Lack of Effect of Sodium Nitroprusside on Insulin-Mediated Blood Flow and Glucose Disposal in the Elderly

Graydon S. Meneilly, Bruno Battistini, and John S. Floras

Insulin increases skeletal muscle blood flow in healthy young subjects by a nitric oxide (NO)-dependent mechanism. Impairment of this mechanism may contribute to the insulin resistance of normal aging, a state characterized by reduced endothelial production of NO, an attenuated effect of insulin on skeletal muscle blood flow, and resistance to insulin-mediated glucose uptake (IMGU). We tested the hypothesis that the NO donor sodium nitroprusside (SNP) would augment insulin-mediated vasodilation and thus increase IMGU in healthy elderly subjects. Experiments were performed with young (n = 9; age, 25 ± 1 years; body mass index [BMI], 24 ± 1 kg/m^2) and old (n = 10; age, 78 ± 2 years; BMI, 25 ± 1 kg/m^2) healthy subjects. Each group underwent two studies in random order. In one study (control), insulin was infused using the euglycemic clamp protocol for 240 minutes at a rate of 40 mU/m^2/min (young) and 34 mU/m^2/min (old). In the other study (SNP), SNP was coinfused with insulin from 120 to 240 minutes. At regular intervals in each study, blood samples were obtained and calf blood flow was measured using venous occlusion plethysmography. Glucose and insulin values were similar in control and SNP studies in both age groups. In the young, SNP had no effect on blood flow to the calf, but its action in calf resistance vessels augmented insulin-mediated vasodilation, since incremental calf vascular conductance was greater during SNP infusion (control *v* SNP, 0.027 ± 0.002 *v* 0.040 ± 0.008 mL/100 mL/min/mm Hg, $P < .0001$). However, SNP had no effect on insulin-mediated glucose disposal. In the elderly, SNP reduced the blood flow to the calf, but this was countered by its effect on calf resistance vessels such that vascular conductance was unaffected (control *v* SNP, 0.012 ± 0.003 *v* 0.011 ± 0.003 mL/100 mL/min/mm Hg, P = nonsignificant [NS]). Steady-state (180 to 240 minutes) glucose disposal (control *v* SNP, 7.47 ± 0.47 *v* 6.54 ± 0.56 mg/kg/min, $P < .01$) rates were significantly lower during SNP infusion. In summary, systemic infusion of SNP did not increase insulin-mediated glucose disposal in either young or old subjects. Thus, the present findings do not support the concept that increasing NO availability will enhance glucose disposal in either age group. However, because the incremental increases in IMGU during SNP infusion paralleled the changes in blood supply to the calf rather than calf vascular conductance, any potential benefits on NO delivery in elderly subjects may have been offset by the direct or reflex effects of systemic hypotension. Other stimuli to NO production that do not cause hypotension must be tested before this therapeutic strategy can be considered as a potential means for enhancing the metabolic actions of insulin in the elderly.

THE BULK OF insulin-mediated glucose uptake (IMGU) occurs in skeletal muscle, possibly through several mechanisms. Initially, insulin was thought to promote glucose uptake into muscle primarily by activating glucose transporters. Recently, it has been demonstrated that insulin increases skeletal muscle blood flow via a nitric oxide (NO)-dependent mechanism.[1-6] However, it is uncertain whether this insulin-induced increase in blood flow is an important element of normal glucose disposal.[1,2,6] Attenuation of insulin-mediated vasodilation has been described in conditions characterized by insulin resistance, but whether this alteration contributes to insulin resistance has not been established.[1-6]

Normal aging is characterized by a progressive impairment in carbohydrate tolerance. One of the major factors contributing to the glucose intolerance of aging is the resistance to IMGU.[7-10] Endothelial production of NO is reduced with age, and we have recently demonstrated that insulin-mediated vasodilation is impaired in the elderly.[11]

We thus undertook the current study with the following hypotheses: (1) in healthy young subjects, the NO donor sodium nitroprusside (SNP) will enhance insulin-mediated blood flow and glucose disposal; and (2) in healthy elderly subjects, SNP will reverse the resistance to insulin-mediated blood flow and to IMGU.

