Influence of Nervous Blockade on Insulin-Mediated Glucose Uptake in the Human Forearm

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This study was undertaken to investigate if a nonpharmacologic increase in forearm blood flow (FBF) could increase forearm glucose uptake (FGU) during hyperinsulinemia. In 10 young volunteers, FBF and the arterial-venous glucose difference were measured in both arms during a 2-hour euglycemic hyperinsulinemic clamp procedure when 1 of the arms was subjected to axillary plexus nervous blockade with local anesthesia. FBF was measured in both arms by venous occlusion plethysmography. Nervous blockade, increasing FBF by more than 3-fold, did not improve insulin-mediated FGU. On the contrary, a tendency towards a reduced FGU compared with the control arm was seen ($P = .07$). Furthermore, while insulin increased FBF to a similar degree in both arms (+ 3.0 and 4.4 mL/min/100 mL tissue, $P < .01$ for both arms), nervous blockade abolished the rapid increase in glucose extraction seen in the control arm when insulin infusion was initiated. The present study showed that an increase in FBF induced by nervous blockade did not increase insulin-mediated FGU. On the contrary, a tendency towards a reduction was seen. Furthermore, insulin induced vasodilation in the blocked arm, but delayed the ability of insulin to promote glucose extraction, suggesting that the well-documented increase in skeletal muscle sympathetic nerve activity seen during acute hyperinsulinemia has metabolic rather than hemodynamic consequences.

PERIPHERAL INSULIN sensitivty in skeletal muscle is usually defined as the amount of glucose uptake in the studied limb during a challenge with insulin, such as seen during the euglycemic hyperinsulinemic clamp. The most widespread method to study limb glucose uptake is to use Fick′s law stating that glucose uptake is the product of limb blood flow and the arterial-venous glucose difference over the limb.

According to that principle, both a reduction in insulin-mediated blood flow and an attenuation of glucose extraction could, to an equal degree and independently of each other, impair peripheral insulin sensitivity. A reduced blood flow to the muscle would limit the delivery of insulin and glucose to the muscle fibers due to a reduced number of perfused capillaries, thereby limiting the availibility of both the initiator (insulin) and the substrate (glucose) for glucose uptake. An attenuated glucose extraction, on the other hand, implies that an event taking place in the insulin receptor or downstream the insulin signaling pathway is impaired. Thus, measurements of both insulin-mediated blood flow and glucose extraction could provide insight into physiologic and pathologic mechanisms regarding insulin sensitivity.

Now more than a decade ago, Laakso et al[1] presented in a cross-sectional study that an attenuated increase in blood flow induced by insulin was a powerful determinant of skeletal muscle glucose uptake in obese insulin-resistant subjects, implying that the hemodynamic actions of insulin were of major importance. Although these investigators have presented evidence that this hemodynamic action of insulin could be a mechanism involved in the insulin resistance regarding glucose uptake also in diabetics,[2-4] the importance of insulin-mediated vasodilation as a determinant of glucose uptake has been challenged. In a number of reports, Yki-Järvinen et al[5-8] presented evidence that insulin-mediated vasodilation and insulin-mediated limb glucose-uptake are parallel phenomenon rather than being causally related. However, most of these studies both speaking in favor and against the importance of insulin-mediated vasodilation have used a cross-sectional approach by which a clear separation between the hemodynamic actions of insulin and its more direct metabolic actions are hard to distinguish, because they are parallel phenomenon.

One way to further study this controversy is to increase limb blood flow by other means than insulin. However, this approach has also produced conflicting results. While the nitric oxide-dependent vasodilator, methacholine, induced both vasodilation and an increased glucose uptake in the leg during hyperinsulinemia,[4] other investigators using adenosine or bradykinin as vasodilators could not confirm that an increased limb blood flow increased glucose uptake.[6,9] We have recently shown that vasodilation in the human forearm by means of methacholine, but not by nitroprusside, induced an increased glucose uptake in the fasting state, despite that both of these vasodilators increased forearm blood flow (FBF) to a similar degree.[10] It therefore seems that different vasodilators could have different direct actions on glucose metabolism on their own. Thus, studies to evaluate the impact of vasodilatation on insulin-mediated glucose uptake should preferably use methods to change blood flow that have no direct effects on glucose metabolism.

