Peripheral blood flow and noradrenaline responsiveness: the effect of physiological hyperinsulinemia

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Abstract

Objective: Insulin seems to have vasodilator properties, but it is unclear if insulin in postprandial concentrations is a specific vasodilator of skeletal muscle resistance arterioles only or that various types of vessels are affected. The aim of the present study was to determine the direct effects and the time course of regional/local physiological hyperinsulinemia on skeletal muscle arterioles, skin blood flow and peripheral venous tone and the responsiveness of these different vascular beds to noradrenaline. Methods: In protocol 1 (n = 12) we infused insulin into the brachial artery for 180 min (3.5 mU/min) and evaluated the effects on forearm muscle blood flow (FBF) and skin blood flow (SBF). Furthermore, noradrenaline (0.025, 0.01 and 0.4 μg/min) was infused (i.a.) at baseline, at 90 and 180 min after the start of insulin. In protocol 2 (n = 10) the same regional forearm hyperinsulinemia was achieved, but now the local venous responsiveness to noradrenaline (1.7–55 ng/min, at baseline and at 90 and 180 min) was measured in a dorsal hand vein. In protocol 3 we evaluated the local effects of different doses of insulin (1–100 mU/min) infused directly into hand veins preconstricted with phenylephrine. Results: Forearm hyperinsulinemia (50 mU/l) led to a significant increase in FBF after 180 min median 26%, interqu. ranges 5–50, P < 0.05, while SBF was not altered. Forearm hyperinsulinemia did not affect the noradrenergic responsiveness in skeletal muscle or skin. Infused locally into hand veins only the highest dose of insulin (100 μU/min) caused a minor venodilation (7% ± 2.4–12.5, P < 0.05). Conclusion: Regional forearm physiological hyperinsulinemia has a vasodilator effect on resistance vessels in skeletal muscle, but is slow in onset (180 min). However, skin vasculature and peripheral veins are not affected by this hyperinsulinemia.

Keywords: Insulin; Blood flow, forearm; Blood flow, skin; Noradrenaline; Human

1. Introduction

Epidemiological studies demonstrate an association between obesity, non-insulin-dependent diabetes mellitus (NIDDM), and hypertension [1,2]. Several experimental studies suggest that insulin not only lowers blood glucose levels but also acts as a vasodilator [3–7]. Impaired insulin vasodilation has been observed in insulin-resistant states such as NIDDM, hypertension and obesity [3,5,8]. The impaired vasodilator properties of insulin could result in elevated vascular tone and augmented responses to vasoconstrictors culminating in increased peripheral resistance. Although insulin might play an important role in blood pressure homeostasis, the mechanism of insulin-mediated vasodilation is still unclear. Several studies suggest that during systemic hyperinsulinemia as well as regional administration in the forearm, insulin-mediated vasodilation occurs primarily in skeletal muscle [3,4,8,9]. Skeletal muscle is one of the main targets of the blood glucose lowering effects of insulin. Furthermore, in lean and obese subjects insulin-mediated vasodilation is tightly coupled to glucose uptake [8], which is compatible with the hypothesis that insulin-mediated vasodilation is the result of the metabolic effects of insulin. In contrast, other studies suggest that insulin is a more direct vasodilator that stimu-
lates the production of endothelium-derived nitric oxide (EDNO) [10,11]. Both in vivo and in vitro studies indicate that supraphysiological doses of insulin can induce vasodilation in blood vessels from various vascular beds, including resistance arterioles, conduit arteries and veins [12–15]. Therefore, it is still unresolved whether insulin, in concentrations as can be found in humans after a meal (postpran-
dial), is a specific vasodilator of skeletal muscle resistance arterioles or, alternatively, that various types of vessels are affected. Besides its direct vasodilating properties insulin (in healthy subjects) also seems to attenuate the constrictor response of the forearm vasculature or peripheral veins to vasoconstrictors, such as noradrenaline and angiotensin II, which could affect blood pressure homeostasis [12,14–16]. However, other authors could not observe any effect of insulin on noradrenergic responsiveness [17,18].

Apart from the type of vessel affected by insulin, the time span in which insulin can accomplish its hemodynamic effects in the various vascular beds has received relatively little attention. During systemic hyperinsulinemia the increase in forearm as well as leg flow seems to have a relatively slow onset (a few hours) [8,19–22]. In contrast, local infusion of insulin directly into veins resulted in marked vasodilation within minutes [14,23,24]. If insulin, in physiological concentrations, is indeed a potent and rapidly acting venodilator, it could play a pivotal role in postprandial volume and blood pressure regulation.

