Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent

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Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E692–E699, 1998.—The purpose of this study was to investigate whether in vivo nitric oxide synthase (NOS) inhibition influences insulin-mediated glucose disposal in rat peripheral tissues. The NOS inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (\textit{L-NAME}) or saline was infused constantly during a hyperinsulinemic-euglycemic clamp in normal rats. Glucose utilization rates of insulin-sensitive tissues (individual muscles, heart, and adipose tissues) were simultaneously determined using tracer infusion of 2-deoxy-D-[\textsuperscript{3}H]glucose (2-[\textsuperscript{3}H]DG). NOS blockade with \textit{L-NAME} resulted in significant (P < 0.05) reduction in both whole body glucose disposal (−16%, P < 0.01) and plasma 2-[\textsuperscript{3}H]DG disappearance rate (−30%, P < 0.05) during hyperinsulinemic-euglycemic clamp. \textit{L-NAME} significantly decreased insulin-stimulated glucose uptake in heart (−62%, P = 0.01), soleus (−42%, P = 0.05), red (−53%, P < 0.001) and white (−62%, P < 0.001) gastrocnemius, tibialis (−57%, P < 0.01), and quadriceps (−33%, P < 0.05) muscles. The NOS inhibitor also decreased insulin action in brown interscapular (−47%, P < 0.01), retroperitoneal (−52%, P = 0.07), and gonadal (−66%, P = 0.06) adipose tissues. In contrast to in vivo NOS blockade, \textit{L-NAME} failed to affect basal or insulin-stimulated 2-[\textsuperscript{3}H]DG transport in isolated soleus or extensor digitorum longus muscles in vitro. These results support the hypothesis that the action of insulin to augment glucose uptake by skeletal muscles and other peripheral insulin-sensitive tissues in vivo is NO dependent.

endothelial-type nitric oxide synthase; neuronal-type nitric oxide synthase; endothelium; vascular beds; soleus; extensor digitorum longus; nitric oxide

Insulin markedly stimulates glucose utilization in skeletal muscles, heart, and adipose tissues. The hormone increases glucose transport in these tissues mainly by activating the translocation of GLUT-4 glucose transporters from an occluded intracellular tubulovesicular reservoir to the cell surface (15, 18). Moreover, it has been proposed that insulin also enhances skeletal muscle glucose disposal by vasodilating the muscle vasculature. Indeed, insulin has been shown to increase skeletal muscle blood flow in both humans and rats (3, 26). More recent studies further showed that insulin dilates arterioles isolated from skeletal muscle (9). The increased muscle perfusion is thought to represent a physiological mechanism that amplifies insulin's overall action to promote the disposal of glucose and other substrates (3, 4).

Previous studies have shown that the enhancing effect of insulin on the muscle vasculature is mediated by the release of nitric oxide (NO) by the endothelium. Indeed, abrogation of NO release by the NO synthase (NOS) inhibitor \textit{N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA)} prevented the action of insulin to increase blood flow to skeletal muscle (30, 32). Moreover, removal of the endothelium or NOS inhibition prevents the vasodilating action of insulin on skeletal muscle arterioles (9). However, the effect of NOS inhibition on insulin-mediated glucose uptake in skeletal muscle is less clear. Baron et al. (5, 6) reported that ~25–40% of insulin's ability to enhance leg glucose uptake could be accounted by an increase in muscle perfusion. In contrast, others found that the ability of insulin and insulin-like growth factor I (IGF-I) to stimulate whole body glucose disposal was not dependent on the effects of these hormones to increase blood flow (10, 30). Therefore the question of whether the hemodynamic effect of insulin and IGF-I contributes to their overall action to stimulate glucose uptake in skeletal muscle and other insulin-sensitive peripheral tissues (heart and adipose tissues) is still under debate. Importantly, there are no studies in which the effects of in vivo NOS blockade on insulin-stimulated glucose uptake was investigated at the tissue level. Skeletal muscles are composed of a mixture of individual myofibers that differ in their functional properties and cellular responses to mechanical, hormonal, and neural factors as well as in their vascularization (25). It is known that muscles composed primarily of oxidative fibers (red) have greater rates of blood flow and a higher capacity for glucose transport and show a higher sensitivity to insulin than muscles mainly composed of glycolytic fibers (white) (12, 14, 17, 22). It is therefore important to study the effects of NOS inhibition on insulin-mediated glucose disposal in individual muscle types.

