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# Effect of Transdermal Nitroglycerin on Insulin Resistance in Healthy Young Men

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## Abstract

**Objective:** There are evidences indicating transdermal nitroglycerin changes the sensitivity of peripheral tissues to the hypoglycemic effect of insulin. In this study we determined effect of continuous application of transdermal nitroglycerin patches in healthy volunteers on development of tolerance to the hypotensive effect of nitroglycerin and hypoglycemic effect of insulin.

**Materials and methods:** The effect of transdermal application of nitroglycerin, as NO donor was studied during 24 hours on blood insulin and glucose level and on blood pressure in healthy, young volunteers. Patches of 0.2 mg/hour nitroglycerine were administered to young healthy volunteers along 24 hours and Venous blood samples were taken early before and 24 hours after the nitroglycerin. Serum was separated and free zed (-80°C) for insulin and glucose determination. Blood pressure also was determined in 6 hours intervals. Results: Before nitroglycerin patches application mean serum insulin level determined level $9.53 \pm 4.37$ and after nitroglycerin were $9.18 \pm$ μU/ml which is statistically significant (P>0.05). Fasting blood glucose levels were increased by $6.63 \pm 1.9$ mg/dl determined 24 hours after nitroglycerine application in comparison with before treatment. The changes observed in blood glucose levels also were statistically significant (P<0.001). Insulin resistance calculated by Homa formula was 1.445 before treatment and 1.540 after nitroglycerin treatment. Conclusion: Transdermal nitroglycerin by doses of 0.2 mg/h causing blood glucose level changes more than change of insulin level indicating change in tissue sensitivity to insulin. The present work was therefore concerned with the possibility that nitrate tolerance impairs the sensitivity of tissues to the hypoglycemic effect of insulin.

**Keywords:** Nitroglycerine; Insulin resistance; Blood pressure

## Introduction

Nitrates producing their effect by releasing nitric oxide (NO) and this substance playing important roles in different organ systems [1]. Nitric oxide have regulatory effects on insulin and carbohydrate metabolism [2,3]. No playing a regulatory role in hypothalamus-hypophysis axis and inhibition of prolactin release and regulating secretion of steroids and catecholamines [4].

There are many reports about role of nitric oxide on insulin resistance, hypertension, and obesity. In this regards some reports indicating pharmacological inhibition of nitric oxide synthesis increased insulin resistance in human subjects [5]. Marcus et al. studies also showed a straight relationship between plasma levels of asymmetric dimethylarginine (AMDA) an endogenous inhibitor of nitric oxide synthesis and insulin resistance in healthy human subjects. Furthermore in Marcus et al. study rosiglytazone application was also accompanied with enhancement in insulin sensitivity and decrease in plasma AMDA level [6].

Regarding relationship between role of NO in insulin sensitivity Kaneki, et al. reported NO playing an important role in the pathogenesis of insulin resistance. Actually NO providing a link between cardiovascular and metabolic homeostasis [7]. In this regards there are also another reports indicated that elevation of nitric oxide activity is a mechanism for lower incidence of arterial hypertension in females [4,8]. Shangjian, et al. also in a study reporting higher levels of serum nitric oxide level as a compensatory mechanism in insulin resistant rats comparing with control group [9]. Other studies in healthy human subjects also indicating a direct relationship between nitric oxide production in endothelial vascular cells and insulin sensitivity [10]. In this study we determined the effect of continuous application of transdermal nitroglycerin patches as a nitric oxide donor in healthy volunteers on development of tolerance to hypoglycemic effect of insulin.

## Materials and Methods

### Subjects

Sixteen healthy male volunteer (18-22 years old) were included in our study. They were nonsmoking, without any history of hypertension and diabetes, and receiving same diet from two days before until termination of examination.

## Materials

Nitroglycerin transdermal patches 0.2 mg/hr (8 cm$^2$) Mylan pharmaceuticals INC. morgan town, wv26505 NDC0378-9104-93. Commercially available Insulin (human) ELISA kit, (EIA-2935) manufactured by DRG instruments GmbH, Marburg Germany was used for insulin determination. The kit sensitivity for human insulin was 99% and has no cross reactivity for pro-insulin. The respective inter-assay and intra-assay co-efficient of variation of kit were 5.2% and 4.8%. Glucose PAP kit manufactured by Man were used serum glucose determination in this study.

## Methods

Patches of nitroglycerine were applied at 8 pm and remained for 24 hours. Blood samples were taken early before and 24 hours after nitroglycerin application. Serum was separated by centrifuge and then was divided into separate aliquots and kept frozen at -80°C for glucose and insulin determination. Serum insulin levels were determined by ELISA method and glucose by enzymatic method [11]. Insulin resistance was calculated by homeostasis model assessment (Homa) equation: Fasting serum insulin micro unit/L × Fasting serum glucose mmol/L 22.5 [12]. Blood pressure also was determined in 6 hours intervals during 24 hours of nitroglycerin patch application.

