Dissociation of Insulin Absorption and Blood Flow During Massage of a Subcutaneous Injection Site

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Nine healthy volunteers with normal body weights were injected subcutaneously with ^{125}I-labeled soluble human insulin (10 U) in one thigh and ^{133}Xe in the contralateral thigh for the measurement of subcutaneous blood flow on 2 consecutive mornings. On one of the days, standardized massage of both injection sites was performed for 30 min starting 30 min after insulin injection. Serum insulin and plasma glucose were determined intermittently before, during, and after massage, and elimination of radioactivities was monitored continuously by external detectors. During massage, the first-order elimination rate constants of ^{125}I increased approximately sixfold compared with the rise during control (0.19 ± 0.04 to 0.88 ± 0.15%/min during the last 15 min of the massage vs. 0.21 ± 0.03 to 0.32 ± 0.03%/min during control). Serum insulin increased from 13.8 ± 1.8 mU/L before massage to a maximal value of 56.4 ± 8.7 mU/L 10 min after massage (vs. 15.3 ± 3.0 and 19.7 ± 2.2 mU/L during control). Plasma glucose fell significantly faster on the massage day, from 10 min after massage onward. No significant alteration in the subcutaneous blood flow was found during or after massage. The results suggest that the pronounced enhancement of insulin absorption induced by massage of the injection site is mainly not blood flow mediated. DIABETES CARE 1986; 9:570–74.

It has previously been demonstrated that massage of subcutaneous insulin injection sites enhances the absorption of insulin.[1–3] Increase in blood flow at the injection site has been suggested as an explanation,[2] but the subcutaneous blood flow has not been determined in connection with massage. Because basic mechanisms regulating the absorption of drugs, including insulin, from subcutaneous tissues are not fully understood, it may be valuable to study situations in detail in which pronounced alterations of the absorption rates take place. Massage was chosen as a suitable test situation because it markedly increases insulin absorption. The aim of this study was thus to investigate the mechanisms underlying the effect of massage on insulin absorption by concomitantly studying the subcutaneous blood flow responses.

SUBJECTS AND METHODS

Subjects. Nine healthy volunteers in the postprandial state (8 women and 1 man) whose mean age was 28 yr (range, 21–35) were investigated. Their body weight and body mass index were 60 ± 1 and 20.9 ± 0.7 kg/m^2, respectively. Coffee, tea, and smoking were prohibited on the day of the experiment. The subjects took no medication. The studies were conducted after approval of the Ethics Committee at Huddinge Hospital, and informed consent from the subjects was obtained.

Procedure. To reduce thyroid uptake of radioactive iodide, 100 mg potassium iodide were given orally 2 h before the first experiment and 30 mg on each of the three following mornings. Two cannulas were inserted percutaneously in antecubital veins for blood sampling and glucose administration. Radiolabeled soluble human insulin (10 U, 20 kBq ^{125}I-Actrapid, Novo, Bagsvaerd, Denmark)[4] was injected subcutaneously in one thigh within 2 s by an injection device (Department of Biomedical Engineering, Huddinge Hospital) at a standard rate and depth. A 0.4-mm-wide cannula was inserted perpendicularly to the skin to 7 mm beneath the skin surface. Thirty minutes before insulin injection in one thigh, 0.1 ml ^{133}Xe (40 kBq, Mallincrodt Diagnostica, Petten, The Netherlands) was injected within 2 min by the same technique as insulin in the same location in the contralateral thigh. Blood samples were drawn intermittently from one of the anticubital veins for analysis of plasma glucose and serum immunoreactive insulin.

Thirty minutes after insulin injection, massage of the two

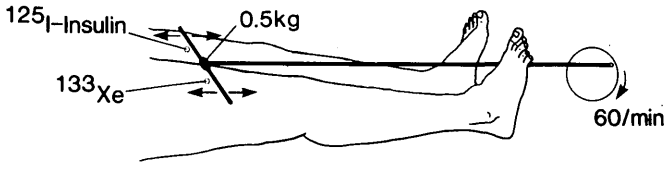

FIG. 1. Device used for massage over the injection sites.