The aim of the present study was to determine the effect and time course of local physiological hyperinsulinemia during a few hours on different types of vessel in the human forearm, skeletal muscle arterioles, skin (micro-
vascular) blood flow and hand veins, and their constrictor responses to noradrenaline. To minimize influences of systemic neurohormonal and metabolic changes on regional hemodynamics, insulin was infused regionally into the brachial artery or directly into a single vein.

2. Methods

2.1. Subjects

Three series of experiments were performed in a total of 36 healthy normotensive male subjects with a mean age of 22 ± 2 years (mean ± s.d.), body mass index of 21.8 ± 2.7 kg/m² and (intra-arterial) blood pressure of 116/74 mmHg. Fasting blood glucose values were 4.0 ± 0.3 mmol/L. No subject had evidence of metabolic or cardiovascular disease and no subject had a family history of diabetes mellitus, hypertension or cardiovascular diseases. Subjects did not use any medication (including NSAID’s) and refrained from smoking and caffeine for at least 12 h before the experiment. All studies were conducted in the morning after an overnight fast and subjects were studied supine in a hospital bed, in a quiet, temperature-controlled room.

The nature, purpose, and potential risks of the study were carefully explained to each subject before informed consent to participate was obtained. All participants gave written informed consent and the studies were approved by the Ethical Review Committee of the University Hospital Maastricht. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Techniques

2.2.1. Intra-arterial forearm study

Before starting the experiment forearm volume was measured by water displacement. Subjects were studied supine; mean room temperature was 24.4 ± 0.5°C. Fore- arm (muscle) and skin blood flow were measured as described previously [25]. In short, thermoregulatory skin blood flow (SBF) was measured using laser-Doppler fluxmetry (Periflux PF3; Perimed, Jarfalla, Sweden). The probes were placed on the ventral side of both forearms near the wrists. The probes remained in the same position throughout the experiment. Flux values are expressed as arbitrary perfusion units (PU), calibrated against an external standard. Forearm blood flow (FBF) was deter-

2.2.2. Venous studies: Linear variable differential trans-

form-

Venous responsiveness was assessed by the linear variable differential transformer (LVDT) technique, which measures alterations in the distension of superficial hand veins during drug infusions [26]. Subjects were studied supine in a temperature-controlled room, mean temperature 26.0 ± 0.5°C which was necessary in order to minimize sympathetic tone. In the non-dominant arm a dorsal hand vein was cannulated with a 23-gauge butterfly needle. This arm was resting on a padded support, 30 degrees above the horizontal, so that the vein, which was above heart level, was completely collapsed. A cuff was attached around the upper arm. Changes in vein diameter were measured using the LVDT (model 100 HMR, Schaevitz Engineering, Pennsauken, NJ) and an ATA signal conditioner (Schae-

vitz Engineering). The LVDT was mounted on the back of the hand using a tripod. The tip of the steel core of the LVDT was positioned over the center of the vein approximately 1 cm proximal to the tip of the butterfly needle. After an acclimatisation period of 30 min, 3 baseline measurements of venous distension were performed with a
cuff pressure of 40 mmHg. The difference between the collapsed and fully distended reading represented the maximum vein diameter.

2.3. Experimental protocols

2.3.1. Protocol 1 (FBF, forearm hyperinsulinemia)

In this first series of experiments the vasoactive effects of regional hyperinsulinemia on thermoregulatory skin perfusion and forearm blood flow were studied (n = 12). Two catheters were inserted in the cubital fossa of the non-dominant arm: a 20-gauge catheter into the brachial artery (retrogradely, after local anesthesia with lidocaine) for infusion of insulin (Actrapid, Novo-Nordisk, Denmark) and noradrenaline (Centrafarm, Ettten-Leur, The Netherlands), and a 21-gauge catheter into an antecubital vein (antegradely) for blood sampling. After an acclimatisation period of 30 min baseline measurements of SBF, FBF and intra-arterial blood pressure were performed. After 15 min, these measurements were repeated before and during infusion of 3 cumulative doses of noradrenaline: 0.025, 0.1 and 0.4 μg/min, each dose for 3 min. Thereafter, an intra-arterial infusion of insulin (3.5 μU/min) was started (t = 0) for 180 min. At t = 45, 90, 135 and 180 min the hemodynamic measurements were repeated; at t = 90 and 180 min, noradrenaline was again administered in 3 cumulative doses. Before each hemodynamic measurement, venous blood from the infused arm was drawn to determine local insulin values. Systemic blood glucose levels were determined in arterial blood from the same arm.