Another unsolved issue in the field of vasodilation and skeletal muscle glucose uptake is the role of the sympathetic nervous system. It is well established that hyperinsulinemia induces an increase in sympathetic nervous outflow to skeletal muscle, as both directly recorded skeletal muscle sympathetic nerve activity (MSNA)[11-13] and norepinephrine levels in the forearm rapidly increase during acutely induced hyperinsulinemia.[14] In rats, it has been shown that this increase in sympathetic nerve activity is centrally mediated, as destruction of the ventrolateral part of the thalamus abolished this activity during hyperinsulinemia.[15] However, the physiologic importance of

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Table 1. Plasma Glucose Measurements and FBF in Both Arms Before any Interventions in the Intervention Group and in the Time-Control Group

	Intervention Group	Time-Control Group
Fasting arterial plasma glucose (mmol/L)	5.0 ± 0.4	5.1 ± 0.3
Fasting venous plasma glucose (control arm)	4.8 ± 0.4	4.9 ± 0.3
Fasting venous plasma glucose (blockade arm)	4.8 ± 0.4	—
FBF (mL/min/100 mL tissue) (control arm)	5.0 ± 2.0	3.9 ± 1.1
FBF (mL/min/100 mL tissue) (blockade arm)	4.8 ± 1.5	—

NOTE. Data are means ± SD.

this increased activity of the sympathetic efferents to skeletal muscle is unclear, as it is supposed to cause vasoconstriction. It has been suggested that this vasopressor activity is a way to counterbalance the vasodilatory action of insulin.

The primary aim with the present study was to evaluate if a profound increase in FBF would influence peripheral insulin sensitivity with the hypothesis that insulin-mediated glucose uptake would increase. To overcome the problem that different vasodilators might have direct effects on glucose metabolism on their own, the present study induced vasodilation by a nonpharmacologic way by blocking nerve traffic in the forearm using axillary plexus blockade with local anesthetics. With this approach, we studied the influence of profound vasodilation in 1 arm, while the other forearm served as a control. Furthermore, this experimental set-up could also be used to study the influence of forearm nerve activity on insulin-mediated vasodilation and glucose uptake, constituting the secondary aims of the study.

MATERIALS AND METHODS

Subjects

Ten young subjects were included in the present study (range, 20 to 25 years; mean, 22 ± 2 SD years; mean body mass index [BMI], 22.3 ± 0.3 kg/m^2). All subjects were male. None had a history of cardiovascular or metabolic disorders, and all were free from regular medication. In a time-control protocol, another 10 young healthy males of a similar age were investigated (mean age, 23 ± 2 years; BMI, 23.1 ± 1.0 kg/m^2).

The study was approved by the local ethics committee, and all participants gave their informed consent.

All experiments were performed in the morning after an overnight fast. All subjects received an arterial catheter in the brachial artery in 1 of the arms. Both the dominant and nondominant arms were used in a random order. A deep venous catheter was inserted in both of the arms in the anticubital fossa. Venous occlusion plethysmography was applied on both arms for determinations of FBF. After a resting period following cannulation of at least 60 minutes, blood samples for plasma glucose were drawn in all 3 catheters followed by determinations of FBF in both arms. Thereafter axillary nerve blockade was applied in 1 of the arms by injection of 20 mL 1% mepivacain (Carbocain, AstraZeneca, Södertälje, Sweden) mixed with 20 mL 0.75% ropivacain (Narop, AstraZeneca). Three injections of this mixture were made within the sheeth surrounding the brachial plexus and the artery through a 24-gauge cannula. A proper nerve blockade lasting throughout the experimental period was evaluated by lost motor function, vasodilation of the arm, and by absence of sensory stimuli, such as lost ability to discriminate between cold and heat, as well as absent sensation of pain. This was found to be satisfactory in all of the cases.

Sixty minutes after the nerve blockade, blood sampling for glucose and measurements of FBF were again performed. Thereafter the euglycemic hyperinsulinemic clamp was initiated, and blood sampling for glucose from both of the arms and measurements of FBF were performed every 15 minutes during the first hour and thereafter after 90 and 120 minutes following the start of the clamp procedure.

In the time-control experiments, no nervous blockade was performed, but FBF, forearm arterial-venous (A-V) differences for glucose and forearm glucose uptake were measured in 1 of the arms during a slow saline infusion instead of the clamp procedure.

The euglycemic hyperinsulinemic clamp technique was performed according to DeFronzo et al[16] with slight modifications. The insulin (Actrapid; Human, Novo, Copenhagen, Denmark) infusion rate during the clamp was 56 mU/m^2 body surface area per minute in all subjects. Glucose was infused intravenously and the target level of plasma glucose during the clamp study was 5.1 mmol/L. Arterial glucose was measured every 5 minutes by a Beckman glucose analyzer II (Fullerton, CA) The glucose disposal (M) during the euglycemic insulin clamp was calculated on the basis of the amount of glucose infused and is expressed per kilogram body weight (mg · kg BW^{-1} · min^{-1}) during the last 60 minutes of the clamp. The variation in arterial glucose concentration was less than 5% during the last hour of the clamp.

