Exogenous Nitric Oxide Increases Basal Leg Glucose Uptake in Humans

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This study addressed the role of blood flow and nitric oxide in leg glucose uptake. Seven subjects (5 men, 2 women) were studied during conditions of resting blood flow and increased blood flow, achieved by infusion of the nitric oxide (NO) donor sodium nitroprusside (SNP) into the femoral artery. Femoral arterial and venous blood samples were obtained and blood flow was determined by infusion of indocyanine green dye. SNP infusion significantly increased leg blood flow (769 ± 103 *v* 450 ± 65 mL · min^{-1} · leg^{-1}, *P* < .001), but did not affect arterial (4.68 ± 0.13 mmol/L control, 4.63 ± 0.09 mmol/L SNP) or venous (4.60 ± 0.14 mmol/L control, 4.54 ± 0.10 mmol/L SNP) glucose concentrations. Glucose uptake was significantly (*P* < .01) higher during SNP infusion (65 ± 6 μmol · min^{-1} · leg^{-1}) than during the basal period (34 ± 6 μmol · min^{-1} · leg^{-1}), whereas lactate release was unaffected (rest, 45 ± 11 μmol · min^{-1} · leg^{-1}; SNP, 42 ± 14 μmol · min^{-1} · leg^{-1}). We conclude that blood flow and/or NO increase basal leg glucose uptake.

BOTH INSULIN and exercise increase skeletal muscle glucose uptake.[1] In lean, insulin-sensitive subjects, both of these stimuli also cause vasodilation in skeletal muscle, whereas in insulin-resistant states the hemodynamic response to insulin is often impaired.[2] As insulin- and exercise-induced vasodilation are both thought to represent recruitment of previously underperfused capillaries,[3,4] it is possible that capillary recruitment is necessary for normal insulin sensitivity.[6]

A related issue is the possibility that adequate production of and/or sensitivity to nitric oxide (NO) plays a necessary role in glucose uptake, as insulin-induced vasodilation is NO-dependent[6] and mice lacking the genes for endothelial and neuronal NO synthase display marked insulin resistance.[7] In addition, NO may play a role in contraction-induced glucose uptake.[1] This effect is at least partially blood flow independent, as inhibition of NO production during aerobic exercise reduces glucose uptake although normal blood flow is maintained.[9] Thus, in the present study we tested the hypothesis that NO, in the absence of accompanying exercise or insulin stimulation, increases glucose uptake in humans in vivo.

MATERIALS AND METHODS

Subjects

Seven subjects (5 men and 2 women) consented to participate in this study after receiving a detailed written and verbal description of the study. The mean age, height, weight, and body mass index (BMI) [mean ± SE (range)] were, respectively, 24 ± 1 years (21 to 29), 1.71 ± 3 m (162 to 181), 68 ± 4 kg (61 to 82), and 23 ± 1 kg/m^2 (20 to 27). Subjects were not taking medications. The study was approved by the Institutional Review Board of The University of Texas Medical Branch in Galveston.

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Experimental Design

Each subject was studied during two 3-hour periods. During one period, the endothelium-independent vasodilator sodium nitroprusside (SNP) was infused into the femoral artery of one leg to increase blood flow to that leg. During this infusion, femoral arterial and venous blood samples were obtained for measurement of the arteriovenous (A-V) glucose and lactate differences. In addition, leg blood flow was determined by indocyanine green dye (ICG) infusion. During the other 3-hour period, the same measurements were made under conditions of resting blood flow (no SNP infusion).