Thus the principal objective of the present study was to investigate the effects of in vivo NOS inhibition on insulin-stimulated glucose uptake in individual skeletal muscles and various adipose tissues. Our results support the hypothesis that insulin-stimulated glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent.

MATERIALS AND METHODS

Animals and surgery. This study was approved by the Animal Care and Handling Committee of Laval University. Male Sprague-Dawley rats (200–250 g) purchased from Charles River (Montreal, PQ, Canada) were used in these studies. Rats were randomly assigned to \textit{N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME)} or control groups and housed in individual cages, maintained on a 12:12-h dark-light schedule, and fed ad libitum with Purina rat chow. Three days before the experiment, animals were anesthetized with a ketamine-xylazine solution (80 and 10 mg/kg, i.p., respectively) for catheterization of the carotid artery and the jugular vein. The right carotid artery was isolated, a PE-50 (polyethylene
tubing, 0.40 mm ID, 0.80 mm OD) catheter was inserted 2.5 cm down the vessel, and the cephalic end of the artery was ligated. Thereafter, the left jugular vein was isolated, and three PE-10 catheters (0.28 mm ID, 0.61 mm OD) were inserted 3 cm into the vessel and sutured in situ. All the catheters were passed subcutaneously to exit dorsally at the midscapular region and were covered with a stainless steel extension spring. The spring was secured to the rat by a rigid polyethylene neck (90° angle) that was sutured subcutaneously at the exit incision. The spring and catheters were then suspended from a contort holder directly above the cage, ensuring the rat 360° of free and unrestricted motion. Heparinized saline (50 IU/ml) was infused into the catheters to prevent coagulation.

Experimental protocol. Three days after cannulation, the overnight fasted rats were weighed and transferred to a quiet isolated room. The extension spring and catheters were suspended directly above the cage (providing free and unrestricted movement), and the ends of the jugular catheters were attached to a syringe pump (Razd, CT) with a 5-ml syringe for glucose, insulin, and L-NAME infusion. Heparinized saline was taken out of each catheter and replaced by fresh saline before the start of the experiment. The carotid artery catheter was occluded with a 23-gauge needle and sterile tuberculin syringe. Once installed, the rat was allowed 60 min to acclimate to its new surroundings. Once acclimatized, a first blood sample was collected via the carotid artery catheter for basal levels of glucose, lactate, and insulin, and blood glucose was monitored at 5-min intervals using a One Touch II Hospital glucometer (Lifescan; Burnaby, BC, Canada) to prevent hypoglycemia. Glucose (50% wt/vol) infusion was normally started 5–10 min after the start of the insulin infusion according to blood glucose levels, and the rate of glucose infusion was then adjusted to maintain normoglycemia throughout the study. A 300-µl sample of blood was collected at 20-min intervals to measure blood glucose levels, plasma glucose and radiolabeled 2-DG and sucrose (see Fig. 1). Twenty-five minutes after the bolus, the animals were rapidly killed by decapitation, and hindlimb muscles (soleus, tibialis, gastrocnemius, and quadriceps), heart, and adipose tissues were quickly excised, cleaned of extraneous tissues, and immediately frozen into liquid nitrogen and stored at −80°C.

Subsequently, tissue samples (30–50 mg) were dissolved in 1 ml of 0.5 M ammonium hydroxide (Solvable) at 55°C for 16–18 h. Thereafter, hydrogen peroxide (30% solution) was added for 60 min at 55°C to decrease quenching, followed by the addition of 10 ml of scintillation fluid (BCS; Amersham, Mississauga, ON, Canada). The samples were then passed as plasma (20 µl) were then counted in a liquid scintillation counter (Wallach 1409). The accumulation of 2-[3H]DG in muscle, corrected for the extracellular space with [14C]sucrose, was used as an index of glucose uptake rates as described by others (31, 34).