2.3.2. Protocol 2 (LVDT, forearm hyperinsulinemia)

In protocol 2, protocol 1 (intra-arterial infusion of insulin) was repeated, but now the venodilator effect of 3 h hyperinsulinemia on the dorsal hand vein was determined. Insulin (n = 10) or saline (n = 8) was infused into the brachial artery of the non-dominant arm. In this arm also a dorsal hand vein was cannulated with a butterfly needle and the LVDT was positioned as described above. After an acclimatisation period of 30 min, basal distension and the vasoconstrictor response of the hand vein to a stepped infusion of NA (1.7–5.5–17–55 ng/min), administered directly into the vein, was measured at t = 0, 90 and 180 min. At these time-points arterial blood glucose and venous insulin levels of the infused arm were also determined.

2.3.3. Protocol 3 (LVDT, local hyperinsulinemia)

In order to study the local effect of different doses of insulin, a third series of experiments was performed in protocol 3 (n = 14). First, a cumulative stepped infusion of phenylephrine (PE) was administered directly into a dorsal hand vein (10–10000 ng/min, each dose for 6 min). After a wash-out period (35 min) vessels were preconstricted to approximately 80% (ED₅₀) of the maximal PE constriction, calculated using non-linear curve-fitting (Graphpad Software Inc.). Subsequently, a cumulative stepped infusion of insulin (1–3–10–30–100 mU/min) was administered. Each insulin dose was infused for 6 min and venous distension was determined during the last 2 min. In these experiments PE instead of NA was used, as in pilot studies using NA it was more difficult to obtain a stable preconstriction. During all experiments the infused volume was kept constant (0.3 ml/min). Blood pressure and blood glucose levels were measured before the start of the PE infusion and during both the highest PE and insulin dose.

2.4. Analytical and statistical procedures

Blood glucose was determined by the glucose-oxidase method on the YSI Model 2300 Stat (Yellow Springs, OH, USA). Total insulin was determined by a commercial radio-immunoassay (Pharmacia, Uppsala, Sweden). Noradrenaline, phenylephrine and insulin were dissolved in saline. In addition, a small amount of blood was added to the insulin solution to prevent the adhesion of insulin to the plastic syringe and tubing. All solutions were freshly prepared immediately prior to the experiment.

For each measurement period the ratios of SBF and FBF were calculated (infused arm divided by the contralateral arm). These calculated ratios correct for all systemic factors that affect the regulation of blood flow in both arms (e.g., changes in blood pressure, level of arousal, hormonal changes etc.), and ensure that only the direct effects of locally infused substances on the forearm blood flow are taken into account [27]. To determine the effect of hyperinsulinemia on SBF, FBF and blood pressure the mean value of the last minute of each period was used for calculations. The distribution of the FBF, SBF and hand vein data was not normal, so a non-parametric Friedman two-way ANOVA test was used to evaluate differences in the dose–response curves, followed by a post-hoc non-parametric Wilcoxon test to examine the differences between the various parameters. Shaffer correction was used for multiple comparisons [28]. Data are expressed as medians and interquartile ranges, unless indicated otherwise. Statistical significance was set at a value P < 0.05%.

3. Results

During all studies systemic blood glucose concentration did not change, nor did systemic blood pressure and heart rate (data not shown).

3.1. Protocol 1 (FBF, forearm hyperinsulinemia)

During intra-arterial infusion of insulin (3.5 mU/min) the local insulin concentration (in the infused arm) increased from 6 (4–7) mU/l at baseline (t = 0), to 45 (28–74) at t = 90 min and 52 (39–82) mU/l at t = 180 min (all P < 0.01) of forearm hyperinsulinemia. After 180
min of hyperinsulinemia FBF ratio had increased significantly by 25.8% (5.0–49.8, \( P < 0.05 \), Fig. 1). Forearm vascular responses to insulin showed a wide variability: at \( t = 180 \) min the change in FBF ratio was between –13 and 70% in all subjects. No changes in FBF were found in 3 subjects (non-responders, increase in FBF ratio \( \leq 0\% \)), although no differences were observed in forearm insulin concentrations between responders (\( n = 9 \)) and non-responders (\( n = 3 \)). The forearm vasoconstrictor response to noradrenaline (expressed as percentage decrease of FBF ratio) was not modified at \( t = 90 \) and \( t = 180 \) min of hyperinsulinemia (Fig. 2). Neither basal SBF nor SBF constrictor responses to noradrenaline changed in any of the subjects during hyperinsulinemia (Figs. 1 and 2).