FBF was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden). A mercury silastic strain gauge was placed at the upper third of the forearm, which rested comfortably slightly above the level of the heart. The strain gauge was connected to a calibrated plethysmograph. Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg by a rapid cuff inflator. FBF was determined from the mean of at least 5 consecutive recordings.

Forearm glucose uptake (FGU) was calculated as FBF (mL/min/100 mL tissue) times the forearm A-V difference for glucose (mmol/L).[17]

Serum insulin was measured in the fasting state and at the end of the clamp procedure in EDTA-plasma using an enzymatic-immunologic assay (Enzymmun; Boehringer, Mannhein, Germany) performed in an ES300 automatic analyzer (Boehringer).

Changes during the clamp procedure and by nerve blockade in the fasting state were evaluated by analysis of variance (ANOVA) for repeated measurements. $P < .05$ was considered significant.

Table 2. FBF, A-V Glucose Difference, and FGU in Both Arms at the Baseline Measurement and After Nervous Blockade in the Fasting State

	Baseline	After Blockade
FBF (mL/min/100 mL tissue) (control arm)	5.1 ± 2.1	4.0 ± 1.6
FBF (mL/min/100 mL tissue) (blockade arm)	4.8 ± 1.5	15.6 ± 5.5*
A-V glucose difference (mmol/L) (control arm)	0.19 ± 0.10	0.20 ± 0.11
A-V glucose difference (mmol/L) (blockade arm)	0.20 ± 0.13	0.06 ± 0.02*
FGU (mmol/L · mL/min/100 mL tissue) (control arm)	1.0 ± 0.77	0.78 ± 0.15
FGU (mmol/L · mL/min/100 mL tissue) (blockade arm)	1.0 ± 0.75	1.0 ± 0.53

NOTE. Data are means ± SD.
*$P < .001$ *v* baseline.

RESULTS

Basic characteristics of the intervention group and the time-control group are shown in Table 1.

After axillary nervous blockade FBF increased by more than 300% ($P < .001$), while no significant change in FBF was seen in the control arm (see Table 2 for details). This increase in FBF was accompanied by a substantial reduction in the A-V glucose difference ($P < .001$), while no effect on glucose extraction was seen in the control arm. Thus, the net result of the nerve blockade in the fasting state was an unchanged FGU, just as seen in the control arm (see Table 2).

During the hyperinsulinemic clamp, serum insulin increased from 8.8 ± 6.6 SD in the fasting state to 85 ± 8.9 mU/L after 2 hours. Arterial plasma glucose remained constant (5.0 ± 0.4 and 5.1 ± 0.5 mmol/L, respectively).

As can be seen in Fig 1, a significant increase in FBF was seen in both of the arms during the 2-hour hyperinsulinemic clamp ($P < .001$). In absolute terms, this increase in blood flow was similar in both of the arms, being +4.2 mL/min/100 mL tissue in the nerve blockade arm and +3.2 mL/min/100 mL tissue in the control arm. However, the effect of hyperinsulinemia on A-V glucose difference was significantly different in the 2 arms ($P < .001$). While a rapid increase in glucose extraction was seen after 15 minutes ($P < .01$) in the control arm, no such increase was seen in the arm being subjected to nervous blockade. In the blocked arm, no significant increase in A-V glucose difference ($P < .05$) was seen before 30 minutes. Therefore, an increase in insulin-mediated FGU was seen just after 15 minutes in the control arm ($P < .002$), while insulin did not promote any increase in glucose uptake in the arm subjected to nerve blockade during this period (Fig 2). However, after 30 minutes, a significant increase in insulin-mediated FGU was seen also in the nerve blockade arm ($P < .01$). As can be seen in Fig 2, FGU in the nerve-blockade arm was at all time-points during the hyperinsulinemic clamp period lower than that seen in the control arm. When total insulin-mediated FGU was calculated as the area under the curve during the 2-hour long clamp procedure, there was a tendency that a reduced FGU was seen in the arm subjected to nervous blockade compared with the control arm ($P = .07$).

As can be seen in Table 3, no changes in FBF, glucose extraction, or FGU were seen during the time-control protocol. Only data from baseline and 2-hour measurements are shown.

