# Comparative effects of nebivolol and metoprolol on oxidative stress, insulin resistance, plasma adiponectin and soluble P-selectin levels in hypertensive patients

Turgay Celik[a], Atila Iyisoy[a], Hurkan Kursaklioglu[a], Ejder Kardesoglu[e], Selim Kilic[b], Hasan Turhan[a], M. Ilker Yilmaz[c], Omer Ozcan[d], Halil Yaman[d], Ersoy Isik[a] and Francesco Fici[f]

***Objectives*** **To determine the effects of nebivolol on oxidative stress, insulin resistance, adiponectin and plasma soluble P-selectin levels in hypertensive patients in comparison with metoprolol.**

***Material and methods*** **Eighty newly diagnosed hypertensive patients in grade 1 hypertension according to the European Society of Hypertension and European Society of Cardiology guidelines were enrolled in this prospective, blinded, randomized study. Seventy-two patients completed the study. After baseline assessment, each patient was randomly allocated to a 5 mg daily dose of nebivolol ($n = 37$, 20 male) or a 100 mg daily dose of metoprolol ($n = 35$, 18 male) and treated for 6 months. Blood pressure, heart rate, oxidative stress (malonyldialdehyde), homeostasis model assessment: insulin resistance, adiponectin and plasma soluble P-selectin levels were measured before and after treatment.**

***Results*** **At the end of treatment, nebivolol and metoprolol significantly decreased blood pressure and heart rate, with a more pronounced bradycardic effect of metoprolol. Nebivolol, but not metoprolol, significantly lowered oxidative stress ($P = 0.03$), the insulin resistance index ($P = 0.003$) and plasma soluble P-selectin levels ($P = 0.008$), and increased adiponectin levels ($P = 0.04$).**

***Conclusion*** **Nebivolol, in contrast to metoprolol, improved oxidative stress, insulin sensitivity, decreased plasma soluble P-selectin and increased adiponectin levels in hypertensive patients. These beneficial effects of nebivolol may contribute to a reduction in cardiovascular risk in hypertensive patients. *J Hypertens* 24:591–596 © 2006 Lippincott Williams & Wilkins.**

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[a]Departments of Cardiology, [b]Epidemiology, [c]Nephrology, [d]Clinical Biochemistry, Gulhane Military Medical Academy, Etlik, Ankara, [e]Department of Cardiology, Gulhane Military Medical Academy Haydarpasa Training Hospital, Kadıkoy, Istanbul, Turkey and [f]Excellence Research Center for Cardiovascular Disease, 2nd University of Naples, Italy

Correspondence and requests for reprints to Turgay Celik, MD, GATA Kardiyoloji A.D, 06018 Etlik, Ankara, Turkey
Tel: +90 312 3044268; fax: +90 312 3043300; e-mail: benturgay@yahoo.com

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## Introduction

An insufficiency of antioxidant activity and decreased insulin sensitivity have been shown in patients with essential hypertension [1–4]. Free oxygen radicals and an insufficiency of antioxidant enzymes have been implicated in the pathogenesis of hypertensive disease [3,4]. Oxygen radicals are known to cause membrane peroxidation and malonyldialdehyde formation, which are detrimental to cellular function. Peroxidation can increase membrane permeability, whereas malondialdehyde can inactivate membrane transporters, by forming intramolecular and intermolecular crosslinks [5,6]. Such events represent an immediate risk to cell viability, although the carcinogenic effects of malondialdehyde may be more damaging in the long term. To minimize free radical damage, there is a complex antioxidant defence system, which includes the interception of free radicals with antioxidants to form less reactive compounds [7].

Adiponectin, which is secreted specifically by adipose tissue, has been shown to have novel insulin-sensitizing, anti-atherogenic, and anti-inflammatory properties [7,8]. Clinical studies have shown that adiponectin levels are lower in individuals with hypertension, diabetes, coronary artery disease, as well in obese individuals [7,8].