SUBJECTS AND METHODS

Subjects

The studies were performed in healthy non-obese young and elderly (age >65 years) subjects recruited on the basis of a normal medical history and physical examination, normal laboratory tests (including hepatic and renal function), a normal electrocardiogram, and a normal glucose tolerance test as defined by National Diabetes Data Group criteria. None of the subjects were on medication. None had symptoms of claudication. The pedal pulses were normal and there were no clinical signs of peripheral vascular insufficiency. In addition, all subjects had blood pressure values in both ankles that were at least as high as the blood pressure in their arms (as measured with a sphygmomanometer). The study was approved by the Committee on Human Investigation at the University of British Columbia. All subjects provided written informed consent prior to participation (Table 1).

Protocol

Each subject underwent two glucose clamp studies in random order performed according to the method of Andres et al.[12] Each study was separated by at least 2 weeks. The studies commenced at 7:30 AM in our

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Table 1. Subject Characteristics at Baseline

Characteristic	Young (n = 9)	Old (n = 10)
Age (yr)	25 ± 1	78 ± 2
Sex ratio (male/female)	5/4	5/5
BMI (kg/m²)	24 ± 1	25 ± 1
MABP (mm Hg)	73 ± 1	97 ± 2*
Pulse rate (bpm)	55 ± 2	62 ± 3
Calf blood flow (mL/100 mL/min)	3.65 ± 0.19	2.55 ± 0.18*
Glucose (mmol/L)	5.0 ± 0.1	5.2 ± 0.2
Insulin (pmol/L)	103 ± 18	91 ± 13
ir-ET (pg/mL)	3.4 ± 1.2	5.7 ± 1.6
Nitrite (μmol/L)	10.6 ± 3.3	11.7 ± 2.2

*$P < .0001$, young *v* old.

Clinical Research Center after an overnight fast. In all studies, intravenous lines were inserted into an antecubital vein for infusion of substrates and into a contralateral hand vein for sampling of "arterialized" venous blood.[13] Three heparinized blood samples were taken at 10-minute intervals from −20 to 0 minutes to measure basal glucose and insulin. At time 0, glucose clamp studies were commenced and continued for 240 minutes. In each study, regular human insulin (Eli Lilly, Indianapolis, IN) was infused at a rate of 40 mU/m²/min in the young and 34 mU/m²/min in the old. These insulin infusion rates were chosen because insulin clearance decreases with age,[14] and we have previously shown that infusing insulin in this way results in equivalent peripheral insulin levels in young and old subjects.[11] During the control study, insulin and glucose alone were infused. During the SNP study, this NO donor (Nipride; Roche Laboratories, Mississauga, Ontario, Canada) was infused from 120 to 240 minutes. To confirm that the infusion was functionally equipotent in each age group, the doses were titrated to reduce the mean arterial blood pressure (MABP) by approximately 10%. Blood samples were obtained at 5-minute intervals to measure plasma glucose and every 30 minutes to measure insulin in each study. Samples were taken to measure the level of immunoreactive endothelins (ir-ETs) and nitrite at baseline and every 60 minutes during the study. The coefficient of variation for plasma glucose did not exceed 5% in any study. Two young and 2 old subjects underwent a determination of peripheral glucose disposal and hepatic glucose production using tritiated glucose methodology as previously described.[15]

During each study, blood pressure was measured at baseline and at 30-minute intervals thereafter using an automated blood pressure cuff (Dinamap; Critikon, Tampa, FL). MABP was calculated from the diastolic blood pressure plus one third of the pulse pressure.

Bilateral calf blood flow was determined by venous occlusion plethysmography using calibrated mercury-in-silastic strain gauges as previously described.[16] This technique was used because it has been shown to reliably measure changes in blood flow in response to insulin infusion.[17-20] Each leg was supported at a level of 15 cm above the right atrium. Venous occlusion pressure was 40 mm Hg at the lower thigh, and the ankle-cuff occlusion pressure was 200 mm Hg. The venous occlusion cuff was inflated for 10 seconds and deflated for 10 seconds over a 3-minute period. The mean of the final 5 measurements of each recording period was used for analysis. Blood flow was measured at 10-minute intervals from −30 to 0 minutes, at 30-minute intervals from 0 to 120 minutes, and at 15-minute intervals from 120 to 240 minutes.