Glucose transport activity in isolated skeletal muscles. Glucose transport in isolated muscles was measured by use of the glucose analog 2-[3H]DG as described previously (11). Male Sprague-Dawley rats (200–250 g) were anesthetized by injection of ketamine-xylazine (90 and 10 mg/kg ip, respectively), and soleus and extensor digitorum longus (EDL) muscles were dissected out and rapidly cut into 20- to 30-mg strips. Animals were then killed by intracardiac injection of ketamine-xylazine. Muscle strips were incubated in a shaking water bath at 30°C for 30 min into a 25-ml flask containing 3.0 ml of oxygenated Krebs-Ringer bicarbonate (KRB) buffer supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA [radioimmunossay (RIA) grade]. Flasks were gassed continuously with 95% O2-5% CO2 throughout the experiment. The muscles were then incubated with or without various concentrations (0.1 µM-2 mM) of L-NAME for 15 min at 30°C in 3 ml of oxygenated KRB buffer. After the initial incubation, muscles were incubated for 30 min in oxygenated KRB buffer in the presence or absence of purified insulin (12 nM). At this concentration, insulin activates glucose transport maximally in both soleus and EDL muscles (data not shown). Muscles were then incubated for 10 min at 29°C in 3 ml of KRB buffer containing 40 mM mannitol and 0.1% BSA. Muscles were then incubated for 20 min at 29°C in 3 ml of KRB buffer containing 8 mM 2-[3H]DG (2.25 µCi/ml), 32 mM [14C]mannitol (0.3 µCi/ml), 2 mM sodium pyruvate, and 0.1% BSA. Insulin and L-NAME were present throughout the wash and uptake incubations (if they were present in previous incubation medium). After the incubation, muscles were rapidly blotted at 4°C, clamp frozen, and stored at −80°C until processed. Muscles were processed by boiling for 10 min in 1 ml of water. Extracts were transferred to an ice bath, vortexed, and then centrifuged at 1,000 g. Triplicate 200-µl aliquots of the muscle extract supernatant and the incubation medium were counted for radioactivity using a Wallach 1409 counter. 2-[3H]DG uptake rates were corrected for extracellular trapping using [14C]mannitol (11).

Tissue fractionation and NOS assay. Overnight fasted Sprague-Dawley rats (200–250 g) were anesthetized by injecting ketamine-xylazine (90 and 10 mg/kg ip, respectively), and hindlimb muscles (soleus, EDL, gastrocnemius, quadriceps femoris, tibialis anterior, and vastus lateralis) were dissected out. Tissues were cleaned of extraneous tissues and homogenized in 20 ml of homogenization buffer containing 25 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 100 µg/ml of phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 20,000 g for 15 min at 4°C. The pellet (total crude membranes) was...
resuspended in half the original volume of homogenization buffer. Protein concentrations of the membrane fractions were determined by the biocinchoninic acid method using BSA as the standard.

NOS activity was quantified by the conversion of \(^{[3]H}\)arginine to \(^{[3]H}\)citrulline as recently described (20), with minor modifications. Aliquots of membrane proteins (100–200 µg) were incubated in 50 mM HEPES (pH 7.4) with 100 nM \(^{[3]H}\)arginine (50 Ci/mmol), 120 µM NADPH, 60 mM L-valine, 12 mM \(^{[3]H}\)citrulline, 1.2 mM MgCl\(_2\), 0.2 mM CaCl\(_2\), 10 µg/ml calmodulin, 3 µM BH\(_4\), 1 µM FAD, and 1 µM FMN. The reaction was carried out for 1 h at 37°C without or with increasing doses (0.1 µM to 2 mM) of the NOS inhibitors L-NAME or N\(^{6}\)-monomethyl-L-arginine (L-NMMA) and terminated by adding 2 ml of 20 mM HEPES (pH 5.5) containing 2 mM EDTA. Samples were applied to 1-ml columns of Dowex AG50W-X8 (Na\(^{+}\) form), which were eluted with 2 ml of water. \(^{[3]H}\)citrulline was quantified by liquid scintillation spectroscopy of 4.0 ml flow through.

Plasma glucose, lactate, insulin, and L-NAME levels. Plasma glucose and lactate levels were measured using a YSI type 2300 Stat Plus automatic glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Basal plasma insulin levels were determined by RIA with a rat insulin-specific RIA kit from Incstar (St. Charles, MO) using rat insulin as standard. Clamped plasma insulin levels were determined using a human insulin-specific RIA kit from the same company.