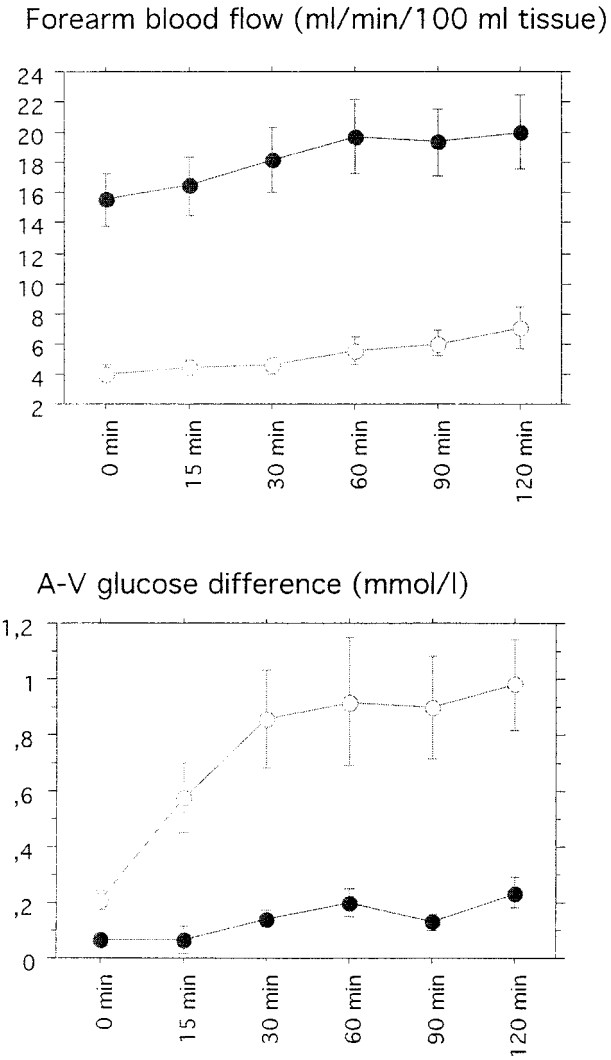

Fig 1. (A) Development of FBF and (B) A-V glucose difference (mmol/L) during euglycemic hyperinsulinemic clamp in the arm subjected to nervous blockade (●) and in the control arm (○). Means ± SEM are given.

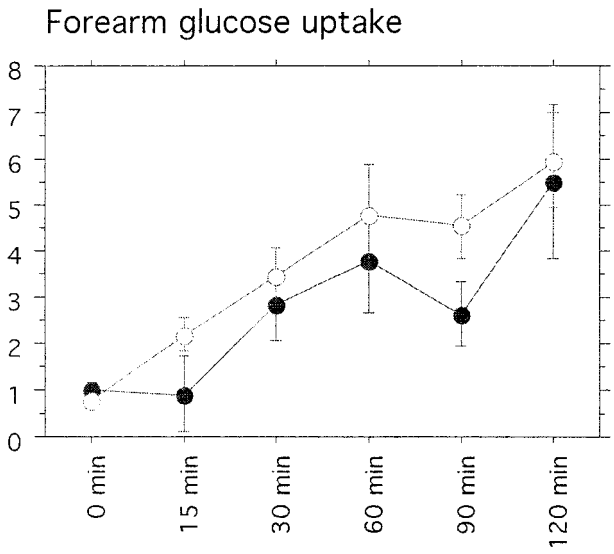

Fig 2. Development of FGU (mmol/L · mL/min/100 mL tissue) during euglycemic hyperinsulinemic clamp in the arm subjected to nervous blockade (●) and in the control arm (○). Means ± SEM are given.

DISCUSSION

The present study disclosed 3 different major findings. First, a substantial increase in FBF, not induced by any drug that could interfere with glucose metabolism in the forearm, did not increase insulin-mediated FGU. In fact, a tendency towards a

Table 3. FBF, A-V Glucose Difference, and FGU at the Baseline Measurement and After 2 Hours of Slow Saline Infusion in the Time-Control Group

	Baseline	2 Hours
FBF (mL/min/100 mL tissue)	4.8 ± 2.2	4.9 ± 2.1
A-V glucose difference (mmol/L)	0.18 ± 0.12	0.20 ± 0.13
FGU (mmol/L · mL/min/100 mL tissue)	0.86 ± 0.66	0.98 ± 0.68

NOTE. Data are means ± SD.

reduction in glucose uptake was seen. Thus, our primary hypothesis was not supported.

