Hypoglycemia Risk During Exercise After Intramuscular Injection of Insulin in Thigh in IDDM

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The influence of bicycle exercise (60% of W_{170} [working capacity at a pulse rate of 170 beats/min]; 40 min) on the absorption of ^{125}I-labeled fast-acting insulin (10 U; Actrapid human insulin) after intramuscular compared with subcutaneous injection in the thigh was studied on 2 consecutive days in 10 insulin-dependent diabetes mellitus (IDDM) patients. Insulin absorption was measured as disappearance of radioactivity (1st-order elimination rate constants) by continuous external monitoring and as appearance of plasma free immunoreactive insulin (IRI). Subcutaneous adipose tissue blood flow (ATBF) and skeletal muscle blood flow (MF) were measured concomitantly in the contralateral thigh with the ^{133}Xe wash-out technique. Plasma glucose was determined intermittently. The rate constant for ^{125}I-insulin increased during exercise from 0.46 ± 0.08 to 1.17 ± 0.14%/min after intramuscular injection ($P < 0.001$) and from 0.31 ± 0.05 to 0.45 ± 0.09%/min (NS) after subcutaneous injection. The rate constant of ^{125}I-insulin from muscle remained elevated during the 80-min recovery period. The peak plasma free-IRI value was 39 mU/L higher, the area under the IRI curve was ~80% greater, and the decrease in plasma glucose was ~2 mM greater after intramuscular injection. Whereas MF increased fivefold, ATBF did not rise significantly during exercise. The results demonstrate that intramuscular compared with subcutaneous thigh injection of insulin followed by bicycle exercise induces a marked increase in insulin absorption and a substantial fall in plasma glucose. Because accidental intramuscular injection of insulin may occur frequently, these findings highlight a previously unobserved risk for an unexpected decrease in plasma glucose levels in connection with leg exercise in IDDM patients. This risk could be minimized by injection into a skin fold or by use of shorter needles for thigh injection. *Diabetes Care* 13:473–77, 1990

Subcutaneous injection is the most common way to administer insulin to insulin-dependent diabetes mellitus (IDDM) patients. The rate at which the injected insulin is absorbed varies considerably not only between but also within regions, and such variability has been shown to negatively influence glycemic control (1). The background to this variability is only partially known. We previously demonstrated that the subcutaneous fat layer in thigh and abdomen is thin even in IDDM patients with normal body weight and that accidental intramuscular injection of insulin therefore probably occurs frequently, especially when the perpendicular injection technique is used (2). In a study carried out under resting conditions, we found that absorption of insulin was more rapid from the superficial parts of the thigh musculature than from the subcutaneous tissue in the thigh (3). Because marked reductions in plasma glucose are common in connection with physical exercise in IDDM patients, we studied the absorption kinetics of insulin in connection with physical exercise after subcutaneous compared with intramuscular insulin injection in the thigh. In contrast to previous studies on the effects of exercise on insulin absorption (4–10), we used computed tomography to correctly locate the injections within the musculature and subcutaneous tissue.

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RESEARCH DESIGN AND METHODS

Ten IDDM patients participated (5 women, 5 men). Their characteristics are given in Table 1. Informed consent was obtained, and the protocol was approved by the local ethics committee of Huddinge Hospital, Stockholm.

All patients were tested on a bicycle ergometer for determination of W_{170} (working capacity at a pulse rate of 170 beats/min) ~1 wk before the study. The patients arrived at the ward the day before the first study. No intermediate-acting insulin was allowed on that day. In the afternoon, the patients received a continuous intravenous infusion of fast-acting insulin (Actrapid human insulin, Novo, Bagsvaerd, Denmark; 30 U mixed with 20 ml albumin and 500 ml Ringer solution). The infusion rate was adjusted to keep plasma glucose between 5 and 8 mM. It was discontinued the next day 30 min before insulin injection. On the morning of the 1st experimental day, computed tomography of both thighs was performed with a Siemens Somatom II (Erlangen, FRG) to determine the depth of the subcutaneous tissue. Potassium iodide (100 mg) was given orally 1 h before insulin injection to prevent thyroid accumulation of radioactive iodide. A 30-mg supplement was given on each of the next 3 days.

