure, in Nonhypertensives and Hypertensives Stratified According to Gender

| Parameter                        | Nonhypertensive | Hypertensive | Male | Female | Male | Female | Male | Female |
|----------------------------------|-----------------|-------------|------|--------|------|--------|------|--------|
| Total cholesterol (mmol/L)       | 5.42 (1.25)     | 5.81 (1.07) | 0.08 | -0.01  | 0.04 | 0.08   |
| LDL-cholesterol (mmol/L)         | 3.24 (0.82)     | 3.56 (0.73) | 0.04 | -0.01  | 0.04 | 0.08   |
| HDL-cholesterol (mmol/L)         | 1.52 (0.67)     | 1.56 (0.39) | 0.04 | -0.01  | 0.04 | 0.08   |
| Fasting blood glucose (mmol/L)   | 5.14 (1.02)     | 5.72 (0.96) | 0.04 | -0.01  | 0.04 | 0.08   |
| Insulin (U/mL)                   | 7.14, 5.08–20.68 | 9.28, 6.75–21.16 | 0.04 | -0.01  | 0.04 | 0.08   |
| HOMA-IR (mU/mL mmol/L)           | 1.40, 0.50–2.10 | 1.35, 0.50–2.70 | 0.04 | -0.01  | 0.04 | 0.08   |
| Albumin (g/L)                    | 4.21, 2.49–7.32 | 5.33, 3.66–9.69 | 0.12 | 0.02   | 0.04 | 0.08   |
| ACR (mg/mmol)                    | 3.33, 1.88–17.26 | 4.02, 2.98–25.33 | 0.12 | 0.02   | 0.04 | 0.08   |

Notes: Values are presented as median and interquartile range or mean (standard deviation).

Adiponectin values are inversely correlated to BMI and visceral fat accumulation which is in line with our results. It was observed that after weight loss adiponectin values increase in obese subjects. Oxidative stress and low-grade inflammation are consequences of visceral fat growth and it was discussed whether those disturbances could secondary increase adiponectin concentration. While some authors found lower levels of adiponectin in patients with metabolic syndrome, others reported that higher adiponectin levels are protective for incident metabolic syndrome. This complex and paradoxical association of adiponectin values was also observed in patients with CKD who have increased global cardiovascular risk. Adiponectin values were significantly higher in patients with CKD and end-stage renal disease which was explained partly with decreased clearance but mostly with metabolic disease.
disturbances and increased adipose tissue production as the 
response to the inflammatory environment and probably due 
to increased sympathetic activity.44–47

Aiming to exclude influence of kidney impairment on 
adiponectin values we did not include subjects with 
eGFR < 60 mL/min/1.73 m² which was not done by other 
authors.8,49 This made our group more homogenous and 
our results probably more credible, but could also explain why our 
results differ from other authors. The next possible explanation 
for observed difference between our results and data from other 
studies is the definition of insulin resistance. Wishing to exclude 
subjects who might be in the ‘‘grey zone’’ of insulin resistance 
we used higher cut-off value in ROC curves for HOMA-IR 
when comparing subjects by insulin resistance, while majority 
of authors used lower cut-off values.7,8,50 However, we obtained 
same results when we performed additional analyses using 
lower cut-off values for HOMA-IR.

It could be speculated that rural population differs from 
urban in some characteristics which might have impact on 
the adiponectin values. One could argue that rural population might 
be healthier than urban with less obesity, more physical activity 
etc. Indeed, it was reported by Milošević et al that in general 
Croatian population urban residents are probably less physically 
active than rural.53 On contrary, in nationwide survey ‘‘Epi-
demiology of Hypertension in Croatia, EH-UH study’’ Jelaković 
et al failed to find differences between urban and rural popu-
lation (unpublished data). However, relationship between adi-
oponectin and physical activity is still unclear. Dvorakova et al 
showed that adiponectin values did not significantly change in 
obese women subjected to a weight loss regime and physical 
activity.52 Ring-Dimitriou et al reported that after 12 months of 
physical activity increased adiponectin in men but not women 
with pre-disposition for metabolic syndrome.53 Contrary to this, 
Kozakova et al found that moderate to vigorous physical 
activity is related to a decrease in adiponectin.54

Independent reverse association between smoking and 
adiponectin was reported.55,56 In our group we found that 
smokers never smoked had borderline significantly 
higher adiponectin values than ex-smokers and current smokers, 
and observed a negative association with log adiponectin where 
every pack-year decreased adiponectin concentration for 1 mg/ 
L. However, we were not able to confirm that smoking is an 
independent predictor of adiponectin concentration in normo-
tensives and untreated, newly diagnosed hypertensives.

This is the first study to the best of our knowledge that 
includes only subjects with eGFR > 60 mL/min/m². This is an 
important point as adiponectin-kidney relationship is known to 
be complex. This could be the reason why many studies which 
cluded patients with impaired kidney function have shown 
conflicting results. Our aim was to exclude this impact which 
could modulate effects of adiponectin and to establish if there is 
an effect of adiponectin on BP in subjects with normal kidney 
function. Another important point is the inclusion of only 
untreated hypertensives which enabled us to correctly assess 
associations of adiponectin with BP.

Finally, based on obtained results it could be concluded 
that in the group of normotensives and non-treated hyperten-
sives without comorbidity and normal kidney function adipo-
nectin is not associated with BP. This finding does not discount 
the possibility that adiponectin and BP relationship is not the 
same in normotensives, pre-hypertensives and early stages of 
hypertension compared to patients in advanced stages of hy-
pertension and/or to patients with target organ damage particu-
larly those with CKD. Obviously, the relationship between 
adiponectin and BP may not be the same in all patients’ 
subgroups. Our results obtained in a population of untreated 
hypertensives and normotensives with normal kidney function 
should not be extrapolated to other patients or groups and should 
only be compared with similar studies.

**LIMITATIONS**

Our study has several limitations. Firstly, this is a cross-
sectional observational and not a prospective research. How-
ever, it is interesting to note that recent meta-analysis by Kim 
et al found no significant difference between weighted mean 
differences and dose-response relationship between cross-sec-
tional and prospective studies.57 When calculating insulin resist-
ance we used HOMA formula and not insulin ‘‘clamp’’ 
technique. Nevertheless, other authors in epidemiological stu-
dies also pre-dominantly used this method. GFR was estimated 
using abbreviated MDRD formula which was modeled on 
patients with kidney disease and is known to underestimate 
kidney function in healthy subjects. Nevertheless, majority of 
authors used this formula as well. Total adiponectin was 
determined and not high-molecular-weight adiponectin. Impor-
tantly, vast majority of authors used the same isomer as we did, 
thus our results could be compared to those studies. One could
argue whether the number of subjects in our study is a disadvantage, but many other similar studies on adiponectin had similar or even smaller groups. In fact, 25 out of 43 non-prospective studies included in meta-analysis by Kim et al had fewer subjects than our study."}
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