Analytical Methods

Plasma glucose was measured immediately in all studies by the glucose oxidase method on a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was placed in prechilled test tubes containing aprotinin (400 KIU/mL) and EDTA (1.5 mg/mL) and centrifuged at 4°C. The plasma was stored promptly at −70°C until analysis. All samples from each subject were analyzed in the same assay. Insulin assays were performed as previously described.[11]

NO is one of the two products synthesized by the enzyme nitric oxide synthase from molecular oxygen and arginine. Although it is produced in trace quantities and is scavenged within 4 seconds, its metabolites nitrate and nitrite can be measured in biologic fluids. The nitrite level was measured via a modified version of the Greiss reaction as previously described,[21] using a colorimetric assay kit (Cayman Chemicals, Ann Arbor, MI). Plasma samples for measurement of ETs were extracted on a C18 column (Amprep; Amersham Pharmacia Biotech, Mississauga, Ontario, Canada). Samples were loaded onto preconditioned columns with 5 mL ethanol and 5 mL nanopure water and then washed with water: trifluoroacetic acid ([TFA] 0.1% vol/vol) before elution with acetonitrile (80% vol/vol):TFA (0.1%). The collected washout for each sample was concentrated with a speed-vacuum apparatus (Savant Instruments, Holbrook, NY). Extracted plasma samples were then measured for immunoreactive ETs by radioimmunoassay, as previously described.[22]

Data Analysis

All data are presented as the mean ± SEM. Leg blood flow was calculated as previously described.[16] To estimate blood supply to the calf, plethysmographic blood flow values obtained in both legs were averaged to yield the mean value at each time point. To determine the presence or absence of calf vasodilation in the setting of changes in perfusion pressure, calf vascular conductance was calculated by dividing calf blood flow by MABP as previously described.[23] Differences were determined using Student's *t* test for paired and unpaired samples. A *P* value less than .05 was considered significant in all analyses.

RESULTS

Subject characteristics are shown in Table 1. The elderly had a higher baseline MABP and lower baseline calf blood flow. The body mass index (BMI), pulse rate, and fasting glucose and insulin values were similar in the two groups.

Hemodynamic Variables

Steady-state (180 to 240 minutes) MABP was lower in the SNP study in both the young (control *v* SNP, 77 ± 1 *v* 66 ± 2 mm Hg, $P < .0001$) and the old (control *v* SNP, 99 ± 2 *v* 88 ± 2 mm Hg, $P < .0001$). SNP infusion reduced MABP by approximately 14% in both the young (14% ± 1%) and the old (14% ± 2%, P = NS for young *v* old). The steady-state pulse was higher in the SNP study in the young (control *v* SNP, 61 ± 1 *v* 65 ± 2 bpm, $P < .05$). The steady-state pulse did not differ between studies in the old (control *v* SNP, 64 ± 3 *v* 64 ± 3 bpm, P = NS).

Steady-state (180 to 240 minutes) incremental calf blood flow is illustrated in Fig 1. Incremental calf blood flow was similar in the young under both conditions (control *v* SNP, 2.08 ± 0.56 *v* 2.11 ± 0.41 mL/100 mL/min, P = NS). Since incremental calf vascular conductance was greater during the SNP infusion (control *v* SNP, 0.027 ± 0.002, *v* 0.040 ± 0.008 mL/100 mL/min/mm Hg, $P < .0001$), the lack of effect on blood flow to the calf was due to the reduction in calf perfusion pressure caused by SNP. In contrast, in the elderly, incremental calf vascular conductance was unchanged (control *v* SNP, 0.012 ± 0.003 *v* 0.011 ± 0.003 mL/100 mL/min/mm Hg, P = NS), whereas incremental calf blood flow rates were lower

during the SNP study (control *v* SNP, 1.28 ± 0.30 *v* 0.54 ± 0.27 mL/100 mL/min, $P < .05$). This reduction in blood flow to the calf despite maintenance of calf vascular conductance was again due to the decrease in calf perfusion pressure caused by SNP.