Guided by the previous tomograms, ^{125}I-labeled Actrapid human insulin (10 U, 40 U/ml, 20 kBq; 11) was injected to a depth halfway between the skin and the muscle fascia on one of the days and 4 mm deep into muscle tissue on the other day. Injections were made in the upper middle line of the thigh at one-third the distance between the inguinal ligament and the patella. The order of intramuscular and subcutaneous injections was randomized. Injection time was 2 s, and injections were given with a device for adjusting their speed and depth (Dept. of Biomedical Engineering, Huddinge Hospital). ^{133}Xe in saline (0.1 ml; 40 kBq s.c., 400 kBq i.m., Mallinckrodt, Petten, Netherlands) was injected into the same location in the contralateral thigh to determine subcutaneous adipose tissue blood flow (12) and skeletal muscle blood flow (13). Injections were made at time 0 with the patients in a supine position with 0.4 × 20-mm needles. Fifteen minutes after the injections, the patients, still in the supine position, received a breakfast of coffee and two sandwiches with medium-fat cheese (total ~1700 kJ). The breakfast was completed within 10 min. After 60 min in the supine position, the patients performed a 40-min bicycle exercise on an ergometer (Siemens-Elema, Solna, Sweden) at 60% of their predetermined W_{170}. Exercise was followed by an 80-min recovery period in the supine position.

TABLE 1
Individual characteristics of insulin-dependent diabetes mellitus patients

Complications	Sex	Age (yr)	Diabetes duration (yr)	Body mass index (kg/m²)
Background retinopathy	M	34	16	24.5
	M	29	11	26.3
	M	23	17	21.9
Slight cataract	F	27	17	22.0
Background retinopathy	F	25	18	21.9
	M	21	9	23.9
Background retinopathy and absent achilles tendon reflex	F	32	19	24.4
Background retinopathy	F	48	18	23.9
	M	26	15	22.6
	F	21	4	24.1
All F		26*	14 ± 2†	23.2 ± 0.5†
All M		26*	13 ± 2†	23.8 ± 0.8†

*Median.
†Means ± SE.

Disappearances of ^{125}I and ^{133}Xe were monitored externally with 0.5 × 0.5-inch scintillation detectors (LEAB, Mölnlycke, Sweden) mounted directly on the skin over the injection sites. The detectors were fixed to the thighs with elastic bandages and connected to a multichannel spectrometer (ND 62, Nuclear Data, Schaumburg, IL). Counts were accumulated over consecutive 60-s intervals for 180 min.

Heart rate was determined by a one-lead ECG, and systolic blood pressure was measured using a mercury sphygmomanometer. Venous blood samples were drawn at 15-min intervals during rest and 10-min intervals during exercise for analysis of plasma glucose and at 15, 60, 80, 100, 120, and 180 min after insulin injection for determination of free immunoreactive insulin.

Plasma glucose was analyzed by a glucose oxidase method on a glucose analyzer (Beckman, Fullerton, CA). Plasma free insulin was determined on blood immediately centrifuged at 3500 × *g* for 10 min and then precipitated with 250 g/L polyethylene glycol in a phosphate buffer. Between centrifugation and precipitation (5–20 min), the samples were kept at 37°C (14). The temperature in the centrifuge was 25–29°C. The samples were then frozen and analyzed by radioimmunoassay (15).

Residual ^{125}I radioactivity at the injection sites was determined every 20 min. The time until 50% of the radioactivity had been eliminated, $t_{1/2}$, was determined by intrapolation. In four subjects the curve was extrapolated linearly in a semilogarithmic plot because $t_{1/2}$ was >180 min. The velocity constants for ^{125}I and ^{133}Xe, expressed as percent per minute, were calculated with a microcomputer (Apple, Cupertino, CA) with regression analysis of the logarithmically transformed counts per minute accumulated over consecutive 20-min intervals. The rate constant for ^{133}Xe from muscle tissue during exercise could be calculated only for the first 5-min period, after which the ^{133}Xe was washed out of the muscle. On the basis of the rate constants for ^{133}Xe in subcutaneous and muscle tissues, blood flows were calculated with a partition coefficient for ^{133}Xe in subcu-

taneous (12) and muscle (13) tissue of 10.0 and 0.7 ml/g, respectively.

