Effects of Obesity on the Pharmacodynamics of Nitroglycerin in Conscious Rats

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ABSTRACT Literature reports have suggested that hemodynamic response toward organic nitrates may be reduced in obese patients, but this effect has not been studied. We compared the mean arterial pressure (MAP) responses toward single doses of nitroglycerin (NTG, 0.5-50 µg) in conscious Zucker obese (ZOB), Zucker lean (ZL), and Sprague-Dawley (SD) rats. NTG tolerance development in these animal groups was separately examined. Rats received 1 and 10 µg/min of NTG or vehicle infusion, and the maximal MAP response to an hourly 30 µg NTG IV challenge dose (CD) was measured. Steady-state NTG plasma concentrations were measured during 10 µg/min NTG infusion. The E_{max} and ED_{50} values obtained were 33.9 ± 3.6 and 3.5 ± 1.7 µg for SD rats, 33.2 ± 4.1 and 3.0 ± 1.4 µg for ZL rats, and 34.8 ± 3.9 and 5.3 ± 2.8 µg for ZOB rats, respectively. No difference was found in the dose-response curves among these 3 groups ($P > .05$, 2-way ANOVA). Neither the dynamics of NTG tolerance development, nor the steady-state NTG plasma concentrations, were found to differ among these 3 animal groups. These results showed that ZOB rats are not more resistant to the hemodynamic effects of organic nitrates compared with their lean controls. Thus, the acute and chronic hemodynamic effects induced by NTG are not sensitively affected by the presence of obesity in a conscious animal model of genetic obesity.

KEYWORDS: nitrate tolerance, Zucker, noninsulin dependent diabetes, mellitus, nitric oxide, obesity.

INTRODUCTION Organic nitrates such as nitroglycerin (NTG) are used in the management of various cardiovascular diseases. Clinical use of these drugs appears to ignore body weight as a determinant of administered dose, but no study has appeared to provide the scientific basis for this practice. Obesity is now categorized as an epidemic in the United States, about 25% of adult women and 20% of adult men are clinically obese with

a body mass index of at least 30.^{1,2} Obesity is often associated with increased mortality because of the increased risk of a number of diseases including noninsulin dependent diabetes mellitus (NIDDM), sleep apnea, pulmonary dysfunction, and cancer.² The presence of obesity also leads to a number of physiological changes in cardiovascular function, resulting in higher incidence of many cardiovascular diseases, such as atherosclerosis, coronary artery disease, and hypertension.¹⁻⁴

In addition to increased health risks, obesity may affect drug therapy because of changes in drug pharmacokinetics (PK) and pharmacodynamics (PD). The volumes of distribution of many highly lipophilic drugs, such as barbiturates and benzodiazepines, have been shown to be increased in obesity, resulting in much longer plasma half-life.⁵ Dosage adjustments are often made for aminoglycosides in obese patients to account for the altered PK profiles observed with this class of agents.⁶ However, little is known about the effects of this disease state on the PK and PD of NTG. It is therefore unknown whether obesity, as such, may require adjustments in the administered dose of NTG to elicit adequate efficacy.

The Zucker obese rat (ZOB) is an animal model of genetic obesity and has been used extensively to study the possible effects of obesity on the pharmacokinetics and pharmacodynamics of a number of drugs.⁷⁻¹¹ In addition, ZOB has been used as an animal model for NIDDM,¹²⁻¹⁶ since these animals exhibit many symptoms that are observed in NIDDM patients, including insulin resistance, hyperglycemia, and hyperlipidemia. Some reports had surmised (but not demonstrated) that diabetic patients may be more resistant to organic nitrates possibly because of increased oxidative stress,^{17,18} a mechanism that has been suggested as one of the underlying causes of nitrate tolerance.¹⁹ Thus, a study examining the acute and chronic pharmacological effects of NTG in ZOB may not only reveal the effects of obesity on the PK/PD of NTG but may also provide useful suggestions regarding the possible effects of experimental NIDDM.

