# UNIVERSITE DE LAUSANNE - FACULTE DE MEDECINE

## SERVICE DE MEDECINE INTERNE B

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# EFFECTS OF SYMPATHECTOMY AND NITRIC OXIDE INHIBITION ON VASCULAR ACTIONS OF INSULIN IN HUMANS

## THESE

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# Résumé

*Introduction:* L'administration aiguë d'insuline induit des effets cardio-vasculaires par la libération de monoxide d'azote (NO) et la stimulation de l'activité nerveuse sympathique. Cependant, il n'est pas encore établi si la stimulation du flux vasculaire musculaire (et la libération de NO) est le résultat d'une action directe de l'insuline au niveau des vaisseaux sanguins et/ou de la stimulation de mécanismes nerveux vasodilatateurs.

*Méthode* Nous avons étudié 9 patients ayant subi une sympathectomie thoracique pour une hyperhydrose, afin d'examiner les effets vasculaires de l'insuline en présence ou en absence de l'innervation sympathique vasoconstrictrice et vasodilatatrice.

*Résultats:* Pendant un clamp euglycémique de 2 heures, l'administration d'insuline (6 pmol/kg/min) induit une augmentation du flux musculaire d'environ le 40% tant dans le membre innervé que dans le membre dénervé (p<0.01 vs valeur basale pour les deux membres). Cette vasodilatation atteint son maximum dans les 30 à 45 premières minutes du clamp dans le membre dépourvu d'innervation sympathique(bras). Par contre, la vasodilatation maximale dans le membre normalement innervé (jambe) est seulement atteinte 2 heures après le début du clamp (p<0.01, membre dénervé vs membre innervé).

L'administration parentérale d'un inhibiteur de la NO synthase (L-NMMA, 50 µg/kg/min), provoque une augmentation de la pression artérielle basale, abolit la vasodilatation induite par l'insuline dans le membre dénervé et induit une augmentation significative de la pression artérielle durant le clamp hyperinsulinémique euglycémique. Par contre, l'inhibition de la NO synthase ne modifie pas la sensibilité métabolique globale à l'insuline.

*Conclusions:* Ces données indiquent que, dans un membre dépourvu d'innervation sympathique, l'insuline provoque une vasodilatation par une action directe au niveau vasculaire. Cet effet est médié par la libération de monoxide d'azote et semblerait être masqué par le tonus sympathique vasoconstricteur dans un membre normalement innervé.

# Effects of Sympathectomy and Nitric Oxide Synthase Inhibition on Vascular Actions of Insulin in Humans

## Claudio Sartori, Lionel Trueb, Pascal Nicod, Urs Scherrer

*Abstract*—Insulin exerts cardiovascular actions by stimulating nitric oxide (NO) release and sympathetic neural outflow. It is unclear, however, whether insulin stimulates muscle blood flow (and NO release) by a direct action at the vasculature and/or by stimulating neural vasodilator mechanisms. In these studies we used patients with regional sympathectomy to examine the vascular actions of insulin in the presence and absence of sympathetic vasoconstrictor and vasodilator innervation. A 2-hour insulin (6 pmol/kg per minute)/glucose clamp increased muscle blood flow in both innervated and denervated limbs by roughly 40% ($P<0.01$ versus baseline for both limbs). The vasodilation reached its maximum within the first 30 to 45 minutes of insulin/glucose infusion in sympathetically denervated limbs, but only at the end of the infusion in innervated limbs ($P<0.01$, denervated versus innervated limb). Infusion of a NO synthase inhibitor ($N^G$-monomethyl-L-arginine [L-NMMA]) increased baseline arterial pressure, abolished the vasodilation in the denervated limb, and led to a significant additional increase in arterial pressure during the clamp, but did not alter whole body glucose uptake. Our data indicate that insulin stimulates blood flow in sympathectomized limbs by a direct action at the vasculature. This effect is mediated by stimulation of NO release and appears to be masked by the sympathetic vasoconstrictor tone in innervated limbs. (*Hypertension.* 1999;34:586-589.)