The Mann-Whitney *U* test, Student's *t* test for paired data, and one-way analysis of variance were used to determine significance of differences. Data are presented as means ± SE.

RESULTS

Table 2 shows the depths of the subcutaneous tissue in the upper lateral quadrant, the upper middle line, and the upper medial quadrant of the thigh. Both men and women showed a gradient of adiposity with increasing depths from the lateral to the medial aspect of the thighs. In three of the five men, the subcutaneous layer was thinner at all measurement points than the length of the conventional 13-mm needle.

From a basal level of 0.46 ± 0.08%/min during the first 20-min period after insulin injection, the disappearance rate of ^{125}I from muscle increased rapidly at the onset of exercise and reached a level of 0.86 ± 0.11%/min during the first 20-min period of exercise, whereafter it increased further to a maximal level of 1.17 ± 0.14%/min (Fig. 1) during exercise. During the 80-min recovery period, the disappearance rate rather than decreasing tended to increase further. Thus, during the last 20 min of the study, the disappearance rate of ^{125}I-insulin from muscle measured 1.34 ± 0.12%/min. The disappearance rate from subcutaneous tissue also increased but much more slowly than from muscle. Thus, from the basal level of 0.31 ± 0.05%/min during the first 20-min period, the disappearance rate increased to a maximal value of 0.63 ± 0.06%/min at the end of the recovery period ($P < 0.01$). The $t_{1/2}$ of ^{125}I was 180 ± 12 min in subcutaneous tissue and 98 ± 8 min in muscle tissue ($P < 0.001$).

TABLE 2
Depth (mm) of subcutaneous tissue in left thigh in 10 insulin-dependent diabetes mellitus patients

Sex	Upper lateral quadrant	Upper middle line	Upper medial quadrant
M	5	11	14
M	9	16	18
M	2	8	11
F	15	17	25
F	7	14	21
M	3	10	11
F	16	19	24
F	10	17	24
M	4	5	11
F	16	25	28
All F	13 ± 2*	18 ± 2*	24 ± 1*
All M	5 ± 1*	10 ± 2*	13 ± 1*

*Means ± SE.

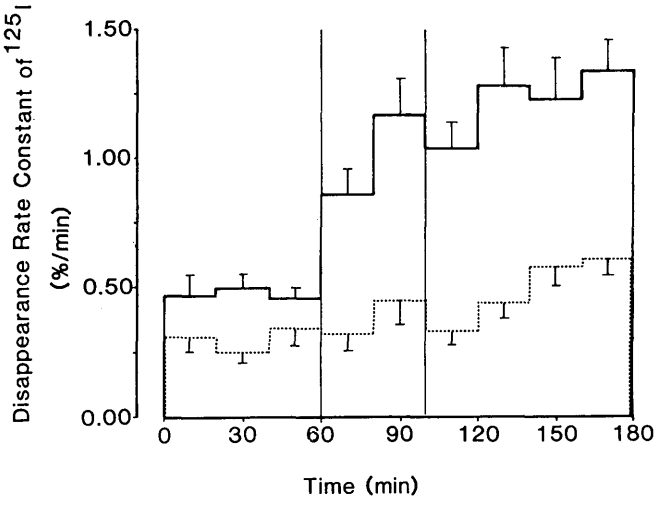

FIG. 1. Disappearance rate constant of ^{125}I in 10 insulin-dependent diabetes mellitus patients after subcutaneous (*dashed lines*) or intramuscular (*solid lines*) thigh injection of 10 U ^{125}I-labeled fast-acting insulin on separate days at time 0. Bicycle exercise (60% of working capacity at pulse rate of 170 beats/min) was carried out between 60 and 100 min. Values are means ± SE.