In the present study we examine the role of obesity in affecting NTG PK/PD, comparing our observed results in ZOB to those obtained from its lean controls (Zucker lean, ZL rats), as well as those from normal Sprague-Dawley (SD) rats. We included the last group of animals for comparison because several previous studies in our laboratory have established basic information on the PK/PD of NTG in this animal strain.^{20,21}

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MATERIALS AND METHODS

Animals

All surgical procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee, University at Buffalo. Male SD rats weighing 300 to 350 g were obtained from Harlan (Indianapolis, IN), male ZL rats (340-440 g) and ZOB rats (490-590 g) were obtained from Vassar College (Vassar, NY). Two days prior to the experiment, animals were cannulated at the left femoral artery for blood pressure measurements or blood sampling, and at the left femoral vein for bolus drug administration and at the right jugular vein for drug infusion.²²

Hemodynamic measurements

All hemodynamic studies were carried out in conscious, unrestrained animals. Food and water were allowed ad libitum. Animals were divided into 2 study groups: (A) NTG dose response group (n = 5-6 in each group) and (B) NTG tolerance group (n = 3-5 in each group). On the day of the experiment, systolic and diastolic blood pressures were recorded continuously via the left femoral artery cannula using a Statham pressure transducer (Ohmeda, Murray Hill, NJ) and a Gould RS3400 recorder (Gould, Cleveland, OH). Basal systolic and diastolic blood pressures were recorded for at least 15 to 30 minutes for each animal prior to the start of the experiment. In group A, intravenous (IV) bolus doses of NTG (0.5, 1.0, 2.0, 4.0, 6.0, 10, 15, 20, 30, 40, and 50 µg) were given in random order via the left femoral vein cannula at 30-minute intervals. The injection volume for all NTG bolus doses was kept at 50 µL, using 5 % dextrose (D5W) as a diluent. The hemodynamic response of each NTG bolus dose was measured as percent maximal change in mean arterial pressure (MAP) vs baseline MAP just prior to the specific dose. In group B, animals received an initial 30 µg of NTG IV bolus challenge dose (CD), and baseline maximal MAP response to this dose was recorded. Fifteen minutes later, rats were infused continuously with 1 of the following 3 regimens using an electronically controlled infusion pump (Harvard Instruments, South Natick, MA): 1 µg/min of NTG, 10 µg/min of NTG, or D5W control. The infusion time was 8 hours for the 1 µg/min NTG and D5W infusion groups, and 1 hour for the 10 µg/min NTG infusion group, as previous data showed that NTG tolerance was readily observed within 1 hour at this higher dose.²³ The infusion volume was kept at a constant rate of 10 µL/min for all 3 groups of animals. To determine the development of NTG hemodynamic tolerance, a 30-µg NTG IV bolus CD was given hourly, and the hemodynamic response produced by the hourly CD was compared with that produced before NTG infusion. A decrease in the maximal MAP response of the bolus CD indicates the development of nitrate tolerance. MAP was calculated as (diastolic pressure + 1/3 [systolic pressure – diastolic pressure]). The time-

averaged maximal MAP response was calculated as the sum of the MAP response induced by NTG bolus CD divided by the number of NTG bolus CD.

NTG pharmacokinetics

Steady-state NTG plasma concentrations were determined in all 3 animal groups (n = 3-4 each). NTG was infused via the right jugular vein at 10 µg/min for 2 hours using a programmable Harvard infusion pump (pump 22, Harvard Instruments). Aliquots of 0.4 mL blood were taken from the left femoral artery catheter at 60 and 120 minutes. Plasma was immediately isolated following each blood sample collection and stabilized in 1N silver nitrate and stored at -80°C until analysis. NTG concentration in plasma was measured using gas chromatography with electron capture detection as described previously.²¹

Statistical analysis

Data are presented as mean ± SD. The E_{max} and ED₅₀ values from NTG dose-response curves were calculated by fitting the curves with an E_{max} model using WinNonlin (Version 2.1, Pharsight, Palo Alto, CA). Statistical analysis was performed, where appropriate, using either Student t-test, 1-way ANOVA, followed by the Student-Newman-Keuls post-hoc test, or 2-way ANOVA. Differences with P < .05 were considered statistically significant.

RESULTS

The body weights of the ZOB, ZL, and SD rats were 536 ± 36, 393 ± 34, and 346 ± 4.5 g, respectively (ANOVA, P < .001 vs ZL or SD rats).

Effects of obesity on NTG dose—MAP response curve after acute dosing

Figure 1 shows the in vivo NTG dose—MAP response curves in SD, ZL, and ZOB rats. Bolus injections of NTG caused a dose-dependent increase in maximal MAP responses in all 3 groups of animals. It was observed that the effects of NTG bolus on MAP were short-lived for all doses examined. Blood pressure returned to baseline values within 30 seconds after NTG bolus dosing. The duration of NTG-induced MAP effect was not dose-dependent (data not shown). The effect of NTG on maximal-MAP dose response was well described by an E_{max} model. **Figure 1** shows that there were no apparent differences in NTG dose-response curves among the 3 animal groups studied, even when NTG doses were not adjusted for body weight (P > .05 vs ZL or SD rats, 2-way ANOVA). The E_{max} and ED₅₀ values obtained from the 3 animal groups are listed in **Table 1**. ZOB animals exhibited E_{max} and ED₅₀ values similar to the ZL controls and the SD rats (P > .05, ANOVA).