P-selectin is an adhesion molecule that is constitutively present in the Weibel–Palade bodies in endothelial cells or alfa granules in platelets, and participates in 'rolling', the first step of neutrophil migration into the postcapillary venules [9]. Raised soluble P-selectin levels have been reported in diabetes [10–12], smoking and hypertension [13,14].

Beta adrenergic blockers have no antioxidant activity *in vivo* [15,16], and treatment with both selective and non-selective beta adrenergic blockers significantly

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increases insulin resistance and basal plasma insulin, despite lowering blood pressure [17,18].

Nebivolol is a new cardioselective beta-blocking agent that has been shown to control blood pressure over 24 h with a single daily dose [19,20]. Nebivolol has novel cardiovascular properties, such as endothelium-dependent arterial and venous dilation via the L-arginine nitric oxide (NO) pathway [19,21,22]. According to the hemodynamic theory of insulin resistance, these hemodynamic properties could favourably modify insulin sensitivity [23]. However, there are no available data regarding the effects of nebivolol on insulin sensitivity in hypertensive individuals until now [24].

Nebivolol has been reported to decrease oxidative stress in healthy volunteers [25] and hypertensive patients [26], but there are no data about the effects of nebivolol on adiponectin and soluble P-selectin levels, which have been suggested to be related to endothelial function in hypertensive patients.

The aim of the present study was to investigate the effects of nebivolol on oxidative stress, insulin sensitivity, plasma adiponectin and soluble P-selectin levels in patients with essential hypertension in comparison with metoprolol.

## Methods

Eighty newly diagnosed hypertensive patients who are in grade 1 hypertension according to the European Society of Hypertension/European Society of Cardiology guidelines [27] were enrolled in this blind, randomized, prospective study. Patients with secondary hypertension, asthma or chronic obstructive lung disease, bradycardia (heart rate $< 55$ bpm), atrial fibrillation or recurrent tachyarrhythmia requiring anti-arrhythmic therapy, diabetes mellitus, impaired glucose tolerance, a history of sensitivity or severe adverse reaction to beta-blockers, pregnancy or nursing, heart failure requiring treatment or valvular disease, myocardial infarction or cerebrovascular accident within the past 6 months, a history of coronary artery disease or confirmed coronary artery disease at coronary angiography or non-invasive tests, body mass index (BMI) 27 kg/m$^2$ or greater, concurrent therapy with medications that could affect blood pressure, severe renal or hepatic failure and a history of smoking were excluded from the study.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice/International Conference for Harmonization guidelines. All patients gave their informed consent before entering the study.

After baseline clinical assessment, patients were randomly assigned to a 5 mg daily dose of nebivolol (group I, $n = 37$, 20 male) or a 100 mg daily dose of metoprolol (group II, $n = 35$, 18 male) for 6 months. The randomization was performed using a table of random numbers. Blood pressure, heart rate, compliance and tolerability of patients to treatment were evaluated every 2 weeks, and laboratory analyses were performed by an investigator unaware of the assigned drugs, at randomization (baseline) and at the end of treatment. If systolic and diastolic blood pressure, measured at the end of the second week of treatment, were not normalized ($< 140$ and $< 90$ mmHg, respectively), patients were considered to be non-responders and were withheld from the study.

Blood pressure was measured three times for each patient with a standard mercury sphygmomanometer on the right arm in sitting position after 10 min resting. Phase I and V Korotkoff sounds were used to determine systolic and diastolic blood pressure measurements. In each patient measurements were performed by the same investigator, in the same room and at the same time of day. The average of three measurements was used for the analyses.

Laboratory tests included lipid profile, fasting plasma glucose, malondialdehyde, fasting plasma insulin, adiponectin and soluble P-selectin levels.

All patients followed the National Cholesterol Education Program step I cholesterol-lowering and salt-restricted diet during the treatment period [28].