At the onset of exercise, plasma insulin measured 22 ± 3 and 21 ± 3 mU/L in the groups receiving intramuscular and subcutaneous injection, respectively (Fig. 2). At the end of exercise, a peak value of 65 ± 8 mU/L was reached after intramuscular injection, whereas after subcutaneous injection, the corresponding level was not >26 ± 4 mU/L. The insulin areas above the basal level measured 402 ± 121 vs. 513 ± 160 mU · L^{-1} · min^{-1} (NS) before exercise on the days of subcutaneous and intramuscular injection, respectively. During and after exercise, the corresponding values were 611 ± 125 vs. 1528 ± 238 ($P < 0.01$) and 1411 ± 260 vs. 2362 ± 242 ($P = 0.001$) mU · L^{-1} · min^{-1}, respectively. Thus, after intramuscular injection, the increases in plasma insulin during and after exercise were more than twice the increases after subcutaneous injection.

Basal plasma glucose measured 10.5 ± 0.5 and 9.4 ± 0.3 mM on the days of intramuscular and subcutaneous injection, respectively. The exercise-induced fall in plasma glucose was considerably greater on the day of intramuscular injection (4.6 ± 0.4 vs. 2.8 ± 0.7 mM, $P < 0.05$; Fig. 3).

From a basal level of 2.0 ± 0.4 ml · min^{-1} · 100 g^{-1}, adipose tissue blood flow increased slightly (~40%), although not significantly, during exercise. Skeletal muscle blood flow increased approximately fivefold (from a basal level of 0.8 ± 0.1 to 4.4 ± 1.1 ml · min^{-1} · 100 g^{-1}, $P < 0.01$) during the first 5 min of exercise.

The basal heart rate measured 67 ± 3 and 70 ± 3 beats/min after intramuscular and subcutaneous injection, respectively. At the end of exercise, the corresponding values were 156 ± 5 and 154 ± 5 beats/min. The systolic blood pressure also rose similarly (from

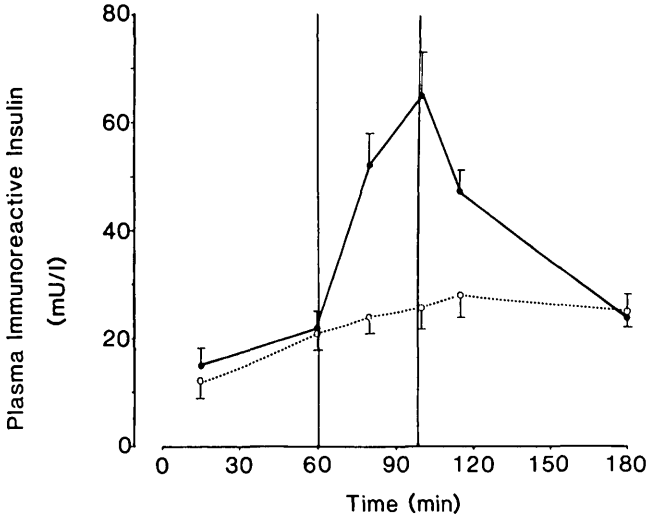

FIG. 2. Plasma free immunoreactive insulin in 10 insulin-dependent diabetes mellitus patients after subcutaneous (○) or intramuscular (●) injection of 10 U ^{125}I-labeled fast-acting insulin on separate days at time 0. Bicycle exercise (60% of working capacity at pulse rate of 170 beats/min) was carried out between 60 and 100 min. Values are means ± SE.

125 ± 5 to 160 ± 10 mmHg) at the end of the exercise period on both days.

DISCUSSION

In this study in IDDM patients, we evaluated the effects of a standardized bicycle exercise on the absorption of fast-acting insulin from subcutaneous compared with superficial intramuscular injection sites in the thigh. Compared with the absorption rate from subcutaneous deposition, the rate from muscle during exercise was more than twice as high, and the area under the plasma insulin curve was much greater, resulting in a greater plasma glucose-lowering effect. When occurring accidentally, intramuscular insulin injection may therefore lead to unexpected reductions in plasma glucose and perhaps even induce hypoglycemia. The hyperinsulinemic state, with increased glucose uptake and low hepatic glucose production, is a possible mechanism behind such a response. In this study, exercise was performed 1 h after the insulin injection. The plasma glucose-lowering effect would probably have been even greater if the exercise had occurred soon after the insulin injection when more insulin remained at the injection site. This would probably also be the case after a higher insulin dose.

