Endogenous Erythropoietin Levels in Patients with Obesity-Related Hypertension

Michal Nowicki, Franciszek Kokot, Michal Kokot, Jan Dulawa, and Edward Franek

The coincidence of increased red blood cell mass with obesity and hypertension has been confirmed in a number of studies. Since erythropoietin (EPO) induces hypertonic effect, we aimed to study the possible involvement of endogenous EPO in the pathogenesis of obesity-related hypertension (ORH). Seventy-two non-obese (body mass index-BMI < 25 kg/m²) and 47 obese (BMI > 30 kg/m²) patients with essential hypertension, and 32 non-obese normotensive control subjects were studied on 120 mmol Na/24 h diets for 3 days and 30 mmol Na/24 h diets for the following 3 days. Serum EPO levels, plasma renin activity (PRA), aldosterone (PAC) and urinary sodium and potassium excretion were estimated under both conditions. Hematocrit, hemoglobin and serum iron, ferritin and TIBC were measured in basal conditions. Patients with ORH showed significantly higher hematocrit values, hemoglobin levels and erythrocyte counts (p < 0.05) than non-obese essential hypertensives and controls, although no differences in serum EPO levels (13.6 ± 1.1 and 12.8 ± 1.0 mU/ml, 15.3 ± 1.2 and 13.3 ± 1.1, 12.2 ± 2.0 and 10.95 ± 1.7, respectively) and log EPO × %Hct product were found between groups. In both hypertensive groups, BMI but not blood pressure positively correlated with hematocrit. A negative correlation between changes in EPO and PRA induced by sodium restriction was found in lean hypertensive subjects only (r = −0.42, p < 0.01). This finding suggests that in contrast to non-obese patients with essential hypertension, in subjects with ORH activity of the renin-angiotensin-aldosterone system does not seem to be related to EPO secretion. Since in both lean and obese essential hypertensive subjects EPO and log EPO × %Hct product are within normal range, the contribution of endogenous EPO to the pathogenesis of hypertension in these patients is unlikely. (Hypertens Res 1994; 17: 43-48)

Key Words: essential hypertension, obesity, erythropoietin, renin-angiotensin-aldosterone system

The coincidence of polycythemia, obesity, and hypertension drew the interest of scientists as early as in 1905 (1). Subsequently, investigators confirmed earlier findings describing patients with Gaisböck's syndrome as having polycythemia, stocky habitus, excessive body weight and hypertension (2,3). Several complications of obesity are associated with altered rheologic characteristics of blood (4). The volume of red blood cells is the main determinant of whole blood viscosity (WBV) (5). Clinical (6,7) epidemiological (8-10) and experimental (11) studies have demonstrated a direct relation between hematocrit and obesity. Moreover, in established hypertension the total peripheral resistance and WBV are elevated (12). Both in established (13) and borderline primary hypertension (14) as well as in some secondary forms of hypertension, e.g., renovascular hypertension (15), increased hematocrit and plasma viscosity have been reported. WBV is an important cardiovascular risk factor since elevation of blood viscosity has been found in several cardiovascular diseases (8-10, 12, 16, 17). In obese persons in contrast to non-obese, the WBV estimated at various rates of shear-stress correlated strongly with 24-h urinary sodium excretion and plasma renin activity (18). Recombinant human erythropoietin (EPO) is widely used in the treatment of renal anemia; however, elevation of blood pressure occurs in 20-30% of patients (19). According to recent findings, EPO induces, besides its primary effect on erythropoiesis, a direct (20, 21) or hormonally-mediated hypertensinogenic effect (22) and it was found to cause antinatriuresis both in experimental studies (23) and in humans (24). Furthermore, several recent investigations have provided evidence of a relationship between the regulation of renal EPO production and sodium metabolism and the renin-angiotensin system (RAS), respectively (23-25).