injection sites was started, on one of the 2 consecutive days, by a specially constructed device (Fig. 1) allowing massage at a standard rate and pressure concomitantly over the symmetrically located injection sites on the two thighs. The other day served as control. Test and control days were randomized. The massage probe consisted of a 0.5-cm-wide bar covered with a rotatable plastic shield to minimize traumatizing friction against the skin. A lead weight (0.5 kg) was placed on the center of the bar. The bar moved along an axis parallel to the length axis of the legs at a rate of 60 beats/min. The massage was performed for 30 min, after which the measurements continued for an additional 60 min. The residual radioactivities over the injection sites were monitored continuously by external scintillation detectors (5 cm diam × 2.5 cm ht; Leab, Mölnlycke, Sweden) mounted ~15 cm above the skin. The detectors were equipped with conical, 14-cm-high lead collimators with internal apertures of 9 and 5 cm diam and were coupled to a four-channel spectrometer (ND 600, Nuclear Data, Schaumburg, IL) set to measure the photopeaks of ^{125}I and ^{133}Xe. Counts were accumulated over consecutive 60-s intervals and displayed on the screen of the spectrometer. After the experiment the recirculating level of ^{125}I was measured over a noninjected part of the thigh and was, if significant, subtracted from the original curve. In two control experiments, ^{133}Xe (40 kBq) was injected together with unlabeled insulin in one thigh and without insulin in the contralateral thigh to investigate whether the effect of massage on blood flow was influenced by the presence of insulin solution. Another control experiment was performed to study whether the increase in insulin absorption during massage takes place mainly via the blood or lymph circulation. Both femoral veins were cannulated on a massage and control day. Insulin was, as before, injected in one thigh. Massage was performed over both thighs in the usual manner on one of the days. Blood samples for the analysis of serum insulin were drawn simultaneously from both femoral cannulas on both days at the times indicated in Fig. 3. Room temperature varied less than 2°C during the experiments.

Analytical procedures. Serum insulin was analyzed by radioimmunoassay.[5] Plasma glucose was determined by the glucose oxidase method on a glucose analyzer (Beckman, Fullerton, CA).

Data analysis. Subcutaneous blood flow was measured by the local ^{133}Xe-clearance technique with a tissue-to-blood partition coefficient of 10 ml/g.[6,7] In the control experiment in which ^{133}Xe was mixed with insulin, only rate constants were determined because the insulin solution probably alters the partition coefficient for ^{133}Xe in the tissue. An Apple II microcomputer was used to calculate the fractional disappearance rates of ^{133}Xe and ^{125}I with linear regression analysis of the natural logarithms of the counts accumulated over consecutive 15-min intervals before, during, and after massage. The area under the serum insulin curve was calculated with trapezoidal integration.

Statistical analyses. The paired *t* test was used to determine significant differences between groups. All data are presented as means ± SE.

RESULTS

^{125}I radioactivity. A clear-cut increase in the elimination rate of the ^{125}I radioactivity from the injection site was noted within 5 min of the start of the massage. During the first 15 min of massage there was a pronounced rise of the elimination rates of the ^{125}I radioactivity to a value ~2.5 times the corresponding value during control (Fig. 2). A further rise to a level three times the value on the control day took place during the second half of the massage. The elimination rate stayed at this elevated level for 30 min after massage, and thereafter it tended to decrease. It was, however, still ~75% above control 60 min after the end of the massage. In the experiment with femoral vein cannulation the disappearance rate of ^{125}I increased from 0.17%/min before massage to 0.50%/min during the second half of the massage, compared with 0.13 and 0.17%/min during control without massage.