Figure 1. A) In vivo acute nitroglycerin (NTG) dose-MAP response curves in Sprague-Dawley (SD) rats (●), Zucker lean (ZL) rats (■), and Zucker obese (ZOB) rats (▲) without correcting for body weight. Data are expressed as mean $\pm$ SD, $n = 5-6$. B) In vivo acute nitroglycerin (NTG) dose-MAP response curves in Sprague-Dawley (SD) rats (●), Zucker lean (ZL) rats (■), and Zucker obese (ZOB) rats (▲) after correcting for body weight. Data are expressed as mean $\pm$ SD, $n = 5-6$.

Effects of obesity on NTG tolerance development

Figure 2 shows the effects of D5W vehicle infusion on maximal MAP response to bolus CD of NTG administered at hourly intervals. Consistent with previous reported studies,²³ a 30- μ g NTG IV bolus CD produced an immediate maximal decrease in MAP of about 23% to 29%. When D5W was infused over 8 hours, repeated bolus CD of 30 μ g NTG produced a consistent maximal drop in MAP of about 24% to 27%, and none of the values observed during the control infusion period was different from that observed prior to D5W infusion ($P > .05$,

ANOVA). There were no apparent differences in the maximal MAP response of the hourly NTG IV bolus CD in the ZOB animals when compared with the ZL and SD rats ($P > .05$, ANOVA). Consistent with these results, the time-averaged maximal MAP response (% change from baseline) of the hourly CD over the entire 8-hour infusion period was $27.1\% \pm 0.8\%$, $26.7\% \pm 4.7\%$, $26.4\% \pm 4.1\%$ for SD rats, ZL and ZOB rats, respectively (**Figure 2B**). ANOVA revealed that this parameter was not significantly different among the 3 animal groups.

Table 1. WinNonlin Fitted $E_{\max}$ and ED_{50} Values from Acute Nitroglycerin Dose-MAP Response Curves in Sprague-Dawley, Zucker Lean, and Zucker Obese rats.*

	$E_{\max}$ (% Maximal Decrease in MAP)	ED_{50} (μ g)	Gamma
Sprague Dawley	33.86 ± 3.64	3.45 ± 1.71	0.75 ± 0.17
Zucker Lean	33.19 ± 4.05	3.02 ± 1.42	0.72 ± 0.20
Zucker Obese	34.75 ± 3.88	5.31 ± 2.76	0.63 ± 0.17
ANOVA Statistics	$P > .05$	$P > .05$	$P > .05$

*Data are expressed as mean $\pm$ SD, n = 5-6.

Figure 2. A) The effect of 10 μ L/min D5W infusion on nitroglycerin-(NTG-) induced maximal MAP effects of the hourly 30 μ g NTG IV bolus challenge dose in Sprague-Dawley (SD) rats (●), Zucker lean (ZL) rats (■), and Zucker obese (ZOB) rats (▲). Data are expressed as mean $\pm$ SD, n = 3-5. B) The time-averaged maximal MAP responses of the hourly 30- μ g NTG IV bolus over 8 hours in the presence of 10 μ L/min D5W infusion in Sprague-Dawley (SD), Zucker lean (ZL), and Zucker obese (ZOB) rats. Data are expressed as mean $\pm$ SD, n = 3-5.

Figure 3. A) The effect of 1 $\mu\text{g}/\text{min}$ NTG infusion on NTG-induced maximal MAP effects of the hourly 30- μg NTG IV bolus challenge dose in Sprague-Dawley (SD) rats (●), Zucker lean (ZL) rats (■), and Zucker obese (ZOB) rats (▲). Data are expressed as mean $\pm$ SD, $n = 3-5$. B) The time-averaged maximal MAP responses of the hourly 30- μg NTG IV bolus over 8 hours in the presence of 1 $\mu\text{g}/\text{min}$ NTG infusion in Sprague-Dawley (SD), Zucker lean (ZL), and Zucker obese (ZOB) rats. Data are expressed as mean $\pm$ SD, $n = 3-5$. * $P < .05$ vs SD rats, ANOVA.