Plasma L-NAME concentrations were determined by ion-exchange high-performance liquid chromatography (HPLC; Ultrapac 8 column, ID 4.0 mm, OD ¼ in., length 272 mm) with ninhydrin as the colorimetric reagent (LKB 4400 amino acid analyzer; LKB Biochrom, Cambridge, UK). Because L-NAME is rapidly converted to N\(^{6}\)-nitro-L-arginine at 37°C, the latter molecule was measured as an index of L-NAME plasma concentrations (35).

Statistical analysis. Values are means ± SE. The effect of L-NAME on all parameters measured in this study was compared by Student’s t-test for unpaired comparisons. The level of significance was P < 0.05.

RESULTS

Effect of NOS blockade on in vivo insulin-mediated glucose disposal. Table 1 and Fig. 1 show physiological data obtained from control and L-NAME-treated animals during the hyperinsulinemic-euglycemic clamp. Body weights and basal or preclamp plasma glucose, insulin, and lactate levels were not significantly different between the two groups. Insulin infusion (postclamp) significantly increased plasma insulin levels by ~50-fold while plasma glucose was kept at preclamp concentrations in both groups of animals (Fig. 1). Insulin infusion also significantly increased plasma lactate levels by 2.5- and 2.1-fold in control and L-NAME-treated animals, respectively (P < 0.05 in both groups; Table 1). Plasma L-NAME levels in treated animals (n = 2) were ~0.02 mM at the end of the clamp period.

Insulin-mediated glucose disposal rate was significantly decreased (~16%, P < 0.01) by L-NAME treatment, indicating that whole body insulin action was impaired during NOS inhibition (Table 1, Fig. 1). Plasma 2-[\(^{3}H\)]DG disappearance rate was also found to be decreased by L-NAME infusion (~30%, P < 0.05). The effect of L-NAME treatment on insulin-stimulated glucose uptake in individual tissues is shown in Figs. 2 and 3. L-NAME infusion during the clamp significantly decreased insulin-stimulated glucose uptake in soleus (~42%, P = 0.05), quadriceps (~33%, P < 0.05), tibialis (~57%, P < 0.01), and red (~53%, P < 0.001) and white (~62%, P < 0.001) gastrocnemius muscles. As shown in Fig. 3, the NOS inhibitor also

<table>
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<tr>
<th>Table 1. Physiological parameters of saline- and L-NAME-treated rats</th>
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<tr>
<td>Treatment</td>
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<tr>
<td>Body wt, g</td>
</tr>
<tr>
<td>Preclamp</td>
</tr>
<tr>
<td>Glucose, mM</td>
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<tr>
<td>Insulin, nM</td>
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<tr>
<td>Lactate, mM</td>
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<tr>
<td>Postclamp</td>
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<td>Glucose, mM</td>
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<tr>
<td>Insulin, nM</td>
</tr>
<tr>
<td>Lactate, mM</td>
</tr>
<tr>
<td>GDR, g·kg(^{-1})·min(^{-1})</td>
</tr>
<tr>
<td>K(_p), min(^{-1})</td>
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</table>

Values are means ± SE from 7 animals in each experiment group. GDR, glucose disposal rate; K\(_p\), plasma 2-deoxy-[\(^{3}H\)]glucose disappearance rate. * Significant difference between preclamp and postclamp treatment, P < 0.05. Significant difference between saline and N\(^{6}\)-nitro-L-arginine methyl ester (L-NAME) groups: †P < 0.001; ‡P < 0.05.
significantly decreased insulin-stimulated glucose uptake in cardiac muscle (262%, \( P < 0.01 \)). We also examined whether L-NAME treatment could influence adipose tissue glucose uptake rates. The NOS inhibitor reduced insulin-stimulated glucose uptake in interscapular brown adipose tissue (247%, \( P < 0.01 \)) as well as in retroperitoneal (252%, \( P < 0.01 \)) and gonadal (266%, \( P < 0.01 \)) white adipose tissues. It should be noted that glucose uptake values were much greater in brown adipose tissue than white adipose tissues in both saline- and L-NAME-treated rats (cf. y-axes in Fig. 3). It has previously been shown that brown adipose tissue is markedly responsive to insulin compared with white fat and skeletal muscles (34).