**Key Words:** nervous system, sympathetic ■ insulin ■ vasodilation ■ hyperinsulinism ■ glucose clamp technique ■ glucose ■ nitric oxide

There is now abundant evidence that insulin exerts cardiovascular actions that are mediated at least in part by the sympathetic nervous system and the L-arginine nitric oxide (NO) system.[1] In lean, healthy subjects, insulin infusion increases both sympathetic neural outflow and blood flow in skeletal muscle tissue.[2] The latter is caused by stimulation of NO synthesis since it is abolished by intra-arterial $N^G$-monomethyl-L-arginine (L-NMMA) infusion.[3,4] Despite much research in the field, it is not clear however, whether insulin stimulates muscle blood flow (and NO release) by a direct action at the vasculature and/or by stimulating neural vasodilator mechanisms.[1] Specifically, studies using local, intra-arterial insulin infusion to probe whether insulin has a direct vasodilator action have produced conflicting results.[1]

To examine the role played by neural versus local vasodilator mechanisms and their interplay with neural vasoconstrictor mechanisms, we compared, in lean subjects who had undergone regional sympathectomy for hyperhidrosis, the vasodilator responses to insulin infusion in the sympathetically denervated and innervated limb. To gain additional insight into underlying mechanisms, we studied effects of NO synthase inhibition on vascular responses to insulin infusion in the denervated limb.

## Methods

Nine lean, healthy subjects (5 men and 4 women; body mass index, $22.5\pm1.2$ kg/m²; mean$\pm$SE age, $34\pm4$ years) who had undergone regional thoracic sympathectomy for hyperhidrosis participated in this study after providing informed written consent. All subjects were normotensive, were taking no medication, and had no evidence of metabolic or cardiovascular disease at the time of the study. Tests were all conducted in the morning after an overnight fast. Subjects had been on a weight-maintaining diet containing at least 40% energy as carbohydrates for 3 days before the tests. The experimental protocol was approved by the Institutional Review Board on Human Investigation.

### General Procedures

Subjects were studied in the supine position. Heart rate (ECG), respiratory excursions (Pneumobelt), blood pressure (Finapres blood pressure monitor, Ohmeda),[5] and limb blood flow (venous occlusion plethysmography) were recorded continuously on an electrostatic recorder. Intravenous catheters were inserted in a right and left antecubital vein, one for substrate and drug infusion and the other for blood sampling.

### Measurement of Muscle Blood Flow

Blood flow in an ipsilateral forearm and calf was measured with venous occlusion plethysmography, with the use of mercury-insilastic strain gauges.[5] Both limbs were elevated 10 to 15 cm above the level of the right atrium to collapse the veins. The circulation to the hand and the foot was arrested by inflating a cuff around the wrist

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and the ankle to suprasystolic pressure during blood flow determinations.

## Experimental Protocols

### Protocol 1: Hyperinsulinemic Euglycemic Clamp Alone

After instrumentation and 1 hour of baseline measurements, the subjects received a primed continuous infusion of crystalline insulin (Actrapid HM, Novo Industri S/A) at a rate of 6 pmol/kg per minute (1 mU/kg per minute) for 2 hours. Euglycemia was maintained by determining plasma glucose concentration every 5 minutes and periodically adjusting a variable infusion of 20% dextrose.[6] Hypokalemia was prevented by administration of KCl infused at a rate of 3 mmol/h. Hemodynamic measurements were recorded for 5 minutes every 15 minutes throughout the study. Blood samples were collected every 30 minutes for analysis of substrate and hormone concentrations.

To demonstrate the efficacy of thoracic sympathectomy, we measured blood pressure and limb blood flow responses to 2 minutes of immersion of the hand in ice water (cold pressor test) and compared vasoconstrictor responses in the denervated and innervated limbs during the second minute of this test. Vasoconstrictor responses to immersion of the hand in ice water in the sympathetically denervated forearm were abolished; during the second minute of the cold pressor test, vascular resistance had increased by 48±20% in the innervated limb, whereas it had decreased by 17±5% in the denervated limb ($P<0.02$ innervated versus denervated limbs).