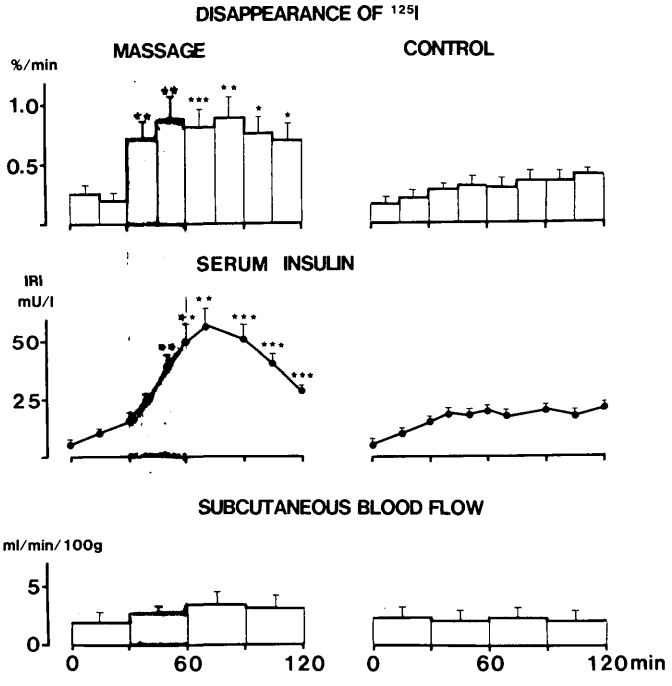

FIG. 2. Disappearance rates of ^{125}I and serum immunoreactive insulin after injection of ^{125}I-labeled soluble human insulin (10 U s.c.) in thigh at time 0. Subcutaneous blood flow in contralateral thigh is also shown. Significant differences between massage and control days are indicated. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

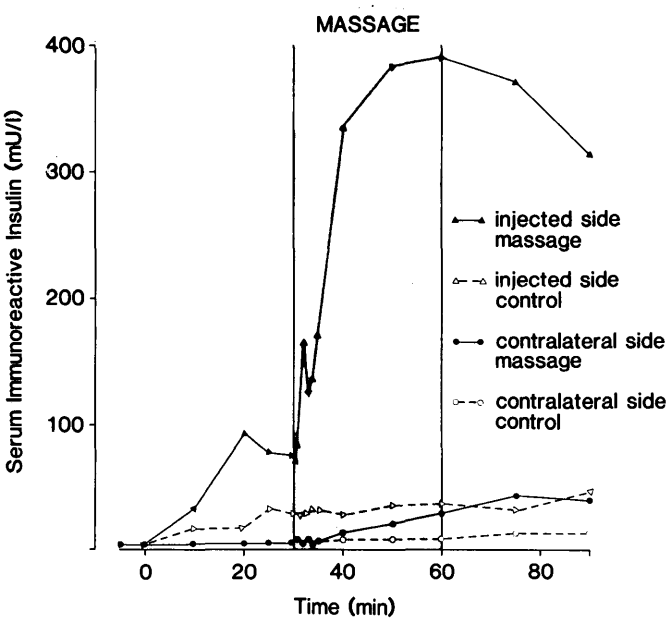

FIG. 3. Serum immunoreactive insulin in both femoral veins of subject injected with soluble human insulin (10 U s.c.) in 1 thigh at time 0 on massage day and on control day. Massage was performed between 30 and 60 min after injection.

Serum immunoreactive insulin. During massage a pronounced rise of serum insulin was found, significant from 20 min of massage onward (Fig. 2). A peak value 35 mU/L above control was found 10 min after massage, and thereafter a continuous decline was seen. Serum insulin was still 85% above the corresponding control level 60 min after the end of massage. The area under the insulin curve was similar on the massage and control days before massage but significantly greater on the massage day, both during and after massage ($P < .01$).

In the experiment with bilateral femoral vein cannulation, the serum insulin concentration from the two legs was similar before insulin injection on both the massage and the control day (Fig. 3). On the massage day, serum insulin had increased to 77 mU/L on the injected side 30 min after injection but was unaltered in the contralateral vein. Two minutes after the induction of massage, a pronounced increase in serum insulin was observed on the injected side, but on the contralateral side no increase was noted until 10 min after the massage had begun. Insulin levels in the injected leg were much higher than those in the other leg even 30 min after massage. Serum insulin levels were generally lower on the control day compared with the massage day, which is in accordance with the lower absorption rate constants on the control day. However, also during control, serum insulin was higher on the injected side during the whole measuring period but did not show the abrupt increase seen on the massage day.

Subcutaneous blood flow. Basal blood flow was similar on massage and control days: 1.9 ± 0.4 and 2.3 ± 0.3 ml · min^{-1} · 100 g^{-1}, respectively (see Fig. 2). Both during and after massage there was a tendency toward increased blood flow versus control, but the changes were not statistically significant. In the two control experiments in which ^{133}Xe was mixed with unlabeled insulin the elimination rate of ^{133}Xe showed only minor changes during massage.