Effect of NOS blockade on in vitro insulin-mediated glucose transport. We and others have shown that skeletal muscle cells express both the neuronal type (nNOS) and endothelial type (eNOS) enzyme isoforms (16, 19, 20, 24). NOS activity is particularly abundant in type IIb-enriched muscles but is also detectable in type I and IIa muscle fibers. It is therefore possible that the in vivo effects of L-NAME on glucose uptake may involve a direct action of the inhibitor on myofiber NOS enzymes. To rule out this possibility, we measured the effect of L-NAME on insulin-stimulated 2-DG transport in both isolated red (soleus) and white (EDL) muscles (Table 2, Fig. 4). This allowed us to study the effects of L-NAME in the absence of any change in muscle perfusion (despite the presence of intact capillaries), which is not limiting under these in vitro conditions. Another well-known inhibitor of NOS, L-NMMA, was also used in these in vitro studies. Both inhibitors were used up to a dose of 2 mM to ensure a maximal inhibition of muscle NOS enzymes, as determined from dose-response curves for the effects of L-NAME and L-NMMA on mixed hindlimb muscle NOS activity (Fig. 5). This was confirmed by the observation that at 2 mM both inhibitors completely abolished basal NO

Table 2. Effect of NOS inhibitors on insulin-stimulated 2-DG transport in soleus and EDL muscles

<table>
<thead>
<tr>
<th>Drug, M</th>
<th>Soleus</th>
<th>EDL</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>l-NAME</td>
<td>l-NMMA</td>
</tr>
<tr>
<td>0</td>
<td>4.12±0.68</td>
<td>3.69±0.44</td>
</tr>
<tr>
<td>10(^{-7})</td>
<td>3.50±0.41</td>
<td>3.12±0.32</td>
</tr>
<tr>
<td>10(^{-6})</td>
<td>3.91±0.56</td>
<td>3.48±0.36</td>
</tr>
<tr>
<td>10(^{-5})</td>
<td>3.70±0.31</td>
<td>3.51±0.48</td>
</tr>
<tr>
<td>10(^{-4})</td>
<td>3.17±0.68</td>
<td>3.06±0.30</td>
</tr>
<tr>
<td>2.0×10(^{-3})</td>
<td>3.71±0.65</td>
<td>3.81±0.61</td>
</tr>
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</table>

Values represent means ± SE of 4–5 individual experiments. NOS, nitric oxide synthase; 2-DG, 2-deoxy-D-glucose; EDL, extensor digitorum longus. No significant effects of L-NAME or N\(^\text{G}\)-monomethyl-L-arginine (l-NMMA) were observed in either muscle type.
hemodynamic effects. Second, we have measured plasma transport in either muscle (Fig. 4, Table 2). Because L-NMMA could produce NO under our experimental conditions. We could only detect a very small production of NO₂⁻ and NO₃⁻ (<0.4 µM) at the highest concentration (2 mM) of L-NAME (but not L-NMMA) used in vitro. Similar production of NO products using the NO donor drug sodium nitroprusside (20 µM) failed to affect glucose transport in EDL muscle (data not shown), strongly suggesting that the release of NO by L-NAME had no impact in this study.

**DISCUSSION**

The present study shows that in vivo NOS inhibition significantly reduces whole body insulin action on glucose disposal (−16%) and 2-[³H]DG disappearance rate (−30%) in conscious unrestrained rats. Although we have not directly assessed NOS activity in the muscle vasculature during in vivo L-NAME treatment, several observations strongly suggest that we have mostly, if not completely, blocked muscle NOS activity under our experimental conditions. First, our protocol of L-NAME infusion was based on that of the pioneering work of Rees et al. (27), who clearly showed, using rats of similar age, that the same dose of L-NAME exerted hemodynamic effects. Second, we have measured plasma L-NAME concentrations by HPLC and found them to be ~20 µM. Rees et al. (27) showed that the acetylcholine-induced relaxation of rat aortic rings is fully inhibited by L-NAME at a concentration of 3–10 µM. Finally, we found that NOS activity in muscle was largely inhibited by L-NAME concentrations (0.01–0.1 mM; see Fig. 5) similar to those found in the plasma of the animals after infusion of the NOS inhibitor.