Metabolic Variables

Glucose and insulin values during the control and SNP studies are shown in Fig 2. Steady-state (30 to 240 minutes) glucose (young control *v* SNP, 5.4 ± 0.1 *v* 5.5 ± 0.1 mmol/L, P = NS; old control *v* SNP, 5.4 ± 0.1 *v* 5.6 ± 0.1 mmol/L, P = NS) and insulin (young control *v* SNP, 728 ± 50 *v* 791 ± 57 pmol/L, P = NS; old control *v* SNP, 791 ± 36 *v* 813 ± 36 pmol/L, P = NS) values were similar in control and SNP studies. Steady-state (180 to 240 minutes) glucose infusion rates (GIRs) are shown in Fig 3. GIRs were similar in young subjects in both studies (control *v* SNP, 11.01 ± 0.96 *v* 10.95 ± 1.09 mg/kg/min, P = NS). However, steady-state GIRs were higher in the elderly during the control study (control *v* SNP, 7.47 ± 0.47 *v* 6.54 ± 0.56 mg/kg/min, $P < .01$). In the two young and two old subjects who underwent a determination of hepatic glucose production, it was suppressed to less than 0.5 mg/kg/min in both the control and SNP studies (data not shown).

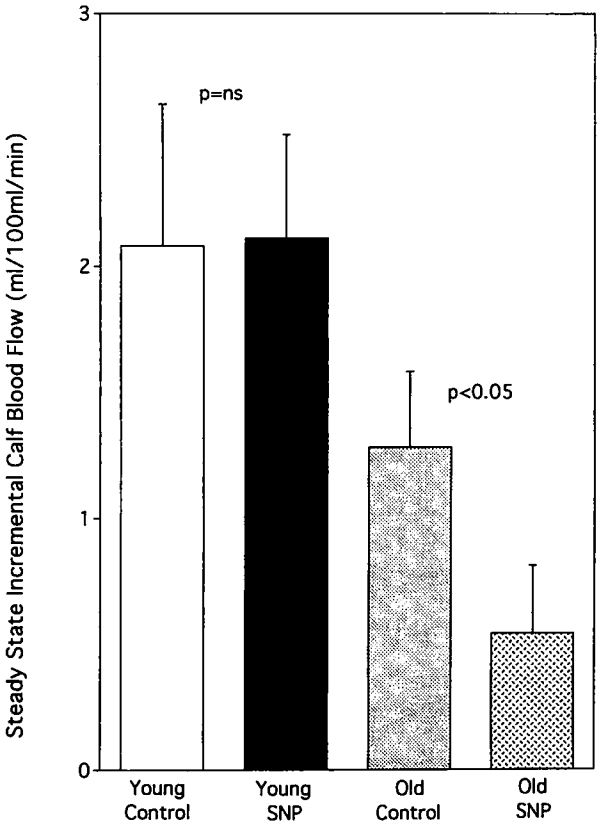

Fig 1. Steady-state (180-240 minutes) incremental calf blood flow in young and old subjects during euglycemic clamp studies. Calf blood flow did not change in the young, but decreased in the old ($P < .05$).