### Blood chemistry

Fasting venous blood samples were withdrawn into both the tubes containing $K_3$ ethylenediamine tetraacetic acid (EDTA) and the tubes containing no anticoagulant agent. After all tubes were spun at 5000 rpm for 15 min, plasma and serum samples were stored at $-80^{\circ}$C until analyses were performed. Plasma adiponectin levels were determined using the enzyme-linked immunosorbent assay (ELISA) method (Human adiponectin ELISA kit; Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). Plasma soluble P-selectin concentrations were analysed using the sP-Selectin ELISA kit (IBL-Immuno-Biological Laboratories, Hamburg, Germany). Plasma malondialdehyde levels were quantified by synchronized fluorometry [29]. For the measurement of the lipid profile, fasting blood samples were withdrawn from hypertensive patients at 0800–0900 h after a 12-h fasting period. Total plasma cholesterol, triglyceride and high-density lipoprotein (HDL)-cholesterol were measured by an enzymatic calorimetric method with the Olympus AU 600 autoanalyser using reagent from Olympus Diagnostics GmbH (Hamburg, Germany). Low-density lipoprotein (LDL)-cholesterol levels were calculated by the Friedewald formula. Blood glucose was measured by the glucose oxidase method, and serum insulin levels were determined with the

immunoenzymatic method (IRMA kit; Immunotech, Beckman Coulter Inc., Prague, Czech Republic). The insulin resistance score, the homeostasis model assessment: insulin resistance (HOMA-IR), assessment was computed using the formula: (HOMA-IR) = [Fasting plasma glucose (mg/dl) × immunoreactive insulin (IRI) (IU/ml)]/405 [30]. None of the patients used vitamin tablets containing vitamin C, vitamin E, or trace elements including zinc and copper during study period, in order to avoid any interference with the analyses.

### Statistical analysis

Results are expressed as mean ± SD and percentages. We used Kolmogorov–Smirnov and Levene tests to determine the distribution characteristics of variables and variance homogenity. With respect to these test results, we used independent samples *t* or Mann–Whitney U tests as appropriate. The statistical differences between groups were tested for significance by chi-squared, Mann–Whitney *U* and independent sample *t*-tests. A two-tailed paired *t*-test was used to compare continous variables before and after drug therapy. Differences were considered statistically significant at $P < 0.05$. Statistical analyses were performed by using the SPSS 11.5 Statistical Package Program for Windows (SPSS Inc., Chicago, Illinois, USA).

## Results

At the beginning of the study 80 patients were enrolled in the study. Eight patients were withdrawn from the study for several different reasons: four patients (three in the metoprolol group and one in the nebivolol group) because of lack of compliance at the third month of therapy, two because they were non-responders (one patient in each group) and two, taking metoprolol, because of the development of severe symptomatic bradycardia. Seventy-two patients completed the study with nebivolol (mean age 50 ± 6 years, 20 male) and metoprolol (mean age 52 ± 6 years, 18 male).

As shown in Table 1 there were no differences between the two groups regarding the baseline demographic and clinical characteristics. The pretreatment biochemical parameters, including plasma glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, malondialdehyde, adiponectin, soluble P-selectin, insulin levels and HOMA-IR index, in patients taking nebivolol were no different from those of patients using metoprolol (Tables 2 and 3).

After 6 months of treatment, systolic and diastolic blood pressures significantly decreased with nebivolol and metoprolol (−22.7/−12.9 and −26.17/−13.0 mmHg, respectively, $P < 0.001$ versus baseline), although there was no difference between two groups (Table 1).