Our finding that NOS blockade decreased whole body glucose uptake in rats is in good agreement with previous studies performed in humans in which NOS blockade resulted in an ~25–40% reduction in insulin-mediated leg glucose disposal (5,6). In addition, the coupling of the 2-[³H]DG technique with the hyperinsulinemic-euglycemic clamp allowed us to assess the effects of NOS blockade on individual tissue glucose uptake rates. Measurements of 2-DG uptake rates in several skeletal muscles confirmed that NOS blockade reduces insulin-stimulated glucose uptake in skeletal muscle. In contrast, Scherrer et al. (30) failed to observe any significant effects of forearm intra-arterial L-NMMA infusion on whole body insulin-mediated glucose disposal. Because in the latter study NOS inhibition totally abrogated the effect of insulin to increase blood flow in the forearm, it was suggested that the vascular action of the hormone may not play a significant role in skeletal muscle glucose metabolism. However, the lack of effect of L-NMMA on insulin-mediated glucose uptake in that study may have been related to the fact that the inhibitor was infused in a forearm (which represents <10% of total skeletal muscle mass), whereas glucose disposal was measured in the whole body. Thus the possible inhibition of forearm glucose uptake by L-NMMA may have been quantitatively too small to have a detectable impact on whole body glucose disposal during insulin infusion. Accordingly, we found that L-NAME caused smaller inhibition of whole body insulin-mediated glucose disposal and 2-[³H]DG disappearance rate (−15 to 30%) compared with its marked effects on individual tissue glucose uptake rates (from 30 to 60% inhibition). Baron et al. (5) also reported greater inhibition of leg glucose uptake than whole body glucose disposal rates in humans infused with insulin and a superimposed infrarenal artery infusion of L-NMMA. It is also possible that NOS blockade may not affect or even increase non-insulin-mediated glucose disposal by organs other than skeletal muscles and adipose tissues, resulting in a smaller absolute decrease of total glucose disposal during NOS blockade. This could also provide an explanation for the previous report that oral administration of L-NAME for 2 wk failed to significantly change insulin sensitivity, grossly assessed by glucose tolerance tests, despite an increase in blood pressure (33). Moreover, it was not clear from the latter study whether L-NAME treatment affected muscle vascular beds and not just the renal beds, causing systemic hypertension.

An important goal of the present study was to examine whether NOS inhibition could affect insulin-stimulated glucose uptake differently in skeletal muscles with varying fiber type compositions. Indeed, it was reasoned that skeletal muscles with a more abundant vascularization (i.e., oxidative myofibers) may be affected more by NOS blockade. Surprisingly,
we found that the inhibition of glucose uptake by L-NAME treatment was not greater in muscles containing a significant proportion of type I oxidative fibers (soleus, red gastrocnemius, cardiac) than muscles enriched in less vascularized type IIb fibers (white gastrocnemius). Moreover, no relationship was found between the contractile (fast twitch vs. slow twitch) or metabolic (oxidative vs. glycolytic) properties of the muscles and their sensitivity toward NOS inhibition. This suggests that the insulin-mediated actions of NO to vasodilate the muscle vasculature and to promote glucose uptake are not dependent on the extent of vascularization or the fiber type composition of the muscle. In this regard, it will be important to determine in future studies whether the extent of vascularization of a given muscle is actually related to its capacity for insulin-mediated NO production and glucose uptake. NO release from the muscle vasculature cannot be measured in isolated muscles in vitro, since both eNOS and nNOS activities are also present within the myocytes (16, 19, 20). Vascular NO production by individual muscles can only be assessed by measuring NO release from both arteries (9) and capillaries (23) of different muscle types and by adjusting this production for the total amount of vascular cells per muscle.

Another finding of the present study is that NOS blockade reduced insulin-mediated glucose uptake in both brown and white adipose tissues. Thus these results strongly suggest that insulin-mediated glucose disposal in adipose tissues is also dependent on vascular NO release. NOS activity is low but detectable in white adipose tissue; in two independent experiments, we measured an activity of 0.14 ± 0.04 pmol·mg⁻¹·min⁻¹ (mean ± SD) in retroperitoneal adipose tissue. This is comparable to NOS activity measured in soleus (0.22 ± 0.02 pmol·mg⁻¹·min⁻¹) but about eight times less than that found in EDL muscle (1.14 ± 0.10 pmol·mg⁻¹·min⁻¹). This is in agreement with the expression of eNOS and the inducible NOS (iNOS) isoform in white adipose tissue (28). Compared with skeletal muscles, adipose tissues contribute to a minor fraction of total glucose disposal after a meal or during insulin stimulation (13). However, in obesity, the increased adipose mass may play a more significant quantitative role in whole body glucose disposal, and possible changes in vascular hemodynamics may be more important in that condition.