To document that differential blood flow responses to insulin/glucose infusion in the forearm and the calf were related specifically to sympathetic denervation (rather than to differential responses of forearm and calf blood flow to insulin infusion), we also studied 3 patients who had undergone both thoracic and lumbar sympathectomy (3 men; mean±SE age, 35±7 years; body mass index, 23.5±1.7 kg/m$^2$) and 4 control subjects (4 men; mean±SE age, 24±1 years; body mass index, 22.7±0.6 kg/m$^2$).

### Protocol 2: Hyperinsulinemic Euglycemic Clamp Performed During Concomitant Systemic L-NMMA Infusion

Six of the 9 subjects (2 men and 4 women; mean±SE age, 38±5 years; body mass index, 21.4±1.0 kg/m$^2$) returned for this protocol. The protocol was identical to protocol 1, except that during the second 30 minutes of baseline and during the entire 2-hour clamp, the subjects received (in randomized order) either a concomitant systemic L-NMMA infusion (50 μg/kg per minute) or a vehicle (normal saline) infusion. To demonstrate that the L-NMMA–induced hemodynamic effects plateau at the time the clamp was commenced, in 10 healthy subjects we infused L-NMMA at the same rate over 60 minutes. We found that mean arterial pressure increased significantly ($P<0.01$) from 77±2 mm Hg at baseline to 84±2 mm Hg after 30 minutes of infusion and then remained unchanged at 86±2 and 85±2 mm Hg after 45 and 60 minutes of infusion, respectively ($P>0.1$, 30 versus 60 minutes). In 4 of these subjects, the L-NMMA infusion was continued for a total of 150 minutes, and, in accordance with data by Dijkhorst-Oei and Koomans,[7] arterial pressure did not increase any further (88±3 and 90±4 mm Hg at 60 and 150 minutes, respectively; $P>0.1$). Consistent with previous findings,[8] limb blood flow and vascular resistance remained unchanged; values for forearm blood flow at baseline and after 60 minutes of infusion were 2.76±0.18 and 2.77±0.19 mL/100 mL per minute, respectively, and those for vascular resistance were 29.0±1.6 and 33.0±2.8 U, respectively. In the 4 subjects in whom the infusion was continued for a total of 150 minutes, forearm blood flow (2.43±0.30 versus 2.52±0.20 mL/min per 100 mL) and vascular resistance (36.3±4.9 versus 37.1 U) did not change between 60 and 150 minutes of infusion.

To examine whether the L-NMMA–induced hemodynamic effects were related specifically to NO synthase inhibition, at the end of the 2-hour clamp, insulin/glucose infusion was continued for another 30 minutes, whereas the L-NMMA infusion was replaced by L-arginine (50 mg/kg over 10 minutes). Hemodynamic measurements were repeated 15 minutes after the end of L-arginine infusion.

**TABLE 1. Effects of a 2-Hour Insulin/Glucose Infusion on Plasma Glucose, Insulin, and Potassium Concentrations, Mean Arterial Pressure, Limb Blood Flow, and Vascular Resistance**

| Variable | Baseline | Clamp |
|---|---|---|
| Glucose, mmol/L | 5.1±0.1 | 5.1±0.1 |
| Insulin, mU/L | 8.4±1.0 | 86.4±4.9* |
| Potassium, mmol/L | 3.6±0.1 | 3.6±0.1 |
| Forearm blood flow, mL (100 mL · min) | 2.15±0.22 | 2.95±0.32* |
| Forearm vascular resistance, U | 46.8±4.0 | 34.2±3.5* |
| Calf blood flow, mL (100 mL · min) | 1.94±0.26 | 2.52±0.32* |
| Calf vascular resistance, U | 55.8±7.4 | 41.9±5.5* |
| Mean arterial pressure, mm Hg | 93±3 | 91±4 |

Entries are for 9 subjects. Data are given as mean±SE.
*$P<0.05$ vs baseline.

## Analytical Methods

Plasma glucose was determined in duplicate by the glucose oxidase method on a Beckman glucose analyzer (Beckman Instruments). Plasma insulin was measured by radioimmunoassay.[2]

## Data Analysis

Mean arterial pressure was calculated as diastolic pressure plus one third pulse pressure. Limb vascular resistance was calculated as mean arterial pressure in millimeters of mercury divided by blood flow in milliliters per minute per 100 mL tissue, expressed in units.