### 3.2. Protocol 2 (LVDT, forearm hyperinsulinemia)

During intra-arterial insulin infusion, venous insulin levels increased from 6 (5–6) to 35 (20–46) mU/ml at \( t = 90 \) min and 40 (22–56) mU/ml at \( t = 180 \) min (all \( P < 0.01 \)). In this protocol venous insulin levels during intrabrachial insulin infusion were somewhat lower compared with protocol 1. The higher room temperature in protocol 2 (26.0 vs 24.5°C) probably resulted in a relative vasodilation, with an increase in intrabrachial blood flow. Very likely, this led to a greater dilution of the infused insulin, with subsequent lower venous insulin levels. No changes were observed in baseline venous diameter during intra-brachial infusion of insulin or placebo (data not shown). Local venous NA administration resulted in a dose-dependent decrease of venous diameter during all experiments. In comparison to \( t = 0 \), no changes in venous diameter were observed during NA administration at \( t = 90 \) and \( t = 180 \) min in either insulin or placebo experiments (Fig. 3).

### 3.3. Protocol 3 (LVDT, local hyperinsulinemia)

In Fig. 4 the dose–response curve of insulin infused directly into the dorsal hand vein is depicted. Local insulin administration resulted in a minor dose-dependent increase in venous diameter, only during the highest dose of insulin (7% [2–13], \( P < 0.05 \)). In 4 of the 14 subjects insulin did not affect venous diameter (increase in venous diameter \( \leq 0\% \)).
4. Discussion

The results of the present study indicate that regional physiological hyperinsulinemia (approx. 40–50 mU/l) induced a moderate increase in muscle blood flow (FBF) with a slow onset. Forearm constrictor responses to noradrenaline were not affected. In contrast to the increase in muscle blood flow, skin blood flow (SBF) was not modified and only minor vasodilator effects were found in hand veins (during the highest dose of insulin).

Impaired insulin-mediated vasodilation could be one of the common characteristics of NIDDM, obesity and hypertension [3,5,8,20]. The mechanism by which insulin exerts its vasodilator effect is not fully understood, especially because of the uncertainty which vascular beds are involved. Another complication is that the studies in which insulin is infused systemically are difficult to interpret, given the complex systemic changes that occur in circulating metabolites/hormones and central nervous system activity [6]. Furthermore, studies with systemic hyperinsulinemia have a duration of several hours and are, therefore, possibly confounded by diurnal rhythms in peripheral blood flow [29]. In an earlier study we showed that FBF gradually increases by approx. 60% during the day without any changes in the ratio of blood flow in both arms [29]. Thus, regional intrabrachial infusion with the use of the contralateral arm as control, enabling the calculation of the ratio of FBF in both arms, probably circumvents these problems. Until now, local studies in which insulin was infused directly into the brachial artery or into a vein have been difficult to reconcile: a very slow vasodilation in forearm studies in contrast to the rapid and very marked vasodilation in dorsal hand vein studies [14,23,24].

4.1. Insulin-mediated vasodilation in the forearm

Laakso et al. reported a dose-dependent increase of peripheral blood flow during systemic hyperinsulinemia (euglycemic clamp) in humans, with a half-maximal response at insulin levels of approximately 40 mU/l [5,8]. However, several other authors did not observe a change in peripheral blood flow during less than 2 h intrabrachial infusion of insulin [7,9,30,31]. Given the slow onset of insulin-mediated vasodilation, as observed in the present study, the time span of these experiments may have been too short.

In the present study local hyperinsulinemia resulted in a modest (26%) increase in muscle blood flow after 3 h, while skin perfusion and venous tone did not change. These data confirm a recent study of Utriainen et al. who concluded that increased peripheral blood flow is based upon vasodilation in skeletal muscle, but not in skin [19]. Baron et al. postulated that this insulin-mediated vasodilation potentiates peripheral glucose uptake [32]. However, postprandial hyperinsulinemia usually has returned to baseline after 3 h. The slow onset of vasodilation in the present study suggests that longer periods of postprandial hyperinsulinemia or possibly higher concentrations are necessary to have more relevant physiological effects on peripheral blood flow. However, after a meal not only insulin but also blood glucose concentration rises. During an euglycemic hyperinsulinemic clamp insulin-mediated vasodilation depends upon the ambient glucose concentration [20,33]. Preliminary data from our group suggest that local hyperglycemia can augment the stimulation of forearm blood flow by insulin (approx. 70% increase in FBF ratio) [34], whereas glucose per se does not affect FBF [35]. Further studies are necessary to determine the role of the postprandial rise in insulin and glucose concentration in the regulation of skeletal blood flow.