Correlations. Disappearance rates of ^{125}I correlated significantly with the subcutaneous blood flow under basal conditions ($r = .77$, $P < .05$; Fig. 4). However, there was no correlation between the changes in the disappearance rate of the radioactivity and the alterations in blood flow during massage (Fig. 4). In fact, a marked increase in the insulin elimination rate of five times the basal value was seen in one subject, despite a reduction in blood flow. Furthermore, there was no correlation between the changes in the serum insulin areas and in blood flow during massage.

There was no significant relationship between the disappearance rates of ^{125}I and the area under the insulin curve during the 30 min preceding massage, but a significant correlation between the two variables was found during massage ($r = .75$, $P < .05$).

Plasma glucose. Basal plasma glucose was similar in the two groups: 4.7 ± 0.3 and 4.9 ± 0.3 mmol/L on control and massage days, respectively. At the end of the massage, plasma glucose tended to be lower than in the control group, but the difference between the groups was not significant

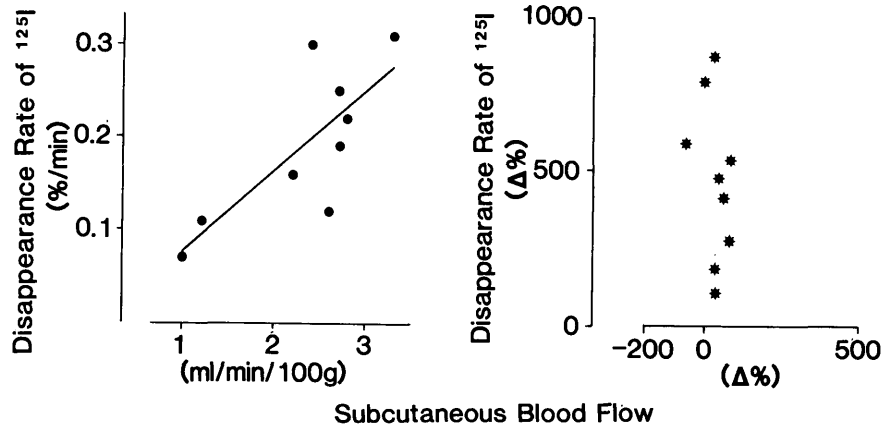

FIG. 4. *Left:* relationship between basal disappearance rate of ^{125}I-labeled soluble human insulin (10 U s.c.) injected in thigh and basal subcutaneous blood flow in contralateral thigh during 30 min preceding massage. Values denote means of 2 experimental days. $y = 0.086x - 0.009$; $r = .77$; $P < .05$. *Right:* relationship between percent change from resting level before massage to that during last 15 min of 30-min massage.

until 10 min after the massage, when values of 2.8 ± 0.4 and 3.4 ± 0.3 mmol/L, respectively, were found ($P < .05$). None of the subjects needed glucose during the control day, but on the day of massage all but one subject received glucose intravenously to alleviate the hypoglycemic response after massage.

DISCUSSION

This study clearly demonstrates that massage of the subcutaneous insulin injection site is a potent way of increasing the absorption of insulin in accordance with previous results.[1-3] In this investigation the effect is documented both as a marked rise in serum insulin and as a pronounced increase in the elimination rates of radioactively labeled insulin, the two methods giving principally the same result. In contrast to previous studies on the effect of massage of subcutaneous injection sites on insulin absorption, this study has also investigated the effect on blood flow. Obviously, during massage, at least of the type and duration used in this investigation, subcutaneous blood flow is essentially unaltered. This seems to be true not only for subcutaneous blood flow in the contralateral thigh but also for blood flow at the site injected with insulin, which may have a different blood flow regulation, because insulin solution has been shown to be vasoactive.[8] The elimination rate of ^{133}Xe was, in fact, essentially unaffected by massage also when injected together with insulin solution. Thus, although the basal absorption rate of insulin correlates significantly with the basal blood flow, as also shown previously,[9-11] the prominent increase in insulin absorption induced by massage of the injection site is mainly due to a mechanism(s) other than an increase in blood flow.