Second, also during nervous blockade, hyperinsulinemia induced vasodilation being in absolute terms similar to that seen in the control arm. Third, during the hyperinsulinemic clamp, a rapid, profound increase in glucose extraction is usually seen within 10 to 15 minutes. This insulin-mediated increase in glucose extraction was delayed in the arm subjected to nervous blockade.

The present study showed that a substantial increase in FBF did not increase FGU neither in the fasting state nor during hyperinsulinemic conditions. On the contrary, there was a tendency that nervous blockade reduced FGU uptake during hyperinsulinemia. Thus, the present study does not support the idea that an increased delivery of insulin and glucose by means of vasodilation would increase skeletal muscle glucose uptake.[1-4] As the increase in FBF in this study was achieved by a pharmacologic intervention that has been shown to not influence glucose metabolism in itself (nervous blockade of a limb),[18] the divergent results previously obtained using different vasodilators might be due to the fact that these vasodilators could have different direct effects on glucose metabolism.[4,6,9,15] However, the present study does not rule out the possibility that the vasodilation induced by insulin might facilitate FGU during the later phases of the hyperinsulinemic clamp, as we have previously shown a close relationship between the degree of vasodilation in the forearm and whole-body glucose uptake during the later phase of the 2-hour clamp procedure in young healthy subjects.[17]

While most studies inducing systemic hyperinsulinemia have shown vasodilation in the limbs, a number of studies using local insulin infusion in the forearm did not.[8] Systemic infusion of insulin is also demanded in order to observe an increase in skeletal muscle sympathetic nerve activity[10-12] or an increase in norepinephrine spillover in the forearm.[13] However, the physiologic role of this increase in skeletal muscle sympathetic nerve activity is not clear. It has been proposed that this anticipated vasoconstricting action might counterbalance the vasodilatory action of insulin, although there seems to be a dissociation between the sympathoexitatory and vasodilatory actions of insulin.[19,20] In the present study, a similar degree of increase in FBF was seen in both the control arm and the arm subjected to nervous blockade. This finding implies that the activation of skeletal muscle sympathetic nerve activity seen during hyperinsulinemia is not the major modulator of the hemodynamic events induced by insulin in the limbs. It is thought that axillary nervous blockade induces vasodilation by means of withdrawal of a tonic α-adrenergic constricting activity of the autonomic nervous system. It is well established that insulin induces vasodilation by means of nitric oxide formation[21,22] The present study suggests that this insulin-mediated vasodilation, presumably caused by nitric oxide, is superimposed on the vasodilation induced by the abolished α-adrenergic activity in the arm subjected to nervous blockade, and that this action of insulin is independent on skeletal muscle sympathetic nerve outflow.

During infusion of insulin, an increased glucose extraction is rapidly seen preceding the vasodilatory action of insulin[8,17] This was confirmed in the control arm in the present study, but in the arm subjected to nervous blockade, the increase in glucose extraction was delayed. This finding implies that the rapid increase in skeletal muscle sympathetic nerve activity seen during hyperinsulinemia rather than being a modulator of hemodynamic effects in the forearm, is involved in the events induced by insulin to promote glucose extraction. Skeletal muscle sympathetic nerve fibers contain efferents that stimulate both β-2 receptors and α-adrenergic receptors. It has been shown that β-2 receptor stimulation will increase the production of nitric oxide,[23] and endothalial nitric oxide synthase has recently been shown to be involved in glucose metabolism.[24] Withdrawal of nerve activity that influences the β-2 receptors in the forearm might therefore interfere with the regulation of glucose metabolism at the cellular level.

In another study some years ago evaluating the glucose uptake in the forearm during the hyperinsulimemic clamp,[17] we also measured the glucose uptake in the leg simultaneously in some of the subjects and found similar results. Also, pilot studies with the positron-emission tomography (PET) technique support the finding that no major differences exist between arm and leg skeletal muscle regarding insulin-mediated glucose uptake.

In conclusion, the present study showed that an increase in FBF induced by axillary nervous blockade did not increase FGU. On the contrary, a tendency towards a reduction was seen. Furthermore, insulin induced vasodilation also in the nerve-blocked arm, but delayed the ability of insulin to promote glucose extraction, suggesting that the well-documented increase in skeletal muscle sympathetic nerve activity seen during hyperinsulinemia rather have metabolic than hemodynamic consequences. Thus, these data do not support the idea that a nonspecific increase in skeletal muscle blood flow would improve insulin-mediated glucose uptake in the clinical setting.

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