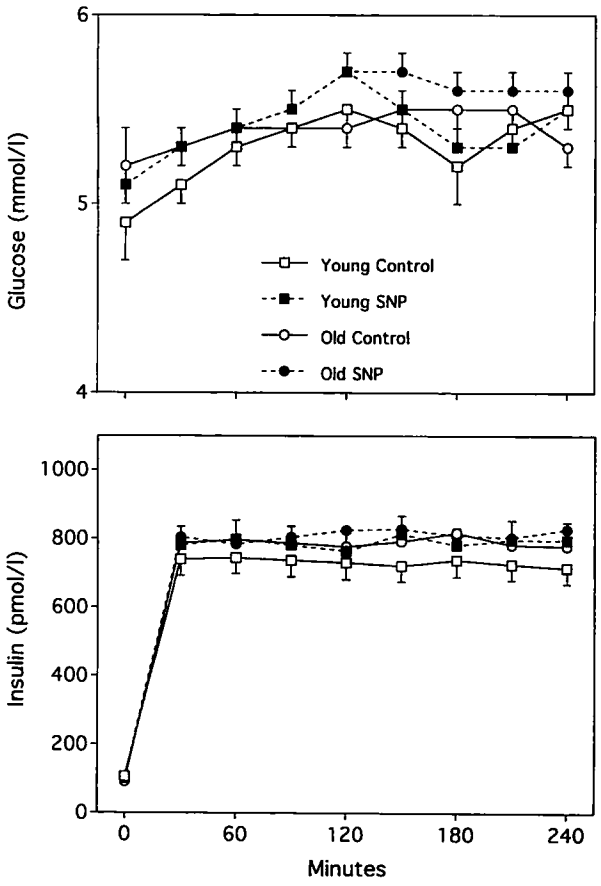

Fig 2. Glucose and insulin values in young and old subjects during euglycemic clamp studies. Insulin and glucose values were similar in the control and SNP studies in young and old subjects.

ET and Nitrite

Basal ET and nitrite levels were not different between young and old subjects (Table 1). ET and nitrite values during the control and SNP studies are shown in Fig 4. Steady-state (180 to 240 minutes) ET (young control *v* SNP, 3.5 ± 1.8 *v* 5.6 ± 2.6 pg/mL, P = NS; old control *v* SNP, 6.9 ± 1.8 *v* 6.9 ± 2.9 pg/mL, P = NS) and nitrite (young control *v* SNP, 18.6 ± 3.5 *v* 19.1 ± 6.0 μmol/L, P = NS; old control *v* SNP, 19.1 ± 3.0 *v* 21.1 ± 2.4 μmol/L, P = NS) values were similar in the control and SNP studies.

DISCUSSION

Several groups have demonstrated that insulin can stimulate blood flow in healthy young subjects by a NO-dependent mechanism.[1,2,6] Insulin-mediated vasodilation is believed by some to be an important component of insulin's effect on glucose disposal, but this hypothesis is controversial.[1,2,6] The present study is the first to evaluate the effect of a systemic infusion of the NO donor SNP on insulin-mediated vasodilation and glucose disposal in healthy young subjects. Furthermore, we replicated this protocol in healthy elderly individuals to determine whether SNP has similar, potentially therapeutically useful effects in a population characterized by reduced endothe-

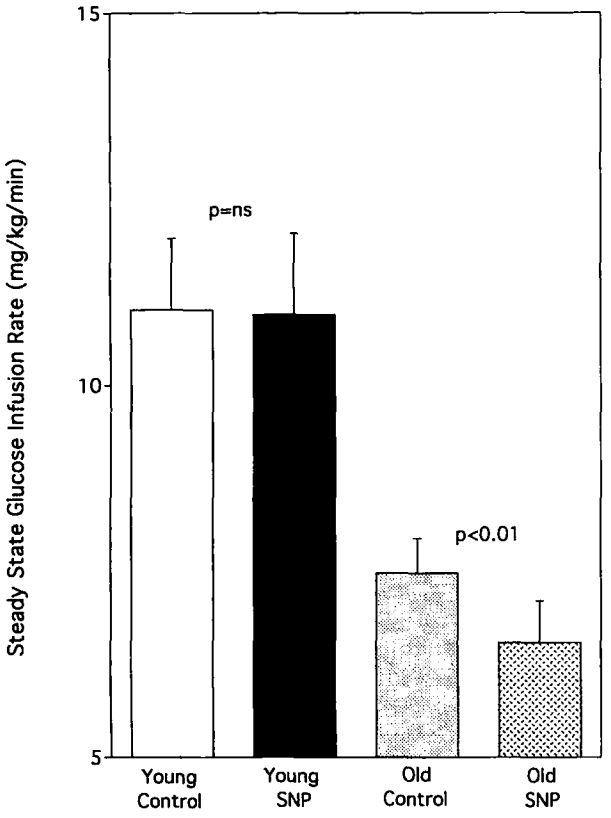

Fig 3. Steady-state (180-240 minutes) GIRs in young and old subjects during euglycemic clamp studies. GIRs did not change in the young, but decreased in the old ($P < .05$).

lial production of NO, an attenuated effect of insulin on skeletal muscle blood flow, and resistance to IMGU.