Despite these findings little interest has been focused so far on the contribution of endogenous erythropoietin to the pathogenesis of essential hypertension (26, 27). Taking the aforementioned findings into account, in the present study we tried to test the hypothesis that endogenous erythropoietin is involved in the pathogenesis of obesity-related hypertension. Assumptioning the existence of EPO
and the renin-angiotensin system feedback loop (25), and the importance of the latter system and sodium metabolism in the pathogenesis of both essential hypertension and obesity, we assessed changes in plasma EPO levels in response to conditions stimulating the renin-angiotensin system, i.e., dietary sodium restriction and upright body position.

Subjects and methods
A total of 119 WHO stage II essential hypertensive (EH) patients were studied: 47 obese (24 males, 23 females) and 72 non-obese (37 males, 35 females). The control group comprised 32 healthy (16 males, 16 females), non-obese subjects without a family history of hypertension. Obesity was assessed according to the patient's body mass index (BMI); hypertensive patients with BMI over 30 kg/m² were included in the group of obesity-related hypertension (ORH) and hypertensive subjects with BMI below 25 kg/m² in the group of non-obese essential hypertensives (NOEH). Diagnosis of arterial hypertension was based on common criteria, and the size of the cuff for blood pressure measurement was adjusted to the patient's arm circumference. Administration of all antihypertensive drugs was discontinued at least 2 weeks prior to hospitalization and smoking was forbidden during the entire study period. Patients were qualified to enter the study after thorough examinations had been performed to exclude secondary causes of hypertension. All patients were otherwise healthy (non-diabetic, without clinical symptoms of circulatory or respiratory insufficiency or any other chronic disease), and maintained a diastolic pressure below 114 mmHg during the entire study period. Informed written consent was obtained from all subjects.

For blood samples obtained after 3 days on a normal sodium diet (NS) (120 mmol sodium/24 h) after overnight recumbency, the following parameters were assessed: sodium, potassium, creatinine, iron, and ferritin serum levels, total iron binding capacity (TIBC), hematocrit value, hemoglobin concentration, erythrocyte count, plasma renin activity (PRA), plasma aldosterone (PAC), and plasma level of EPO (EPO). Urine for the estimation of 24-h excretion of potassium (UK-1) and sodium (UNa-1) and fractional excretion of sodium (FE Na-1) was collected during the third day of administration of a NS diet. Then all examined subjects received a low salt diet (LS) (30 mmol/24 h) for three days. On the third day, urine was collected for the assessment of sodium and potassium excretion, and fractional excretion of sodium (UK-2, UNa-2, FE Na-2, respectively). On the fourth day in the morning, a second blood sample was withdrawn from fasting patients after 3 h of maintaining an upright position for the estimation of PRA (PRA), plasma aldosterone (PAC) and EPO (EPO). PRA and PAC were measured by radioimmunoassay as previously described (28). EPO was measured by RIA by the method developed in our laboratory. In brief, rHuEPO obtained from Boehringer Mannheim was labeled with 125I using the chloramine method. Sera and standards were incubated with EPO antibodies (raised in rabbits, final dilution 1:60,000) for 24 h at 4°C. Then 125I -EPO was added and the incubation extended for a further 24 h. Antibody-bound EPO was precipitated using anti-γ-globulin antibodies with polyethylene glycol. The inter- and intra-assay coefficients of variation were 15% and 9% respectively; the sensitivity was 1.25 mU/tube. Fractional excretion of sodium was calculated as a percentage of sodium to creatinine clearance, the latter being calculated from 24-h creatinine excretion.

Statistical analysis was carried out using one-way analysis of variance with t-test for unpaired samples with Bonferroni correction for post-hoc comparisons between groups. Student's paired t-test to estimate changes within groups, and Wilcoxon test (for hematocrit and ferritin values). Both multiple and linear regression models were used to study the relationship of erythropoietin levels and hematocrit to various predictor variables. Results are presented as means ± SEM if otherwise not stated. p < 0.05 was considered significantly different.