An important point to consider when evaluating the in vivo effects of NOS inhibition on skeletal muscle metabolism is that NOS enzymes are also expressed in the myofibers and not only in the vascular endothelial cells. Indeed, we and others (16, 19, 20) have shown that skeletal muscle cells express nNOS and eNOS isoforms. Although nNOS is confined to fast-twitch glycolytic fibers, eNOS appears to be present in more oxidative muscle cells (16, 19, 20). Thus it may be argued that some of the in vivo effects of L-NAME on glucose uptake by skeletal muscles may be partly explained by inhibition of myofiber NOS enzymes. However, we found that two NOS inhibitors, even at doses which totally inhibit muscle NOS activity (2 mM; Fig. 5) and exceeded L-NAME concentrations measured in the plasma of the rats used in the present study, did not reduce insulin-stimulated glucose transport in isolated soleus or EDL muscles. This is in accordance with recent studies in which L-NMMA was reported not to inhibit insulin-mediated glycogen synthesis in soleus (7) and insulin-stimulated glucose uptake in EDL muscle (2). However, the lack of effect of L-NAME and L-NMMA on basal glucose transport in EDL muscle is in contrast with previous studies in which NOS blockade by L-NAME was reported to reduce basal glucose uptake in this muscle type (1, 2). In this study, we have used muscle strips from 200- to 250-g rats, whereas Balon and Nadler (1, 2) used intact muscles mounted on clips from smaller rats. However, we have also used mounted muscles from smaller rats (50–60 g) in other experiments and failed to detect a reduction in basal glucose uptake with either L-NAME (16) or L-NMMA (unpublished data). Another appreciable methodological difference is that the latter group used animals in which the feeding ad libitum before muscle isolation in their study. More studies are needed to test whether muscle NOS activity is modulated by feeding and fasting. In any case, our results indicate that the inhibitory effects of L-NAME on insulin-stimulated glucose uptake by skeletal muscles (and presumably adipose tissues) are not attributable to a direct action of the inhibitor on muscle NOS enzymes. Rather, our data strongly suggest that the observed reductions of insulin-mediated muscle glucose uptake by L-NAME treatment were caused by the inhibition of insulin’s ability to promote vascular NO release. Although we have not measured the hemodynamic effect of insulin in the present study, others have shown that the hormone causes vasodilation of muscle vascul bed both in rats and humans (3, 6, 26) and that this effect is dependent on NO release by the endothelium (9, 30, 32).

In contrast with the lack of effect of NOS inhibition on insulin-stimulated glucose uptake, increasing NO production has recently been shown to affect glucose transport in isolated muscles and muscle cells. Thus NO donors, such as sodium nitroprusside and spermine nonoate, can increase glucose transport in isolated EDL and soleus muscles (2, 36). Furthermore, we recently found that inducing the expression of the macrophage-type iNOS in skeletal muscle cells by cytokines and endotoxin increases basal glucose uptake (8). In the same cells, the ability of insulin to stimulate glucose transport was impaired, suggesting that NO overproduction may be involved in cytokine-induced insulin resistance in muscle. Thus NO appears to modulate skeletal muscle glucose disposal by two distinct pathways: 1) it promotes in vivo glucose uptake by increasing muscle perfusion during insulin stimulation, and 2) it increases basal glucose transport at the cellular level but can inhibit insulin-stimulated glucose uptake when its production is markedly increased in the muscle cells (e.g., during cytokines and endotoxin challenge).

In summary, the present study shows that NOS blockade in vivo significantly reduces whole body insu-
lin-mediated glucose disposal and insulin-stimulated glucose uptake rates in individual skeletal muscles. The inhibitory effects of NOS blockade on muscle glucose utilization were not related to the vascular, contracile, or metabolic properties of individual fibers. The NOS inhibitor also decreased insulin-mediated glucose uptake in brown and white adipose tissues. However, NOS inhibition in vitro had no effect on insulin-stimulated glucose transport. These results strongly suggest that the stimulatory effect of insulin on peripheral tissue glucose utilization in vivo is NO dependent.

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