Previous investigators have infused vasoactive agents both systemically and locally to examine the effect of alterations in blood flow on insulin-mediated glucose disposal in young healthy subjects. The results of these experiments are conflicting. Systemic infusion of norepinephrine or epinephrine did not reduce insulin-mediated glucose disposal,[24,25] whereas the vasoconstrictor angiotensin II enhanced insulin-mediated glucose disposal,[26] as did N(G)-monomethyl-L-arginine (L-NMMA), which inhibits the synthesis of NO.[27] Calf blood flow actually increased in the latter experiments, probably due to the concurrent increase in mean arterial pressure. Femoral artery infusion of bradykinin increased leg blood flow by 70%, but had no effect on IMGU,[28] whereas infusion of metacholine, which stimulates endothelial production of NO, increased both leg flood flow and IMGU.[29] Conversely, femoral artery infusion of L-NMMA reduced both leg blood flow and insulin-mediated glucose disposal.[2] In middle-aged subjects with obesity, hypertension, and insulin resistance, forearm infusion of SNP increased blood flow but did not affect insulin-mediated glucose disposal.[30] In summary, the results of studies in young and middle-aged subjects that have examined the effect of augmentation or reduction of blood flow on insulin-stimulated muscle glucose uptake are contradictory, but a comparison between studies is difficult due to differences in the site, dose, and duration of infusion, subject characteristics, and the mechanism by which each of these agents cause vasoconstriction or vasodilation.[31]

Our present data indicate that coinfusion of the NO donor SNP with insulin can augment the effect of this peptide on calf vascular conductance in young healthy subjects, but dilation of calf resistance vessels by this mechanism does not cause an increase of glucose disposal. However, because SNP also reduced calf perfusion pressure, resulting in no net effect on the insulin-mediated increase in calf blood flow, these observations do not specifically address the issue of whether enhancing substrate delivery (by increasing blood flow) will augment glucose disposal in this age group.

Several studies have found that normal aging is characterized by resistance to insulin-mediated glucose disposal.[7-10] It has been assumed that insulin resistance in the elderly is due to a postreceptor defect in insulin action. Although early studies found no effect of aging on insulin-mediated blood flow,[20] our group and others have recently demonstrated that insulin-induced vasodilation is impaired in the elderly.[11,32] We postulated that impaired insulin-mediated vasodilation is an important cause of insulin resistance in the aged. Normal aging is characterized by a decrease in endothelial NO production in vitro[33] and a decrease in endothelium-dependent and NO-

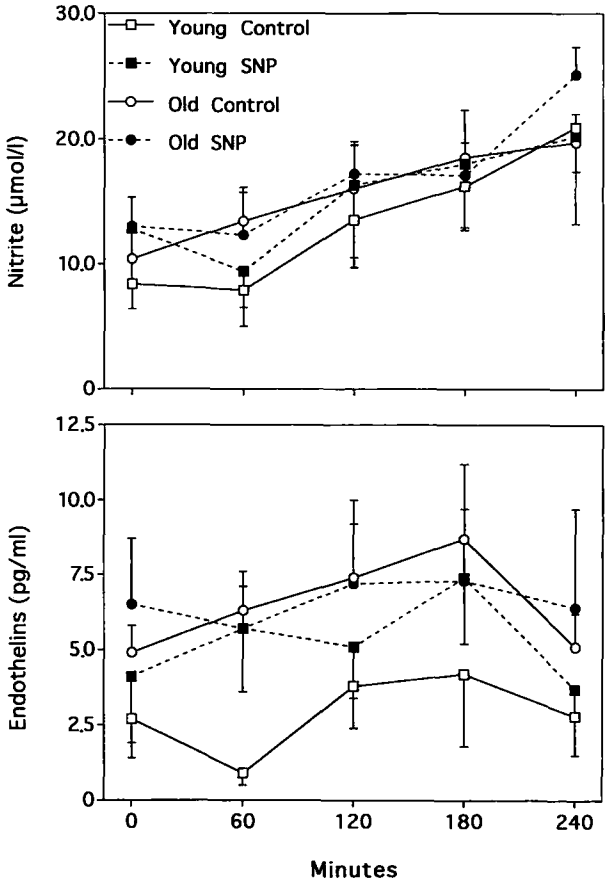

Fig 4. Endothelin and nitrite values in young and old subjects during euglycemic clamp studies. Endothelin and nitrite values were similar in the control and SNP studies in young and old subjects.

mediated vasodilation in vivo.[34,35] Because endothelial NO production may be impaired with aging, we sought to bypass this defect by administering an endothelium-independent vasodilator and NO donor, SNP. We hypothesized that SNP infusion would increase blood flow and enhance glucose disposal in the elderly.