Results
The median BMI was: 31.8 kg/m² (range 30–34.8) in ORH, 23.6 (19.1–25) in NOEH, and 23.4 (18.7–24.8) in control subjects. The median age was: 35.0 years (22–49) in NOEH, 37.5 (23–53) in ORH and 36.5 (22–48) in the control group. Patients of all analyzed groups did not differ in sodium and potassium serum concentrations. Serum creatinine was in the normal range in all subjects and was higher in subjects with ORH than NOEH (97.2 ± 2.7 vs. 90.4 ± 2.1 µmol/l, p < 0.05). When analyzed for family history of hypertension (positive, when at least one sibling or parent in the patient's family developed hypertension before age 60), those with a positive history comprised 45% of ORH and 42% of NOEH. Mean arterial pressure (MAP) was significantly higher in both groups of patients with hypertension than in the control group and higher in patients with ORH than in patients with NOEH on a LS but not a NS diet. Restriction of dietary sodium caused a significant decrease in MAP in ORH and NOEH (119.4 ± 2.1 vs. 114.0 ± 2.6, and 114.3 ± 2.6 vs. 107.0 ± 1.5 mmHg, respectively) as well as in control subjects (100.6 ± 0.8 vs. 92.9 ± 1.2 mmHg) and a decrease in body weight in all groups. The decrease in body weight was comparable in patients with ORH (1.55 ± 0.3kg) to NOEH (1.3 ± 0.3kg) and control subjects (1.2 ± 0.3kg). Patients with ORH had significantly higher hematocrit values, hemoglobin concentration, and erythrocyte count than NOEH and control subjects (Table 1). No differences were found between groups with respect to parameters of iron metabolism assayed by serum levels of ferritin, iron and total iron binding capacity (TIBC) (Table 1). Dietary sodium restriction caused a significant decrease of absolute and fractional urinary sodium excretion in all examined groups without influencing absolute urinary potassium excretion (Table 2). No significant differences

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Table 1. Hematocrit value, hemoglobin concentration, erythrocyte count, plasma ferritin, total iron binding capacity (TIBC) and serum iron levels in hypertensive obese- (ORH) and non-obese (NOEH) patients and normotensive non-obese control subjects. Means ± SEM. *p <0.05 (ORH vs. NOEH and ORH vs. controls)

<table>
<thead>
<tr>
<th></th>
<th>ORH</th>
<th>NOEH</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit %</td>
<td>44.2±0.60</td>
<td>42.8±0.54*</td>
<td>42.8±0.65*</td>
</tr>
<tr>
<td>Hemoglobin mmol/l</td>
<td>8.63±0.11</td>
<td>8.40±0.10*</td>
<td>8.37±0.12*</td>
</tr>
<tr>
<td>Erythrocytes 10⁵/l</td>
<td>4.51±0.06</td>
<td>4.35±0.05*</td>
<td>4.34±0.07*</td>
</tr>
<tr>
<td>Serum iron µg/dl</td>
<td>103.9±3.9</td>
<td>112.4±4.6</td>
<td>106.9±4.3</td>
</tr>
<tr>
<td>Ferritin ng/ml</td>
<td>106.8±19.7</td>
<td>94.9±13.2</td>
<td>104.8±12.9</td>
</tr>
<tr>
<td>TIBC µg/ml</td>
<td>406.2±20.5</td>
<td>425.9±19.9</td>
<td>404.9±24.6</td>
</tr>
</tbody>
</table>

in absolute urinary sodium and potassium and fractional sodium excretion were found between the examined groups.

As presented in (Fig. 1) PRA values were not significantly different between the examined groups. Patients with ORH showed significantly higher basal levels of PAC than did those with NOEH (Fig. 1). Dietary sodium restriction for three days and upright body position for 3 h caused significant increases in PRA and PAC in all groups.

As shown in (Fig.2) EPO levels were not different between groups both on a NS and LS diet. EPO levels tended to be lower on a LS diet in all groups but the decline did not reach a level of statistical significance.

The product of log EPO × %Hct assessed in basal conditions was not different between groups (41.9±2.4 in NOEH, 42.6±2.8 in ORH, and 43.0±1.8 in the control group). In the simple regression analysis no correlations were found between EPO levels and PRA, PAC, serum and urinary sodium and potassium both on a NS and a LS diet in all examined groups with the exception of a negative correlation between changes in PRA (Δ PRA) and EPO (Δ EPO) in response to sodium restriction found in NOEH (r =−0.42, p<0.01) (Fig. 3).