Figure 3 shows the effects of 1 $\mu\text{g}/\text{min}$ NTG continuous infusion on the maximal MAP response of the hourly NTG IV bolus CD. At this low dose, there was no apparent change over the entire 8-hour infusion period. Each of the hourly 30- μg NTG IV bolus CD produced similar maximal MAP response ($P > .05$, ANOVA). ANOVA revealed that the maximal MAP effect of the 30- μg NTG IV bolus CD from the NTG infused group was not different from the corresponding D5W controls for all 3 groups of animals ($P > .05$). The time-averaged values for maximal

MAP response of the hourly CD over the 8-hour infusion period was $25.4\% \pm 3.5\%$, $21.9\% \pm 2.4\%$, and $19.1\% \pm 1.8\%$ for SD rats, ZL, and ZOB rats, respectively. ANOVA revealed that the time-averaged maximal MAP responses (percentage change from baseline) in the ZOB rat were significantly different from the SD rats ($P < .05$), but not from the ZL rats ($P > .05$).

Figure 4 shows the effect of 10 $\mu\text{g}/\text{min}$ NTG continuous infusion on the maximal MAP responses of the hourly

Figure 4. A) The effect of 10- μ g/min nitroglycerin (NTG) infusion on NTG-induced maximal MAP effects of the hourly 30- μ g NTG IV bolus challenge dose in Sprague-Dawley (SD) () Zucker lean (ZL) () and Zucker obese (ZOB) rats (). Data are expressed as mean $\pm$ SD, $n = 3-5$. *** $P < .001$ vs corresponding 0 hr, Student t test. B) The time-averaged maximal MAP responses of the hourly 30- μ g nitroglycerin (NTG) IV bolus over 1 hour in the presence of 10- μ g/min NTG infusion in Sprague-Dawley (SD), Zucker lean (ZL), and Zucker obese (ZOB) rats. Data are expressed as mean $\pm$ SD, $n = 3-5$.

NTG IV bolus CD. Consistent with data obtained from earlier studies,²³ the maximal MAP response of the first hourly NTG IV bolus CD was significantly attenuated. The decreases in maximal MAP response at 1 hour compared with baseline MAP response were 60.5%, 58.7%, and 57.0% for SD, ZL, and ZOB rats respectively ($P < .001$ vs the corresponding baseline response for each group of animals, Student t test). However, there

was no apparent difference in the hemodynamic behavior among the 3 animal groups ($P > .05$, ANOVA). The time-averaged maximal MAP response of the challenge NTG dose after the 1-hour infusion period was $18.7\% \pm 0.8\%$, $17.9\% \pm 2.4\%$, and $18.9\% \pm 4.9\%$ for SD, ZL, and ZOB rats, respectively ($P > .05$, ANOVA).

Figure 5. Steady-state nitroglycerin (NTG) plasma concentrations after 2 hours of 10- μ g/min NTG infusion in Sprague-Dawley (SD) (□), Zucker lean (ZL) (▨), and Zucker obese (ZOB) rats (▩). Data are expressed as mean $\pm$ SD, n = 3-4.

Effect of obesity on steady-state NTG plasma concentrations after 10 μ g/min infusion

Figure 5 shows NTG plasma concentrations at 1 and 2 hours after the start of 10 μ g/min infusion. Consistent with the reported half-life of approximately 5 minutes in rats,²⁰ steady-state NTG concentration was reached at 1 hour of NTG infusion. Our results indicate that NTG plasma concentrations at 1 and 2 hours were similar in all 3 animal groups. The time-averaged steady-state plasma concentrations were 84.9 ng/mL, 85.7 ng/mL, and 102.9 ng/mL for SD, ZL, and ZOB rats, respectively ($P > .05$, ANOVA).

DISCUSSION

In the present study, we showed that acute in vivo NTG hemodynamic responses were not significantly altered in the ZOB rats vs ZL and SD controls (**Figure 1**), whether the MAP response was corrected for body weight or not. In addition, our results indicated that the time-course and extent of NTG tolerance development in the ZOB rats were not different from the ZL and SD controls at the 2 infusion doses of NTG examined. Neither dose was corrected for the body weight difference between ZOB rats

and the ZL and SD controls. Consistent with these data, no difference in the steady-state NTG plasma concentration was observed among these animal groups. These results suggest that NTG PK and PD are not highly sensitive toward obesity in a conscious animal model of genetic obesity.