Procedure

Subjects reported to the General Clinical Research Center (GCRC) at The University of Texas Medical Branch in Galveston the evening prior to the study and were fasted from 10 PM until the completion of the study the next day. At approximately 7 AM, catheters were placed in the femoral artery (5F), femoral vein (5F), and a forearm vein (20-gauge polyethylene) under local anesthesia (lidocaine). After line placement, subjects rested quietly in bed for the remainder of the study. The study consisted of two 3-hour periods, the order of which was randomly assigned. During one period, SNP was infused into the femoral artery at a rate of 8 μg/min to increase leg blood flow. During the other period the subject was studied under conditions of resting leg blood flow. Heart rate and blood pressure were not affected by the local SNP infusion. Over the final 2 hours of each period, blood samples were taken from the femoral artery and the femoral vein for the determination of glucose and lactate concentrations using an automated glucose and lactate analyzer (YSI 2300 STAT, Yellow Springs, OH). In 2 subjects, these samples were taken approximately every 15 minutes during the second hour of the period, whereas in the other 5 subjects the samples were taken during the third hour of the period. There were no differences observed between the second-hour and third-hour sampling protocols. Leg blood flow during each period was measured as previously described.[10] Briefly, ICG dye (0.5 mg/mL) was infused into the femoral artery at a rate of 1 mL/min. Blood samples were taken from the femoral vein of the leg into which the dye was infused, to measure dye dilution, and from a forearm vein, to measure recirculation of dye.

Calculations and Statistics

Leg glucose uptake and lactate release were determined by multiplying the respective A-V differences by the blood flow. Data were statistically evaluated using a 2-tailed (nondirectional), paired *t* test (Microsoft Excel 97, Redmond, WA). A *P* value less than .05 was considered statistically significant. Data are presented as means ± SE.

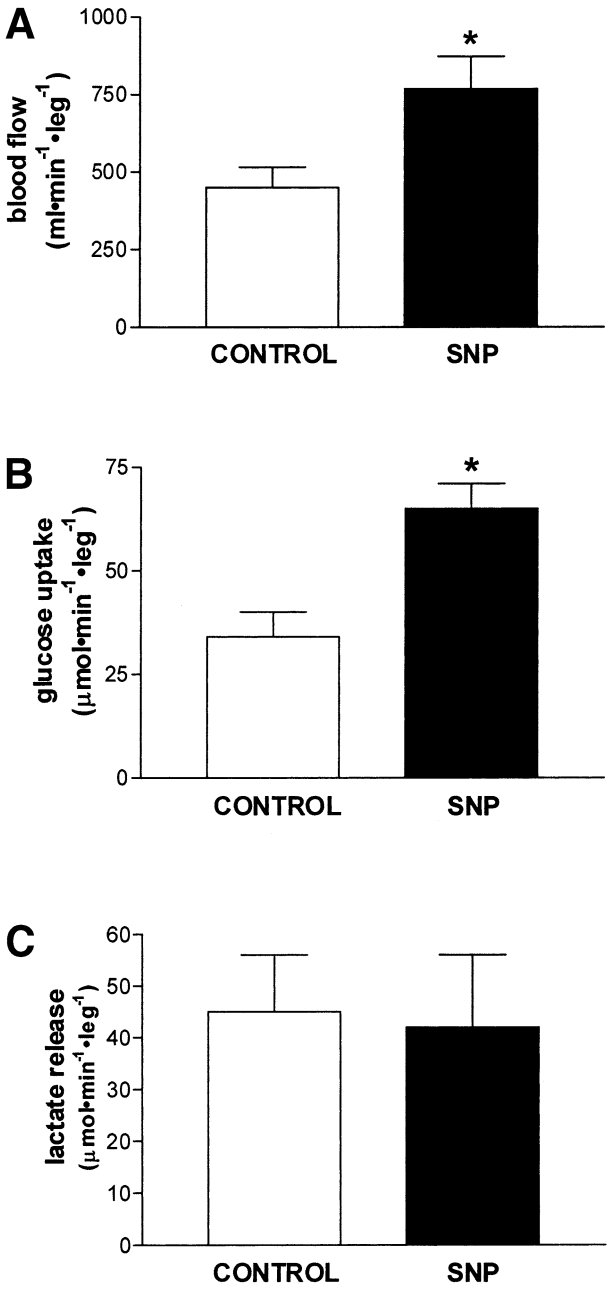

Fig 1. (A) Blood flow, (B) blood glucose uptake, and (C) leg lactate release with (SNP) and without (CONTROL) infusion of SNP. *Significantly different ($P < .005$) *v* control.