The FBF data in the present study indicate that there is a relatively wide variability in insulin-mediated vasodilation in man. In 3 of the 12 subjects no vasodilation could be observed, although the insulin concentrations of the non-responders and responders were similar. Given the very slow onset of insulin-mediated vasodilation, the time span of this experimental protocol could have been too short in the 3 non-responders. Several authors have reported that insulin-mediated vasodilation is impaired in various related disorders such as obesity, hypertension and NIDDM [3,4,8,20]. However, all subjects were normotensive, non-obese and normoglycemic. Furthermore, all had a negative family history for hypertension and diabetes mellitus. The vasodilatory effect of insulin is probably related to stimulation of NO release [11]. Therefore, the observed variability in insulin-mediated vasodilation could be the consequence of variability in NO release.

4.2. Noradrenaline in the forearm

Several authors reported that intrabrachial infusion of insulin into the human forearm results in an attenuated constrictor response to both adrenergic and non-adrenergic stimuli [9,29,30]. However, we could not demonstrate any effect of insulin (approx. 45 mU/l) on the forearm constrictor responses to noradrenaline. Also Tack et al. could not observe an effect of insulin on forearm vasculature responses to either local administered exogenous or systemic endogenous released noradrenaline [17,18]. Differences in experimental protocol could explain some of the discrepancies between the various studies. However, it should be stressed that different results can be obtained when forearm constrictor responses are analysed in another way. In several studies the constrictor response is expressed in absolute terms as change in FBF of the infused arm (ΔFBF). When our data are analysed similarly, we also find significant differences in NA responsiveness during hyperinsulinemia: ΔFBF during the highest dose of noradrenaline was −0.67 ml/dl/min at t = 0 and −1.67 at t = 180 min (t = 0 vs t = 180 min, P < 0.01). Absolute changes in FBF in response to constrictors or dilators, however, do not take into account that the diameter of the
vasculature studied could affect its subsequent response to a vasoactive drug. The data should, therefore, be analysed as the percentage change in FBF, which corrects for differences in baseline vascular tone. Finally, when forearm responses to local infusion of vasoactive drugs are evaluated, both arms should be taken into account [27]. As mentioned before in Section 2, the calculated FBF ratio corrects for all systemic factors that affect the regulation of blood flow in both arms (e.g., changes in blood pressure, level of arousal, hormonal changes etc.), and ensures that only the direct effects of locally infused substances on the forearm blood flow are taken into account. For instance, when expressed as percentage change in FBF ratio, forearm noradrenergic responsiveness remains unaltered during 24 h in healthy volunteers, and is not affected by variations in blood flow or blood pressure [25].

### 4.3. Insulin and noradrenaline in the hand vein

A common disadvantage of most dorsal hand vein studies is the unknown local insulin concentration [14,23,24]. In most of the insulin studies, veins are preconstricted first, as they have very little basal tone. Extremely high local insulin concentrations can easily be reached as the insulin is infused into a very small volume with a low flow. In the present study (protocol 2), insulin was infused into the brachial artery, resulting in comparable physiological insulin concentrations in skeletal muscle, skin and peripheral veins. This hyperinsulinaemia in the hand vein did not affect either the basal venous distension or the venoconstrictor responses to noradrenaline. In contrast, insulin infused directly into dorsal hand veins resulted in a rapid but minor venodilation only during the highest dose of insulin, which is in agreement with other authors [14,23,24]. In a protocol in which higher concentrations of insulin were administered, Feldman et al. found an even more marked venodilatation, in veins preconstricted with phenylephrine [23]. In some studies the achieved insulin concentration must have been supraphysiological, because oral glucose had to be administered to prevent systemic hypoglycaemia [23,24]. An additional confounder is that in most hand vein studies the drugs were dissolved in 5% glucose. When infused in dorsal hand veins, 5% glucose can induce an aspecific venodilation [36]. If insulin were indeed a potent rapid venodilator, one would expect that it lowers venous return, and would probably lead to a decreased cardiac output during hyperinsulinaemia. However, such a decrease in cardiac output has never been reported. Several authors, on the other hand, have found that cardiac output rises during systemic hyperinsulinaemia [37,38]. Thus, from the physiological point of view, it is (in our opinion) unlikely that insulin is a potent venodilator.

### 4.4. Conclusion

In conclusion, the present study suggests that insulin within the physiological range increases peripheral blood flow by vasodilating skeletal muscle resistance arterioles. No effect was observed on skin blood flow or venous tone. Further research is necessary to determine if the postprandial rise in insulin concentrations, in combination with the postprandial rise in blood glucose concentrations, selectively directs peripheral blood flow to skeletal muscle, one of the main targets of insulin action.

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