A multiple regression model with automatic stepwise procedure was built to estimate the relationship of predictor variables, i.e., age, BMI, mean blood pressure, serum creatinine and PRA, to dependent variables, i.e., hematocrit and plasma EPO, respectively. In the multiple regression analysis both age and BMI were correlated with hematocrit but not EPO in lean hypertensive patients (β = −0.43, F =12.4, p<0.01 for age and β =0.33, F =7.5, p<0.01 for BMI) and in ORH (β =−0.32, F =4.9, p<0.05 for age and β =0.10, F =5.1, p<0.05 for BMI).

### Discussion

Epidemiological studies have documented the presence of a strong association between obesity and hypertension regardless of age, race and sex (29). Studies addressing this complex relationship have focused mainly on alterations in sodium metabolism (30), sympathetic overactivity (31), insulin resistance (32), and activity of the renin-angiotensin-aldosterone system (30, 33).

Whole blood viscosity is a major cardiovascular risk factor (5, 12). Volume of red blood cells is a major determinant of WBV, and there is increasing evidence from both experimental and clinical studies that WBV is increased both in obesity and hypertension (4, 6-10, 13, 14). Plasma erythropoietin levels were not measured in any of these studies, however, except for a recent one (34). In this recent study, plasma EPO levels were not elevated in essential hypertensive patients despite higher hematocrit and WBV. Our findings of EPO levels remaining in the normal range in lean essential hypertensive patients are consistent in respect both with this study and the study of Bourgnoigne et al. (26). However in the latter, EPO levels were measured by bioassay, a method which may have been unreliable to measure EPO levels in normal range. In contrast to the study of Linde et al.(34) and Letcher et al.(13), we did not find elevated hematocrit and red blood cell count in NOEH.

### Table 2. Urinary excretion of sodium (UNa), potassium (UK) and fractional sodium excretion (FENa) in hypertensive obese- (ORH) and non-obese (NOEH) patients and normotensive non-obese control subjects on a normal- (NS) and low-sodium (LS) diet. Means±SEM. **p<0.01 (LS vs. NS)

<table>
<thead>
<tr>
<th></th>
<th>UNa (mmol/24h)</th>
<th>FENa (%)</th>
<th>UK (mmol/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORH</td>
<td>114.4±8.8</td>
<td>1.12±0.14</td>
<td>42.5±7.7</td>
</tr>
<tr>
<td>NOEH</td>
<td>106.7±9.8</td>
<td>1.19±0.17</td>
<td>41.2±7.6</td>
</tr>
<tr>
<td>Controls</td>
<td>101.9±9.9</td>
<td>1.23±0.18</td>
<td>31.4±3.8</td>
</tr>
<tr>
<td>ORH</td>
<td>33.5±2.9**</td>
<td>0.47±0.08**</td>
<td>41.7±3.2</td>
</tr>
<tr>
<td>NOEH</td>
<td>34.9±6.1**</td>
<td>0.46±0.09**</td>
<td>40.9±5.2</td>
</tr>
<tr>
<td>Controls</td>
<td>32.1±6.3**</td>
<td>0.60±0.08**</td>
<td>33.9±3.5</td>
</tr>
</tbody>
</table>
patients. In study of Letcher et al. (13), however, patients were not matched for body mass index and mean BMI was 27.4 in hypertensives (clearly indicating overweight) and 23.2 in the control group. Therefore, it is reasonable to postulate that obesity might have been responsible for the elevated WBV and red blood cell volume found in Letcher's study. In fact, in our study body mass index but not mean blood pressure was positively correlated with hematocrit in both lean and obese hypertensives. Further support for the concept comes also from the early observation of Messerli et al. (30) who found elevated red blood cell volume in obese hypertensive but not in normotensive subjects. In a study of patients with "stress polycythemia" (3), EPO levels were in a normal range despite higher hematocrit; however, in this study bioassay was used to measure EPO and therefore the findings may have been subjected to the same methodological problems as the study of Bourgnoigne et al. (26).