Although the rationale for infusing SNP was to exploit its property as a NO donor, we recognized that the systemic blood pressure would decrease, and therefore adjusted the dose of SNP to ensure that this effect would be similar in both young and old subjects. The hypotensive response to SNP coinfused with insulin was similar in the young and the old. To account for this reduction in MABP during SNP infusion, we calculated the calf vascular conductance to estimate the effect of SNP and insulin on calf resistance vessels.[23] In elderly subjects, coinfusion of SNP also caused dilatation of calf resistance vessels because conductance was maintained despite a significant decrease in blood flow to the calf.

Reductions in preload and blood pressure caused by SNP are countered by a reflex increase in sympathetic vasoconstrictor discharge to the calf.[36] The reflex increase in peroneal muscle sympathetic nerve activity in response to these hemodynamic changes is similar in young and healthy elderly subjects.[37] This direct measure of sympathetic outflow is consistent with our previous findings and other studies, in which the norepinephrine and epinephrine response to systemic insulin or SNP infusion was similar in healthy young and elderly subjects.[11,32,38] Because ET levels were also similar before and during SNP, the contrasting effects of SNP and insulin on calf blood flow in the present experiments cannot be attributed to greater reflex vasconstriction in this vascular bed in elderly subjects. Rather, in older subjects, an attenuation of vasoconstrictor responsiveness to sympathetic stimulation in vascular beds other than the calf leading to a redistribution of blood flow[39] and an attenuation of cardiac output responses to nitroprusside are two more likely explanations for this observation. In young healthy subjects, nitroprusside infusion increases cardiac output by increasing the heart rate and the stroke volume does not change,[36] whereas in older subjects, SNP has no effect on stroke volume, heart rate, or blood pressure.[40] In this context, it is worth noting that SNP increased the heart rate in the young but not in the older subjects in the present study. Cardiac output was not assessed, but the absence of this information does not affect our principal conclusions, since the blood flow measured herein represents that proportion of cardiac output directed at the calf vascular bed under each of the experimental conditions.

Insulin-mediated glucose disposal in the elderly was also attenuated when SNP was coinfused with insulin. How can we explain this difference in the effect of SNP on glucose disposal in the young and the old? These different responses could arise from the contrasting effects of SNP on blood flow to the calf in the two groups. The incremental increases in IMGU during SNP paralleled the changes in blood supply to the calf rather than the changes in conductance. This observation is consistent with the concept that SNP either had less effect on cardiac output in the elderly or diverted blood flow away from insulin-sensitive skeletal muscle to vascular beds supplying insulin-insensitive tissue (such as the kidney) in the aged. If so, this would result in a net decrease in whole-body glucose disposal. In our previous experiments involving SNP infusion in healthy young and old subjects, glucose values did not change before and after SNP.[38] Taken together, these observations would indicate that (1) any potential benefits of increased NO delivery by SNP will be offset by its hypotensive action and (2) a NO donor that does not reduce systemic blood pressure may not alter glucose metabolism in the absence of insulin.

In summary, systemic infusion of SNP did not increase insulin-mediated glucose disposal in either young or old subjects. Thus, the present findings do not support the concept that administration of potent vasodilators with the intent of increasing NO availability will enhance glucose disposal in either age group. However, because the incremental increases in IMGU during SNP infusion paralleled the changes in blood supply to the calf rather than the incremental calf vasodilation, any potential benefits on NO delivery in elderly subjects may have been offset by systemic hypotension. To clarify this issue, further experiments must be performed to investigate the effects of hypotension induced by agents that do not alter the NO system, or other approaches to the modulation of the NO pathway that do not cause hypotension must be tested, before this therapeutic strategy can be considered as a potential means of enhancing the metabolic actions of insulin in the elderly.

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