RESULTS

Blood flow was significantly increased by SNP infusion (450 ± 65 *v* 769 ± 103 mL · min^{-1} · leg^{-1}, Fig 1A). Arterial and venous glucose and lactate concentrations were not different in the 2 periods (Table 1). The A-V glucose and lactate differences were significantly differerent from 0 during both the control and SNP infusion periods (Table 1). Glucose uptake was significantly elevated in response to SNP infusion (34 ± 6 μmol · min^{-1} · leg^{-1} *v* 65 ± 6 μmol · min^{-1} leg^{-1}, Fig 1B), whereas lactate release was unaffected (rest, 45 ± 11 μmol · min^{-1} · leg^{-1}; SNP, 42 ± 14 μmol · min^{-1} · leg^{-1}; Fig 1C).

DISCUSSION

Despite intense interest in the role of blood flow and/or NO in insulin-stimulated glucose uptake,[6,11,17] their effects on basal glucose uptake in healthy humans in vivo have received little attention.[13,15,18] In the present study, we have shown that local administration of the NO donor SNP to the leg increases glucose uptake. Moreover, this effect occurs in the absence of exercise or insulin stimulation, the 2 main physiological stimulators of glucose uptake in vivo.

Limb blood flow can be increased by several mechanisms, each of which has implications for the response of limb metabolism to the change in flow. If the increase in flow represents an increase in flow velocity through a fixed number of capillaries, then the A-V difference of substrates taken up or released by the limb would be expected to decrease in response to the increased flow. On the other hand, if the increase in flow represents an increase in the number of perfused capillaries, several responses are possible, depending upon whether the balance between nutritive and non-nutritive flow routes is affected. Nutritive blood flow refers to flow through vessels in which exchange of substrates can occur, whereas non-nutritive flow refers to flow through blood vessels that do not allow exchange of substrates between tissues and the blood.[2] If increased blood flow is achieved predominantly through increased recruitment of nutritive vessels, the A-V difference should either not change or possibly increase. On the other hand, if the increase in flow is caused by recruitment of non-nutritive vessels, the A-V difference would be expected to decrease. As SNP infusion had no effect on the A-V difference in the present study, we contend that increased nutritive flow was at least partially responsible for the increase in total limb flow.

The majority of studies addressing the role of blood flow in muscle glucose metabolism have employed vasodilator infusions during an accompanying infusion of insulin. The results of these studies have varied, with some showing an effect of vasodilation to increase insulin-stimulated glucose uptake,[2,18] whereas others have failed to show this response.[11-17] It is worth noting that only 2 of the studies[15,17] failing to show an increase were conducted in normal, healthy subjects, with the rest conducted in overweight,[13] obese,[11] hypertensive,[13,14] or elderly[12] subjects, populations in which insulin sensitivity is

Table 1. Femoral Arterial and Venous Glucose and Lactate Concentrations With (SNP) and Without (control) Infusion of SNP

	Control	SNP
Arterial glucose (mmol/L)	4.68 ± 0.13	4.63 ± 0.09
Venous glucose (mmol/L)	4.60 ± 0.14	4.54 ± 0.10
A-V glucose (mmol/L)	0.08 ± 0.01*	0.09 ± 0.01*
Arterial lactate (mmol/L)	0.42 ± 0.03	0.46 ± 0.06
Venous lactate (mmol/L)	0.52 ± 0.04	0.53 ± 0.06
V-A lactate (mmol/L)	0.10 ± 0.02†	0.07 ± 0.02‡

*$P < .002$ *v* 0.
†$P < .01$ *v* 0.
‡$P < .05$ *v* 0.

often reduced. The approach of concomitant vasodilator and insulin infusions is reasonable, as insulin is the major physiological stimulator of glucose uptake and, without insulin infusion, an increase in blood flow might narrow the already small A-V glucose difference. However, as discussed above, such a narrowing should only be expected if the vasodilation causes a greater relative increase in flow than in uptake capacity. That is, if the increase in flow represents an increase in flow velocity through a fixed number of capillaries, then a narrowing of the A-V glucose difference would be expected. Alternatively, if vasodilation primarily represents a recruitment of previously underperfused capillaries, as has been previously shown for both exercise- and insulin-induced vasodilation,[3,4] the A-V difference may not change appreciably.