**Table 1 Demographic and clinical characteristics of the patients**

| | Nebivolol ($n = 37$) | Metoprolol ($n = 35$) | *P* |
|---|---|---|---|
| Age (years) | 50.24 ± 6.10 | 52.48 ± 5.59 | 0.10** |
| Sex (M) *n* (%) | 20 (54) | 18 (48) | 0.64*** |
| BMI (kg/m$^2$) | | | |
| Before | 26.89 ± 3.64 | 26.77 ± 3.14 | 0.88** |
| After | 27.02 ± 3.84 | 26.85 ± 3.29 | 0.84** |
| *P* | 0.28* | 0.49* | |
| SBP (mmHg) | | | |
| Before | 153.37 ± 5.78 | 155.00 ± 6.41 | 0.26** |
| After | 130.67 ± 14.58 | 128.85 ± 11.76 | 0.56** |
| *P* | < 0.001* | < 0.001* | |
| DBP (mmHg) | | | |
| Before | 92.16 ± 6.61 | 94.85 ± 6.00 | 0.08** |
| After | 79.18 ± 9.39 | 81.85 ± 5.82 | 0.15** |
| *P* | < 0.001* | < 0.001* | |
| Heart rate (bpm) | | | |
| Before | 75.97 ± 8.72 | 77.51 ± 8.37 | 0.44** |
| After | 69.37 ± 8.89 | 57.88 ± 8.10 | < 0.001** |
| *P* | < 0.001* | < 0.001* | |

BMI, Body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure. Values are mean ± SD or *n* (%). *Paired *t*-test. **Independent samples *t*-test. ***Chi-squared test.

The heart rate of the patients taking nebivolol was no different from that of those taking metoprolol at baseline (Table 1). For both groups, resting heart rates after treatment were significantly lower than pretreatment levels (−6.6 bpm for nebivolol, −19.6 bpm for metoprolol, $P < 0.001$). However, metoprolol caused a greater decrease in heart rate compared with nebivolol at the end of treatment (−11.49 bpm, $P < 0.001$).

After 6 months of drug therapy, nebivolol significantly lowered plasma malondialdehyde, soluble P-selectin, insulin levels and the HOMA-IR index and increased plasma adiponectin levels compared with the metoprolol group (Table 3). No difference between pre and post-treatment values of plasma malondialdehyde, insulin resistance index, plasma insulin, adiponectin and soluble P-selectin was observed in the metoprolol group (Table 3).

**Table 2 Comparison of the basic biochemichal parameters between groups**

| | Nebivolol ($n = 37$) | Metoprolol ($n = 35$) | *P*** |
|---|---|---|---|
| Total cholesterol (mg/dl) | | | |
| Before | 197.62 ± 41.44 | 198.62 ± 43.37 | 0.92 |
| After | 199.18 ± 37.77 | 202.40 ± 36.92 | 0.71 |
| *P** | 0.62 | 0.12 | |
| Triglyceride (mg/dl) | | | |
| Before | 129.13 ± 50.32 | 134.77 ± 51.16 | 0.63 |
| After | 143.70 ± 76.86 | 149.65 ± 67.62 | 0.72 |
| *P** | 0.19 | 0.10 | |
| LDL-cholesterol (mg/dl) | | | |
| Before | 127.47 ± 42.43 | 128.84 ± 47.62 | 0.89 |
| After | 124.69 ± 36.79 | 128.12 ± 43.45 | 0.90 |
| *P** | 0.45 | 0.82 | |
| HDL-cholesterol (mg/dl) | | | |
| Before | 45.75 ± 11.76 | 44.34 ± 10.77 | 0.53 |
| After | 44.32 ± 10.67 | 42.82 ± 9.74 | 0.59 |
| *P** | 0.14 | 0.13 | |

Values are mean ± SD. *Paired *t*-test. **Independent samples *t*-test. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

**Table 3 Comparison of plasma soluble P-selectin, adiponectin, malonyldialdehyde, insulin, glucose and insulin sensitivity index between two treatment arms**