In view of previous studies and measurements herein of the depth of the subcutaneous tissue in the injected regions, these findings have therapeutic implications (2,3). To avoid accidental intramuscular injection and the risk of unexpected reductions in plasma glucose, patients should be instructed to make injections into a skin fold or to use shorter (<8-mm) needles for thigh injection.

The mechanisms behind the enhancing effect of exercise on insulin absorption from muscle are not evident from this study. Certainly, vasodilation plays an important role because capillaries are recruited, and diffusion distances in the interstitial space are reduced during exercise. The degree of vasodilation in muscle tissue during exercise is much greater than in subcutaneous tissue (16), as is also illustrated by the blood flow responses in this study. However, note that, although blood flow in the exercising muscles increased severalfold, the rate of insulin absorption did not rise more than ~100%. Assuming that capillary diffusion capacity is rate limiting for insulin absorption, this agrees with the previously established relationship between capillary recruitment and increase in blood flow in skeletal muscle during exercise (16).

It is unlikely that the high insulin absorption rate in the postexercise period would mainly be associated with changes in the blood circulation (17). Massage-induced enhancement of insulin absorption, which occurs despite unaltered blood flow (18), may be due to insulin oligomers (19) dissociating into dimers and monomers when absorbed (20). Monomeric insulin analogues are absorbed faster in animals (21) and humans (22). Such dissociation, which could be associated with increased paracapillary circulation (16), might partially explain the pronounced enhancement of insulin absorption from muscle during and after exercise. This hypothesis should be tested by studying monomeric insulin analogues in connection with exercise.

The elevation of the ^{125}I disappearance rate from skeletal muscle in the recovery period occurred simultaneously with a sharp decline in the plasma insulin con-

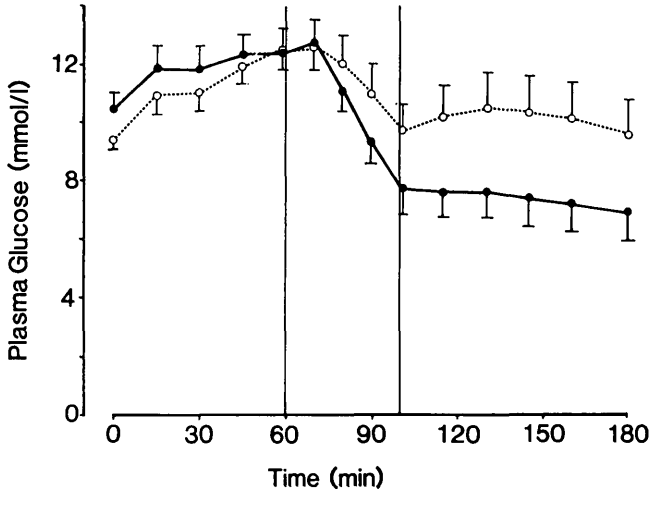

FIG. 3. Plasma glucose in 10 insulin-dependent diabetes mellitus patients after subcutaneous (○) or intramuscular (●) injection of 10 U ^{125}I-labeled fast-acting insulin on separate days at time 0. Bicycle exercise (60% of working capacity at pulse rate of 170 beats/min) was carried out between 60 and 100 min. Values are means ± SE.

centration. This may appear to be a discrepancy. However, note that the plasma insulin levels are influenced not only by the amount absorbed but also by uptake in peripheral tissues, which may change in connection with exercise. Furthermore, much of the insulin was absorbed already during exercise.

Compared with previous data on the effects of bicycle exercise on insulin absorption, the increase in the rate of absorption from the subcutaneous thigh depot in this study was small (4–10). This may be because, in this study, the depot was located in the middle of the subcutaneous layer. In earlier studies, injection sites and depths of injection have seldom been well defined. A deep subcutaneous depot might be influenced by the massagelike effect of the contracting muscle, massage being a potent means of enhancing insulin absorption (9,18,23).

In summary, we have demonstrated a previously unobserved risk for unexpected decreases in plasma glucose in connection with leg exercise in IDDM patients. Thus, after intramuscular injection of insulin in the thigh, which may frequently occur by accident, exercise with the thigh musculature markedly increases the absorption rate of the injected insulin and induces a considerable fall in plasma glucose. This risk can be reduced by injecting into a skin fold or by use of shorter (<8-mm) needles for thigh injection.

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