The 5 minutes of data from forearm and calf blood flow, blood pressure, and heart rate collected every 15 minutes were averaged to a single value. Whole body glucose uptake was assumed to be equal to exogenous glucose infusion necessary to maintain euglycemia during the clamp[2] and was averaged for 30-minute periods. Statistical analysis was performed by ANOVA for repeated measures followed by Fisher's post hoc test. For single comparisons, the 2-tailed paired $t$ test or the Wilcoxon signed rank test was used, as appropriate. A $P$ value <0.05 was considered statistically significant. Data are given as mean±SE.

## Results

### Protocol 1

During the 2-hour euglycemic hyperinsulinemic clamp, muscle blood flow increased (and vascular resistance decreased) in both the denervated forearm and the innervated calf (Table 1). The increase in muscle blood flow was comparable in magnitude (at the end of the clamp, blood flow had increased by 37±5% and 32±6% and vascular resistance had decreased by 28±3% and by 24±3% in the forearm and the calf, respectively) but was markedly different in time course. In the denervated forearm, almost all of the vasodilation occurred within the first 30 minutes of insulin infusion, whereas in the innervated calf, insulin-induced stimulation of muscle blood flow was progressive and occurred mainly during the second hour of the clamp ($P<0.02$, forearm versus calf) (Figure 1).

In the 3 subjects who had undergone both lumbar and thoracic sympathectomy, >80% of the insulin-induced vasodilation in the denervated calf occurred within the first 30 minutes, and the time course was virtually superimposable to the one observed in their denervated forearm (and to the one observed in the denervated forearm of the subjects who had undergone thoracic sympathectomy alone). In the 4 control subjects, as expected, the insulin-induced vasodilation in the innervated forearm and calf was slower in onset compared
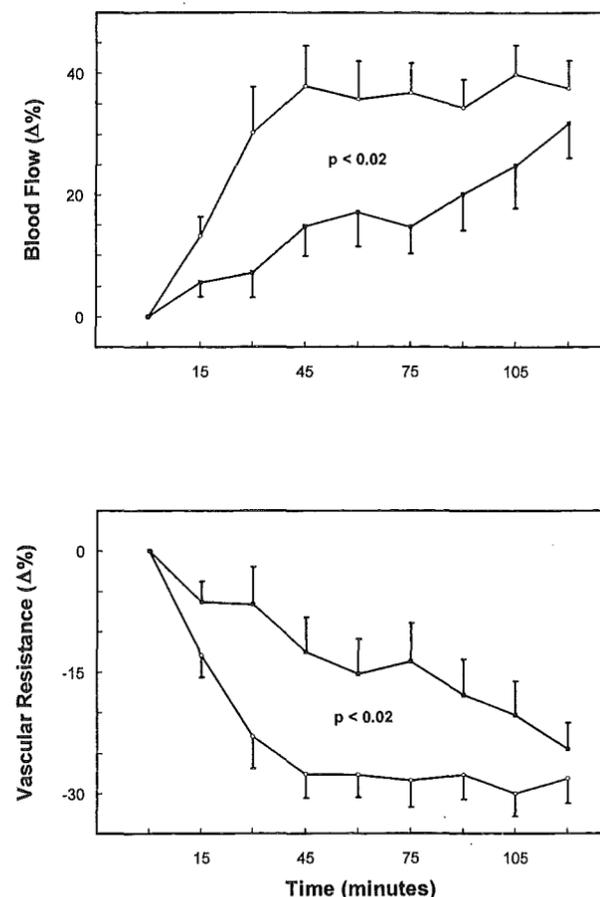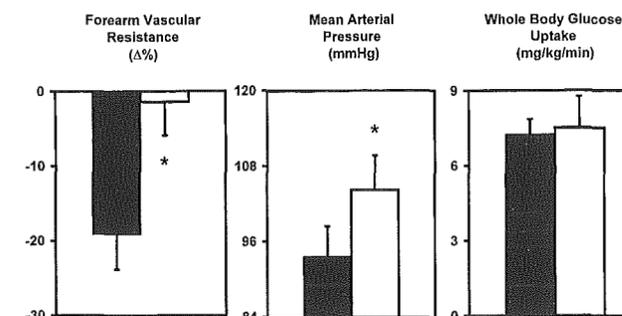
**Figure 2.** Mean±SE effects in 9 subjects of a 2-hour insulin/glucose infusion performed during concomitant saline (■) or L-NMMA (□) infusion on forearm vascular resistance, mean arterial pressure, and whole body glucose uptake. *$P<0.05$ vs saline infusion.