## Results

Administration of nitroglycerine patches decreased mean serum insulin level from 9.53 ± 4.38 μu/ml to 9.18 ± 3.52 μu/ml after 24 hours. But this changes was not statistically significant (P=0.684). At the other hand mean blood glucose levels were increased 6.625 ± 1.90 mg/dl during 24 hours nitroglycerine patches administration and this changes were statistically significant (P value=0.006). In the basis of changes observed in serum insulin and blood glucose levels, Homa index was determined about 1.445 and 1.540 respectively for before and after nitroglycerine patch administration.

| Effect of nitroglycerine patch on mean diastolic and diastolic blood pressure. | Diastolic BP Mean ± S.E | P value | systolic BP Mean ± S.E | P value |
|---|---|---|---|---|
| B.p before nitroglycerine | 16 | 8.35 ± 0.7882 | | 12.81 ± 1.25 | |
| B.p 6 hrs after nitroglycerine | 16 | 7.65 ± 0.5692 | 0.001 | 11.28 ± 0.795 | 0.005 |
| B.p 12 hrs after nitroglycerine | 16 | 7.62 ± 0.5000 | 0.019 | 11.18 ± 0.75 | 0.031 |
| B.p 24 hrs after nitroglycerine | 16 | 7.65 ± 0.4732 | 0.006 | 11.21 ± 0.795 | 0.022 |

**Table 1:** Effect of nitroglycerine patch on mean diastolic and diastolic blood pressure.

Tables 1 of 2 showing the effect of nitroglycerine patches administration on diastolic and systolic blood pressures. Nitroglycerine patches in all cases decreased both of systolic and diastolic blood pressure which was statistically significant. Table 2 showing correlation coefficients between serum insulin, blood glucose level and blood pressure determined before and after nitroglycerine patch administration.

| Type of Data | | Correlation coefficient | P value |
|---|---|---|---|
| Insulin | Before and after NG | 0.663 | 0.006 |
| Blood glucose | Before and after NG | 0.724 | 0.002 |
| Systolic BP | Before and after 6 hrs NG | 0.647 | 0.0005 |
| Systolic BP | Before and after 12 hrs NG | 0.552 | 0.031 |
| Systolic BP | Before and after 24 hrs NG | 0.55 | 0.022 |
| Diastolic BP | Before and after 6 hrs NG | 0.815 | 0.001 |
| Diastolic BP | Before and after 12 hrs NG | 0.561 | 0.019 |
| Diastolic BP | Before and after 24 hrs NG | 0.633 | 0.006 |

**Table 2:** Evaluation of correlation coefficient between data determined before and after nitroglycerine administration.

## Discussion

This study performed on nondiabetic healthy volunteers without a previous history of other diseases, and our HOMA index calculated were less than 2.5 ruling out the presence of insulin resistance before and after nitroglycerine patch application (12). However our results showed a little increase in Homa index from 1.445 to 1.540 after 24 hours nitroglycerine patch application, indicating a little increase in insulin resistance by nitroglycerine or at least without effect on insulin resistance in our study design. Previous studies indicating different effect of acute and chronic nitrates on insulin resistance. In this regards Bajza, et al. studied the antihypertensive and hypoglycemic effect of 7 days nitroglycerin transdermal patch application on rabbit skin. They reported enhancement of blood glucose response to acute (6 hours) application of nitroglycerin. But the effect of nitroglycerine patch application during 7 days were different, and 7 days nitroglycerine application caused a significant increase in insulin tolerance in spite of increase in insulin sensitivity which observed by nitroglycerine patches after 6 hours [10]. But in another animal studies Ayala et al. reported chronic treatment of mice with selective type 5 phosphphodiesterase blocker of sildenafil alone or in combination with L-arginine caused a significant decrease in fasting blood glucose level. Ayala reports also indicate phophodiesterase-5 is a potential target for insulin resistance prevention [12]. Piatti, et al. study also indicating long term application of L-arginine as a precursor of nitric oxide synthesis caused improve in insulin sensitivity in diabetic patients and decreased blood pressure [13]. Bajza, et al. in another study performing in rabbits reported tolerance to nitroglycerine decreased

the effect of food on enhancement of insulin sensitivity [14]. Saydow, et al. also in a study reported a positive correlation between inhibition of NO production and insulin resistance in normotensive, non-diabetic healthy humans [15,16]. In conclusion our results seems in agreement with previous studies indicating straight relationship between NO, insulin and vascular resistance and a little increase in insulin resistance which was observed in our study may be related to some degree of tolerance to NO production by nitroglycerine due to continuous 24 hours patch application.

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