Obesity is often closely associated with NIDDM, and the body mass index has been shown to be a good predictor of the risk of diabetes.^{2,4} Endothelial dysfunction and altered nitric oxide (NO) activity have been observed in obesity²⁴⁻²⁸ and obesity-associated NIDDM^{17,18, 29-32} for endothelium-dependent vasodilators. Moreover, impaired vasodilator responses to endothelium-independent vasodilators such as NTG and sodium nitroprusside have been reported for obesity-associated NIDDM.^{17, 32,33} The results from the in vivo NTG dose-response curve (**Figure 1**) showed that acute NTG pharmacological effect was not significantly changed in obesity even when doses were not adjusted for kg body weight, suggesting that obesity and possibly NIDDM do not lead to any observable resistance to the hemodynamic effects of NTG. These observations are in agreement with several reports that only endothelium-dependent vasodilator responses are altered in obesity.^{27,34,35} Consistent with our data, literature reports

also indicated that endothelium-independent vasodilator responses are not impaired in either animal models of NIDDM, using ZOB rats,^{31,36} or diabetic humans.^{37,38}

However, our results from the acute NTG hemodynamic study (**Figure 1**) are in contrast to an earlier study by Laight et al,³³ which reported impaired NTG-induced MAP effects in the ZOB rats vs ZL controls. This apparent discrepancy may be due to the differences in the animal models employed. Our study was performed in conscious, unrestrained animals, whereas Laight et al³³ performed their study in anesthetized animals, in which cardiovascular regulatory controls are diminished. In addition, the magnitude of change shown in the latter study was quite modest (about 20% decrease in E_{max} , with no apparent change in ED_{50}). Thus, the findings of Laight et al³³ can also be interpreted as demonstrating only a marginal effect of obesity on NTG-induced vascular response.

Figure 2 shows that with vehicle D5W infusion, NTG-induced maximal MAP response was similar in the ZOB rats, ZL controls, and SD rats throughout the 8-hour infusion period. These results suggest that in the presence of D5W vehicle infusion, the 30- μ g NTG IV bolus, when given at 1-hour intervals, did not induce any apparent hemodynamic tolerance. At an infusion rate of 1 μ g/min NTG (**Figure 3**), vascular tolerance development was not observed for all 3 groups of animals ($P > .05$ vs corresponding D5W control group, and $P > .05$ vs preinfusion baseline response). These results were in general agreement with our previous data²³ showing that, in SD rats, administration of a 1 μ g/min regimen for 10 hours produced little hemodynamic tolerance. However, when the time-averaged maximal MAP response was used as an index of NTG hemodynamics, ANOVA revealed that ZOB rats were significantly different from the SD rats but not from the control ZL rats. These results suggest that there may be slight differences in NTG hemodynamics among the different strains of rats. However, these results also indicated that genetic obesity has no apparent effect on NTG hemodynamics, as no differences were observed between the ZL and ZOB rats.

When the NTG infusion dose was increased from 1 μ g/min to 10 μ g/min, hemodynamic tolerance to the maximal MAP lowering effect of the hourly 30- μ g NTG IV bolus was observed within 1 hour of NTG infusion (**Figure 4**). The development of NTG tolerance at this high infusion dose was both rapid and pronounced. However, the extent of NTG tolerance development in the 3 groups of animals was not different. Laight et al had reported a more severe NTG tolerance in the ZOB rats compared with the ZL control; however, the difference reported did not reach statistical significance.³³

The ZOB rats have been shown to manifest pre-existing oxidative stress in both the myocardium³⁹ and the

plasma.⁴⁰ This general state of elevated oxidative stress may lead to in vivo inactivation of NO. The reactive oxygen species (ROS), particularly superoxide anion, can inactivate NO via the formation of the highly reactive radical, peroxynitrite,¹⁹ and deplete intracellular thiols, which are important mediators in NO metabolism.⁴¹ Results from our study would imply that the increased oxidant stress present in ZOB does not affect the animal's vascular sensitivity to NTG. One possible explanation for this observation is that in vivo oxidative stress, even when it occurs generally in ZOB rats, does not affect the vasculature. Plasma 8-epi-prostaglandin F_{2 α} , used in the study by Laight et al³³ as an index of oxidant stress, may not be reflective of the oxidant state in the vasculature. Indeed this explanation was supported by the observation in the same study that although tiron (a superoxide scavenger) effectively restored NTG-induced vasodilation in ZOB rats, it was not able to restore plasma 8-epi-prostaglandin F_{2 α} levels to that of ZL rats. It is currently unknown if higher levels of ROS are indeed present in the vasculature of the ZOB rat since no studies have been performed to address this issue.

Consistent with data obtained from the in vivo pharmacodynamic studies, steady-state NTG plasma concentrations were not changed in the ZOB rats. The mean steady-state plasma concentrations observed in our study were in general agreement with previous reports in normal SD rats and congestive heart failure rats.²¹ Our results also suggest that obesity and possibly NIDDM did not have a discernable effect on NTG plasma pharmacokinetics.

In conclusion, results of the present study do not support the view that NTG-induced hemodynamic effects may be significantly affected by obesity and experimental NIDDM. The PK/PD of NTG are not sensitively dependent on the body weight of conscious rats.

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