There are several mechanisms that might contribute to an increase in insulin absorption during massage despite unaltered blood flow. First, the possibility must be considered that augmented lymph drainage during massage might influence the absorption, as suggested by Dillon.[3] However, because there was a prompt and marked rise in the insulin concentration in femoral venous blood from the leg injected with insulin at the beginning of massage, it appears that the blood and not the lymph circulation is the dominating route of absorption during massage, as it is under resting conditions.[9]

Another possibility is that increases in temperature might be induced by the massage. Diathermy, for instance, has been shown to elevate the subcutaneous temperature.[12] However, a direct effect on the diffusion coefficient of insulin by a change in temperature within the physiological range is small and can probably not explain the pronounced increase in insulin absorption found in this study. In addition, a rise in temperature would probably increase the subcutaneous blood flow,[13,14] but no significant change was found during massage, which opposes a temperature effect. Furthermore, massage of injection sites might also enhance the absorption of drugs by release of vasoactive compounds, e.g., acetylcholine[15]; however, it does not seem likely in this study because the blood flow was essentially unaltered.

The changes in the tissue pressure created by the massage may influence the absorption rate in several ways. Thus, the tissue volume in which the insulin depot is distributed will probably be enlarged. In this way the insulin molecules will reach more capillaries and, according to current concepts of insulin absorption, have greater possibilities for being absorbed.[16] On the other hand, the spreading of the depot will also reduce the concentration difference across the capillary, an effect that will tend to decrease the absorption rate. Furthermore, the changes in tissue pressure will induce stirring in the interstitial space, reducing diffusion gradients that may be rate limiting for the absorption.[17] The importance of the interstitial space as a resistance to absorption from the connective tissue spaces has previously been pointed out by Schou.[15] Moreover, the possibility that increased transvascular absorption of fluid might influence insulin transport across the capillary membrane by convection, i.e., a solvent-drag mechanism, cannot be excluded. This mechanism is thought to be of little or no importance for the transport of diffusible solutes under ordinary rates of absorption and filtration,[18] but may operate when these processes are markedly augmented, which is probably the case during massage.

An entirely different mechanism by which increased absorption of insulin might occur during massage would be alteration of the structure of the insulin molecule. In concentrated neutral solution insulin is considered to be present in hexameric form.[19] Currently it is not known how soon after injection and by which mechanism(s) dissociation of insulin occurs. Possibly the dilution by tissue fluid contributes to this process, which might be enhanced by massage. Theoretically this mechanism has a potential of increasing the transcapillary transport by at least a factor of 10, i.e., considerably more than the enhancement obtained by massage in this study. The importance of a change in the molecular structure of insulin for the massage-induced enhancement of insulin absorption should be tested experimentally, e.g., by injecting monomeric insulin. Thus, it is reasonable to rule out increased blood flow as an underlying factor for the enhancement of insulin absorption by massage. Increased lymph transport also seems to be an unlikely explanation. Further studies are needed to elucidate the relative importance of changes in the diffusion conditions and in the degree of dissociation of the insulin molecule for the enhanced insulin absorption by massage.

These findings have implications for previous studies of insulin pharmacokinetics showing that absorption of insulin may already be enhanced soon after the start of a physical exercise and that the effect is more marked when the injection site is located close to the exercising muscles.[20,21] The early enhancing effect of exercise on insulin absorption has been shown to occur without increase in the subcutaneous blood flow.[21,22] This flow-independent increase of insulin absorption induced by exercise is probably due, at least partly, to the massage effect of the contracting muscles on the insulin depot, as previously suggested by Ferrannini et al.[21]

Regardless of the mechanism(s) behind the enhancement of insulin absorption by massage of the injection site, it is

evident that this is a procedure of great potency. In fact it is the most potent method currently known to enhance the absorption of subcutaneously injected insulin, with obvious therapeutic implications. This technique is one example of a practical arrangement that might be used not only for further studies of mechanisms for the absorption kinetics of insulin but perhaps also for testing massage in the clinical situation.

In summary, this study shows that massage of subcutaneous insulin injection sites markedly increases the absorption rate of insulin without significantly elevating the subcutaneous blood flow. Enlargement of the capillary surface area in contact with the insulin solution, improved transport within the interstitial space, and accelerated dissociation of the insulin molecule may contribute to the enhanced insulin absorption during massage.

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