| | Nebivolol ($n = 37$) | Metoprolol ($n = 35$) | $P$ |
|---|---|---|---|
| Malonyldialdehyde (mmol/l) | | | |
| Before | $0.61 \pm 0.46$ | $0.64 \pm 0.37$ | 0.75** |
| After | $0.47 \pm 0.30$ | $0.64 \pm 0.34$ | 0.03** |
| $P$ | 0.007* | 0.76* | |
| Adiponectin (μg/ml) | | | |
| Before | $2.56 \pm 0.89$ | $2.52 \pm 0.77$ | 0.84 |
| After | $2.81 \pm 0.91$ | $2.46 \pm 0.75$ | 0.04 |
| $P$ | $< 0.001$ | 0.08 | |
| Soluble P-selectin (ng/ml) | | | |
| Before | $1.29 \pm 0.46$ | $1.45 \pm 0.46$ | 0.16** |
| After | $1.21 \pm 0.36$ | $1.46 \pm 0.39$ | 0.008** |
| $P$ | 0.002* | 0.59* | |
| Glucose (mg/dl) | | | |
| Before | $93.67 \pm 9.17$ | $95.14 \pm 11.38$ | 0.54** |
| After | $94.43 \pm 7.13$ | $97.17 \pm 7.83$ | 0.12** |
| $P$ | 0.71* | 0.36* | |
| Insulin (μU/ml) | | | |
| Before | $12.19 \pm 5.20$ | $11.51 \pm 4.75$ | 0.56** |
| After | $9.72 \pm 5.13$ | $11.84 \pm 1.62$ | 0.001*** |
| $P$ | 0.006* | 0.69* | |
| HOMA-IR | | | |
| Before | $2.79 \pm 1.16$ | $2.67 \pm 1.07$ | 0.66** |
| After | $2.29 \pm 1.24$ | $2.83 \pm 0.42$ | 0.003*** |
| $P$ | 0.008* | 0.39* | |

HOMA-IR, The homeostatic model assessment of insulin resistance. Values are mean ± SD. *Paired *t*-test. **Independent samples *t*-test. ***Mann–Whitney U test.

## Discussion

The results of our study show that in mild to moderate hypertensive patients, nebivolol, in contrast to metoprolol, decreases malondialdehyde, soluble P-selectin, insulin concentrations, the HOMA-IR index and increases plasma adiponectin levels.

Essential hypertension is associated with endothelial dysfunction, and with greater oxidative stress producing free oxygen radicals that contribute to the decrease in NO bioavailibility. Elevated plasma levels of malondialdehyde, a final product of lipid peroxidation and an index of oxidative stress, have been demonstrated in patients with essential hypertension [2,31].

Although few types of beta-blockers, such as labetolol and carvedilol, have been shown to have a significant antioxidant property *in vitro* [15,16], no favorable effects of the other types of beta-blockers were shown on oxidative stress [31,32].

It has been shown that nebivolol decreases systemic oxidative stress in healthy volunteers [25] and hypertensive patients [26], in whom, in contrast to atenolol, it significantly decreased plasma and LDL hydroperoxides, plasma 8-isoprostanes and the concentration of reactive oxygen species in endothelial cells, exposed to oxidative stress, and incubated with the plasma of nebivolol-treated patients. The reduction in malondialdehyde in our study confirms the antioxidant activity of nebivolol, which prevents NO breakdown, as demonstrated by basal and stimulated NO concentrations in endothelial cells exposed to oxidative stress [26].

The reduced insulin sensitivity in hypertensive patients seems to be related to vascular changes in skeletal muscles induced by the shear stress [23] on the endothelial cells, which could lead to a change in the production or release of vasoactive substances such as NO [33]. Endothelial dysfunction is considered an intrinsic component in the insulin resistance in type II diabetes and hypertension [34,35], and can lead to an 'activated state', partly characterized by increased platelet adhesion, aggregation and increased expression of P-selectin on platelet membranes [36]. In our study, nebivolol, but not metoprolol, decreased plasma insulin and improved the insulin resistance index in patients with no established insulin resistance and normal BMI. In hypertensive patients with type II diabetes, neither nebivolol nor atenolol impaired insulin sensitivity [37], whereas in patients with impaired glucose tolerance and elevated BMI (mean BMI $30.4 \pm 3.3$ kg/m$^2$), insulin sensitivity was impaired by atenolol and was not modified significantly by nebivolol [24]. The unaltered insulin sensitivity reported by Fogari *et al.* [37] was observed with a relatively low dose of atenolol (50 mg), which produced a similar reduction in heart rate compared with nebivolol, whereas in another study [24], a relatively high dose of atenolol (100 mg) reduced heart rate more than nebivolol and caused an impairment in insulin sensitivity. Therefore the extent of insulin resistance induced by beta-blockers might be related not only to the presence of established insulin resistance [37], but also to the degree of beta adrenergic blockade [38]. In our study, metoprolol, at a similar extent of blood pressure reduction of nebivolol, lowered heart rate more significantly, suggesting a less pronounced beta adrenergic blockade of nebivolol. Therefore the less pronounced beta adrenergic receptor blockade of nebivolol, associated with the favourable effects on the endothelium, might explain the improvement in insulin sensitivity in patients with normal BMI and no insulin resistance.