**Figure 1.** Mean±SE blood flow and vascular resistance responses to a 2-hour insulin/glucose infusion in the sympathetically denervated forearm (○) and the innervated calf (■) in 9 subjects. The insulin-induced vasodilator response occurred significantly more rapidly in the denervated than in the innervated limb.

with the one observed in the patients with denervated limbs, and blood flow continued to increase progressively throughout the clamp. Blood flow values at time 0, 30, 60, 90, and 120 minutes of the hyperinsulinemic clamp in the innervated forearm were 2.0±0.2, 2.5±0.4, 3.0±0.3, 3.3±0.4, and 3.6±0.4 mL/min per 100 mL and in the innervated calf were 2.0±0.2, 2.6±0.6, 2.9±0.7, 3.1±0.7, and 3.3±1.0 mL/min per 100 mL, respectively.

## Protocol 2

L-NMMA infusion did not have any detectable effect on plasma glucose and insulin concentrations at baseline and during the insulin/glucose infusion (Table 2).

L-NMMA infusion significantly ($P<0.05$) increased baseline mean arterial pressure and markedly altered the blood pressure and forearm blood flow responses to insulin/glucose infusion (Table 2). While mean arterial pressure remained unchanged during the clamp plus vehicle infusion, it increased significantly when the clamp was performed during concomitant L-NMMA infusion (Table 2). In the denervated forearm, L-NMMA infusion attenuated the insulin-induced vasodilation. At the end of the clamp, forearm blood flow had increased by 30±6% during vehicle infusion but by only 10±5% during L-NMMA infusion, whereas the forearm vascular resistance had decreased by 19±5% during vehicle infusion but remained unchanged (−1±4%) during L-NMMA infusion (Figure 2). When, at the end of clamp, L-NMMA infusion was replaced by L-arginine infusion, the altered hemodynamic responses to insulin were restored. Mean arterial pressure decreased from 104±5 to 95±5 mm Hg ($P<0.01$), and forearm blood flow increased from 2.37±0.23 to 2.73±0.33 mL/100 mL per minute ($P=0.07$).

In contrast to these marked hemodynamic actions, L-NMMA did not have any detectable effect on whole body glucose uptake. During the last 30 minutes of the clamp, whole body glucose uptake was 7.3±0.6 and 7.5±1.2 mg/kg per minute during vehicle and L-NMMA infusion, respectively (Figure 2).

**TABLE 2. Comparative Effects of a 2-Hour Insulin/Glucose Infusion, Performed Alone or With Concomitant L-NMMA Infusion, on Plasma Glucose, Insulin, and Potassium Concentrations, Mean Arterial Pressure, Limb Blood Flow, and Vascular Resistance**

| Variable | Baseline | Clamp | Baseline | L-NMMA Alone | Clamp+L-NMMA |
|---|---|---|---|---|---|
| Glucose, mmol/L | 5.0±0.1 | 5.0±0.1 | 4.9±0.1 | 4.9±0.1 | 5.0±0.1 |
| Insulin, mU/L | 7.7±0.9 | 77.1±3.8* | 6.1±1.5 | 6.0±1.0 | 76.3±5.8† |
| Potassium, mmol/L | 3.8±0.1 | 3.6±0.1 | 3.8±0.1 | ... | 3.6±0.1 |
| Forearm blood flow, mL (100 mL · min) | 2.05±0.13 | 2.66±0.18* | 2.17±0.21 | 2.15±0.22 | 2.37±0.23 |
| Forearm vascular resistance, U | 45.4±4.4 | 36.3±3.3* | 43.3±5.7 | 47.7±6.2 | 46.7±6.3 |
| Mean arterial pressure, mm Hg | 91±5 | 94±5 | 90±7 | 96±6 | 104±5† |

Entries are for 6 subjects. Data are given as mean±SE.
*$P<0.05$, clamp vs baseline.
†$P<0.05$, clamp+L-NMMA vs L-NMMA alone.