The product of log EPO $\times$ Hct is relatively constant in non-renal anemia and in healthy persons, remaining in a range of 40–60, and therefore can be used as a marker of the appropriate relationship between EPO and hematocrit (35). In the present study, despite higher hematocrit values in ORH, in the presence of a normal concentration of EPO, the calculated factor was in the normal range, thus providing no evidence that in ORH levels of EPO are inappropriate to the degree of hematocrit.

The reason for the elevation of blood pressure seen in patients with renal failure treated with recombinant EPO is still a matter of controversy. While early studies linked EPO-induced hypertension solely with its primary effect on erythropoiesis, i.e., as a consequence of increased hematocrit, improved tissue oxygenation, and inadequate cardiac output (36), several recent reports related the hypertensionogenic effect of EPO to its direct effect on resistance vessels (20), stimulation of vasopressor hormones (22), and antinatriuresis (23, 24). The question, however, arises whether these "secondary" effects of EPO are operative in a normal range of levels since in the above-mentioned studies concentrations of EPO exceeded many times those found in humans both in physiologic and pathologic conditions. In this respect it is noteworthy that in a recent study by Brier et al. (24) a concentration of EPO of 100 mU/ml (which is close to the normal range) already resulted in antinatriuresis.

Taking into account the central role of sodium in hypertension of obesity (29, 30) and the antinatriuretic properties of EPO on the one hand (23, 24), and the existence of EPO and the RAA feedback loop (25) on the other, in the present study we assessed EPO levels under different sodium intakes in parallel with the activity of RAS. Our findings of normal PRA and elevated PAC on a normal sodium diet in hypertensive obese patients are consistent with those of Rocchini et al. (33) and may reflect the increased response of aldosterone secre-
tion to angiotensin II in ORH. Contrary to what has already been reported (33), we did not find any differences in urinary sodium excretion between the ORH and NOEH groups. Consistently, similar decreases in body weight and blood pressure were found in response to sodium restriction in all analyzed groups. This indirectly shows that there were no differences in the salt-sensitivity of patients between the studied groups, an finding important with respect to the study of Naomi et al.(37) who found that sodium-sensitivity index may determine EPO response to changes in dietary sodium intake. It is noteworthy that we found a slight decline in EPO levels in response to sodium restriction in all groups, a finding which we reported not only in essential but also in some secondary forms of hypertension (27). These results are, however, difficult to explain because in agreement with the hypothesis of Kurtz and Eckardt (38) we should expect that the decrease in the fractional sodium excretion indicating increased tubular reabsorption of sodium, i.e., “metabolic strain” for proximal tubules, should result in the stimulation of EPO production. However, contrary to these expectations we found a slight decrease in EPO levels when patients remained for 3 days on a low sodium diet and, moreover, we found a negative correlation between changes in EPO and PRA responses to dietary sodium restriction in NOEH but not in ORH or healthy controls. Our data contrast in this respect with the hypothetical existence of a feedback loop between EPO and the RAS put forward by Yaqoob et al. (25). This hypothesis, based on evidence of a suppression of the RAS in patients given rHuEPO (19) and a decrease in EPO levels during treatment with ACE inhibitors (39, 40), has been challenged, however, by the results of some experimental studies (24, 41).

In conclusion, our results provide evidence that elevated hematocrit is a characteristic feature of obesity-related hypertension. Hematocrit was correlated to body mass index but not to blood pressure in both groups hypertensive patients. Both in lean and obese essential of hypertensive patients plasma EPO levels remain within a normal range and therefore the contribution of endogenous EPO to the pathogenesis of hypertension in both groups of patients is unlikely. A negative correlation between changes in EPO and PRA in response to sodium restriction was found only in lean hypertensive patients. This finding suggests that the patients with essential hypertension, in subjects with ORH, activity of the renin-angiotensin-aldosterone system dose not seem to be related to EPO secretion. The pathogenetic significance of these differences remains to be clarified.

Fig. 3. Correlation between changes in plasma EPO levels (EPO) and PRA (PRA) in response to sodium restriction in non-obese patients with hypertension. \( r = -0.42, p<0.01 \)

References
9. Garn SM, Ryan AS: Relationship between fatness