The present finding that the A-V glucose difference did not change does not agree with previous studies showing a narrowing of the A-V difference with increases in perfusion.[2,11,15] However, those studies differ from the present one in that vasodilator infusion was superimposed on an existing insulin infusion. Thus, if insulin had already caused some capillary recruitment, further increases in blood flow may have reflected increased capillary recruitment, increased flow velocity through previously perfused capillaries, and/or increased non-nutritive blood flow.[2,17]

Two previous studies have evaluated the effects of blood flow on basal glucose uptake in healthy individuals. In one study,[15] the effects of bradykinin infusion on basal glucose uptake were investigated. In contrast to the present study, bradykinin infusion increased blood flow but reduced the A-V glucose difference, with the result that glucose uptake was not changed. Bradykinin, like NO, may have direct effects on skeletal muscle glucose uptake[19] and has been suggested as a regulator of exercise-induced glucose uptake.[19,20] Thus, the inability of bradykinin infusion to increase glucose uptake in the study of Nuutila et al[15] suggests that bradykinin infusion alone increases non-nutritive blood flow, making any direct effects of bradykinin inconsequential. In a second study, low-dose insulin-like growth factor (IGF)-1 infusion significantly increased forearm blood flow but had no effect on forearm glucose uptake.[16] As for bradykinin, one possibility is that low-dose IGF-1 infusion increases flow through non-nutritive blood vessels.

Insulin-induced vasodilation is NO-dependent.[6] As a result, endothelium-dependent (eg, methacholine chloride) or -independent (eg, SNP) vasodilators that increase NO levels have been administered along with insulin in previous studies addressing the role of blood flow in insulin-stimulated muscle glucose uptake.[2,11,14,17,21] Although used to modulate blood flow in those studies, it is important to note that NO has been reported to affect whole body energy expenditure[22] and may play an important role in muscle glucose uptake apart from its vasodilatory actions. NO release is detectable from incubated skeletal muscle preparations and increases with contraction.[23] Inhibition of NO production in these preparations reduces basal and contraction-induced glucose uptake.[8,23,24] Inhibition of NO production also reduces glucose uptake, without affecting blood flow, during contractions in vivo.[9] In addition, mice in which the genes for endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) have been knocked out are insulin-insensitive.[7] However, the role of NO in insulin-stimulated glucose uptake is still unclear, as NO donors have been reported to reduce insulin-stimulated glucose uptake.[25] Thus, our results may also be explained by an increase in muscle NO levels during SNP infusion. Indeed, our results are consistent with recent reports suggesting that NO increases in vitro glucose uptake in rat skeletal muscle by a mechanism distinct from the insulin and exercise pathways.[26,27]

Several previous studies have investigated the possibility that vasodilation and/or NO provision might increase glucose uptake in insulin-resistant states. In most[11-14,16] of these studies, vasodilator infusion did not affect insulin-stimulated glucose uptake. However, in 2 studies in which glucose uptake was studied without coinfusion of insulin,[13,28] infusion of the vasodilators adenosine[13] and methacholine chloride[28] significantly increased both blood flow and basal glucose uptake. In the latter study,[28] infusion of methacholine chloride more than doubled forearm glucose uptake in hypertensive patients. In the same study, the endothelium-independent vasodilator SNP had no effect, consistent with a previous study of hypertensive patients in which SNP did not improve insulin-stimulated glucose uptake.[14] Thus, at least in hypertensive patients, it appears that endothelial NO (produced in response to methacholine chloride) effectively increases glucose uptake whereas non-endothelial NO (from SNP) does not. Taken together with the results of the present study, it appears that in some insulin-resistant states muscle does not respond normally to exogenous NO and/or increases in blood flow.

In the present study, lactate release across the leg was not affected by SNP/increased blood flow. This result is consistent with a previous study in which post-exercise leg lactate release was not affected by light exercise during the recovery period.[29] We are not aware of any previous studies investigating the response of basal leg lactate release to NO. However, SNP has been found to increase both lactate release and glucose oxidation in incubated rat soleus muscle, without affecting glycogen synthesis.[30] It is difficult to compare the in vivo human results with the in vitro results from the rat; however, one tentative possibility is that SNP-induced glucose uptake does not stimulate glycolysis to the same extent in humans as in rats.

In conclusion, we have shown for the first time in humans that exogenous NO increases leg glucose uptake in the absence of exercise or insulin stimulation. The relative importances of NO-induced capillary and glucose transporter recruitment in this response deserve further investigation.

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