## Discussion

It is now clear that insulin exerts cardiovascular effects by stimulating NO release, but the underlying mechanisms are not known.[1] Here we provide the first evidence that insulin stimulates muscle blood flow in sympathectomized limbs by a direct action at the vasculature in vivo. In healthy subjects having undergone regional thoracic sympathectomy for hyperhidrosis, a 2-hour insulin infusion increased muscle blood flow in the denervated forearm by roughly 40%, an increase that was of comparable magnitude to the one observed in the innervated calf.

The experimental model of regional sympathectomy not only allowed us to study the potential contribution of sympathetic vasodilator (nitrergic) nerves to insulin vasodilation, but also permitted us to examine whether sympathetic vasoconstrictor tone modulates such vasodilation. In innervated limbs, as in the present study, insulin-induced vasodilation consistently has been found to be slow in onset and to occur progressively.[1] In contrast, in the denervated forearm vasodilation rapidly reached its maximum (during the first 30 to 45 minutes of insulin infusion) and remained stable thereafter. This finding is specific for denervation and not related to differential vasodilator responses to insulin infusion in the forearm and the calf, as evidenced by the studies in the patients who had undergone both lumbar and thoracic sympathectomy and in the control subjects. Consistent with the rapid onset of the vasodilation in sympathetically denervated limbs in vivo, insulin rapidly stimulates NO release in cultured human vascular endothelial cells[9] and in vascular ring preparations in vitro.[10]

Previous findings indicated that prevention of the insulin-induced sympathetic activation by dexamethasone abolishes the stimulation of muscle blood flow during insulin infusion in humans.[11] Taken together with the present findings, these earlier findings suggest that the baseline sympathetic vasoconstrictor tone prevents the direct vasodilator action of insulin in innervated limbs, because it can only be demonstrated in the absence (surgical sympathectomy), but not in the presence (dexamethasone studies), of sympathetic innervation. Consistent with this hypothesis, in patients with autonomic failure (sympathetic denervation as an experiment of nature), insulin infusion evokes vasodilation and hypotension.[1] Second, in innervated limbs, stimulation, by insulin, of sympathetic vasodilator outflow is necessary to induce vasodilation, because the suppression of the insulin-induced sympathetic activation also abolishes the vasodilation.

To examine whether NO contributes to the direct local vasodilator action of insulin, we examined the effects of systemic inhibition of NO synthase by L-NMMA infusion on insulin-induced stimulation of blood flow in sympathectomized limbs. We found that L-NMMA infusion markedly attenuated the insulin-induced stimulation of muscle blood flow and decrease in vascular resistance in sympathectomized limbs. This effect was related specifically to NO synthase inhibition, as evidenced by the L-arginine studies.

Insulin resistance is a common feature of essential hypertension,[12,13] and preliminary evidence suggests that endothelial dysfunction may contribute to impaired muscle glucose uptake.[14] In rats, arterial hypertension induced by L-NMMA infusion impairs insulin-stimulated glucose uptake.[15] In contrast, the present findings suggest that in humans, a clinically relevant impairment in NO release (as evidenced by the increase in baseline arterial pressure) does not alter insulin stimulation of glucose uptake.

In summary, we have used an experimental model (regional sympathectomy) to study vascular actions of insulin in the presence and absence of sympathetic vasodilator and vasoconstrictor innervation in humans. We found that insulin has a direct vasodilator action in the skeletal muscle vasculature in vivo. This direct vasodilator action is mediated by stimulation of NO release and appears to be masked by the sympathetic vasoconstrictor tone in innervated limbs.

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