Adiponectin, a protein secreted by adipocytes [7], has been shown to improve insulin sensitivity [7,8], and there is growing evidence that decreased adiponectinemia is involved in the pathogenesis of insulin resistance [8]. In our study, plasma adiponectin significantly increased after treatment with nebivolol in comparison with metoprolol. Therefore the improvement of insulin sensitivity cannot be explained solely by the lesser degree of beta-blockade induced by nebivolol, but may be partly related to the increase in plasma adiponectin, through the activation of NO synthase [39]. However, the exact mechanisms of the improvement in insulin sensitivity after nebivolol treatment still remain to be elucidated.

The adhesion molecule P-selectin plays an important role in modulating interactions between blood cells and the endothelium. Increased levels of soluble P-selectin have been reported in diabetes [11], smoking [40], hypertension [13] and in a variety of cardiovascular disorders, including coronary artery disease and atrial fibrillation, with some relationship to prognosis and the prediction of adverse cardiovascular events [41]. Nebivolol, but not metoprolol, lowered plasma soluble P-selectin, suggesting a favourable effect on platelet and endothelium activation, which are early events in the inflammatory cascade involved in the atherosclerotic process.

Although standard beta-blockers seem to having a hard time at present [42,43], perhaps the new generation beta-blockers such as nebivolol might yet 'rescue' the reputation of beta-blockers in the future.

In conclusion, we have shown that nebivolol, in contrast to metoprolol, decreases oxidative stress, improves insulin sensitivity, reduces plasma soluble P-selectin levels and increases plasma adiponectin. These data suggest some potential beneficial cardiovascular protective effects of nebivolol during the long-term treatment of hypertensive patients.

### Study limitations

The most important limitations of the current study are as follows.

The study population was a very homogeneous hypertensive population in whom a number of risk factors and co-morbidities had been excluded. Therefore the results of the current study may not be extrapolated in a whole hypertensive cohort.

There were no hard clinical outcomes in the current study. However, we considered that the follow-up period of the patients was not long enough to examine the major clinical outcomes accurately.

Plasma nitrite/nitrates and peroxynitrite levels were not evaluated, therefore, it is impossible to correlate the results with the effect of nebivolol on the L-arginine/NO pathway.

Although a number of different markers of oxidative stress have been described up to now, no clear 'best' marker of oxidation has yet emerged. We analysed plasma malondialdehyde levels, an index of oxidative stress, to evaluate the oxidative status in our study. Malondialdehyde is the principal and most studied product of polyunsaturated fatty acid peroxidation. Malondialdehyde action on lipoproteins has been related to atherogenesis. Its reactivity towards collagen is probably responsible for the stiffening of cardiovascular tissue. Arterial stiffness in the cardiovascular system is one of the main pathophysiological mechanisms of hypertension. Therefore we analysed malondialdehyde levels as an index of oxidative stress in the current study.

The hyperinsulinemic-euglycemic clamp technique was not utilized to evaluate insulin resistance; however, a significant correlation between the insulin resistance index, calculated by the HOMA-IR and the hyperinsulinemic-euglycemic clamp has been reported [44].

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