Interstitial Insulin During Euglycemic-Hyperinsulinemic Clamp in Obese and Lean Individuals

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Transcapillary insulin transport has been considered a rate-limiting step of insulin action. However, direct measurement of interstitial insulin levels during physiologic levels of insulinemia have not been performed. We determined changes in interstitial insulin in eight healthy non-obese men and seven healthy obese men by microdialysis during a euglycemic-hyperinsulinemic clamp. Interstitial insulin was determined in the subcutaneous tissue of the abdomen and thigh. Steady-state insulin concentrations were reached approximately 10 minutes after the start of insulin infusion in the subcutaneous tissue of the abdomen and thigh and returned to basal levels approximately 10 minutes after the infusion was discontinued. There was no difference in the rapidity of change in interstitial insulin between obese and lean individuals at either site studied, irrespective of the pattern of fat distribution. The relative change in dialysate insulin concentration during the euglycemic clamp did not differ between obese and lean individuals at either site studied. It was also unaffected by the waist to hip ratio. The rapid change in interstitial insulin concentration could be of physiologic significance in determining the effects of changes in circulating insulin concentration. We conclude that transcapillary insulin transport in adipose tissue is unaffected by obesity and the pattern of fat distribution in healthy men. It is also concluded that when interstitial insulin is determined directly, transcapillary insulin transport is rapid and does not demonstrate a significant lag phase.

INSULIN RESISTANCE is an important feature of several disease states, including type II diabetes mellitus and syndrome X. Insulin resistance is usually defined by the relationship between circulating insulin and glucose uptake. However, there is a poor correlation between the dynamics of arterial insulin levels and the dynamics of glucose uptake.[1-3] Mathematical modeling of insulin dynamics data suggests that interstitial insulin, rather than plasma insulin, is a more direct determinant of insulin action in vivo.[4] Since the interstitial fluid is in direct contact with the insulin-responsive cells, measurement of the interstitial fluid insulin level is likely to provide a better understanding of the relationship between insulin concentration and insulin action. Few studies have directly measured the concentration of insulin in interstitial tissue. Since lymph is formed by the movement of interstitial fluid into the lymph capillaries through gaps between overlapping lymphatic capillary endothelial cells, the concentration of insulin in the lymphatics has been used as an indirect measure of interstitial insulin. These studies have indicated that the changes in lymphatic insulin are different from those seen in the plasma, suggesting that the capillary endothelium is a barrier to insulin transport.[5-7] Further studies of lymphatic insulin in humans and dogs have shown that lymphatic insulin levels are lower and achieve steady state significantly later than plasma insulin levels during euglycemic-hyperinsulinemic clamp studies.[8-12] The reported delay in achieving steady-state insulin levels in the lymph was similar in magnitude to the delay in reaching steady-state glucose uptake in these studies. These results suggest that transendothelial transport of insulin may be a rate-limiting step in insulin action and that defects in insulin transport could have pathophysiologic significance. However, changes in lymph insulin may not provide accurate information about changes in actual interstitial insulin concentrations, the rate of change, and insulin action. A more direct measure of interstitial insulin may well provide additional and useful information. It is now possible to determine interstitial insulin by the technique of microdialysis.[13-15] We have used this technique to measure changes in interstitial insulin concentrations in healthy non-obese and obese men during a euglycemic-hyperinsulinemic clamp. The use of an ultrasensitive insulin assay made it possible to measure dialysate insulin concentration during the basal state and during physiologic levels of hyperinsulinemia. Changes in interstitial insulin concentrations during physiologic levels of insulinemia have not been previously reported in human subjects.

There is significant correlation between obesity, specifically abdominal obesity, and insulin resistance.[16] The kinetics of insulin action have also been noted to be delayed in obese subjects.[17,18] It has been postulated that reduced skeletal muscle capillary density in the obese might result in altered tissue insulin concentrations and "apparent" insulin resistance.[19] The reason for insulin resistance in individuals with abdominal fat distribution remains unclear. It must be noted that thigh fat is relatively inert, whereas abdominal fat is metabolically active.[20] Thus, differences in regional insulin exposure may be a factor in overall insulin action and resistance. Blood flow through human adipose tissue, measured by the clearance rate of radioactive xenon, decreases significantly with increasing thickness of fatty tissue.[21] It is possible that differences in blood flow and/or transendothelial transport of insulin in the subcutaneous adipose tissue between the abdomen and thigh could lead to differences in interstial insulin levels in subjects with different patterns of fat distribution, which would not be apparent in lymph samples obtained from thoracic duct lymph. The measurement of interstitial insulin levels at

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different sites in subjects with different patterns of fat distribution has not been previously studied and may provide important new information regarding insulin resistance and obesity, especially if a change in interstitial insulin concentration, via altered transcapillary insulin transport, precedes the development of insulin resistance. We measured changes in interstitial insulin in the subcutaneous tissue of the abdomen and thigh during a euglycemic-hyperinsulinemic clamp. The present study indicates that when directly measured, transcapillary insulin transport is more rapid than previously hypothesized, and suggests that there may be no significant differences in the rate or time required to reach steady state in plasma and interstitial fluid in normal individuals despite differences in adiposity.

SUBJECTS AND METHODS

Fifteen healthy men, eight non-obese and seven obese, without glucose intolerance, endocrine disorders, cardiac disease, or hypertension were recruited for the study. These disorders were excluded by a medical history, physical examination, and laboratory evaluation. All subjects had a normal glucose tolerance test result. A chest x-ray and electrocardiogram were used to confirm the absence of overt cardiac disease. Results of these investigations were verified before subjects were included in the study. Clinical characteristics of the study group are shown in Table 1. Obesity was assessed by body mass index (body weight in kilograms divided by height in meters squared) and percent body weight. The study was approved by the Human Studies Subcommittee at the Louisville Veterans Administration Medical Center. Studies were conducted in random order after obtaining informed consent from all volunteers.

Euglycemic-Hyperinsulinemic Clamp

The procedure for the euglycemic clamp has been previously described.[1] After an overnight fast, a cannula was inserted retrogradely into a dorsal wrist vein, and the hand was maintained at 68°C to obtain arterialized venous blood. A primed insulin infusion was used for the study. Crystalline biosynthetic human insulin (Squibb Novo, Princeton, NJ) was infused at a rate of 287 pmol/min/m^2. Two milliliters of the patient's blood was added to 50 mL infusate to reduce insulin adsorption to the infusion apparatus. Computer-based algorithms for maintaining euglycemia during clamp studies may be associated with an initial decrease in plasma glucose levels. The following modifications were therefore incorporated to minimize fluctuations in plasma glucose. The glucose infusion was initiated 2 minutes after beginning the insulin infusion. Plasma glucose values were obtained every 5 minutes, and manual adjustment of the glucose infusion rate was initiated if judged appropriate by the investigator. A basal period of 2.5 hours and a hyperinsulinemic-euglycemic clamp of 2 hours was used. During the basal period, intravenous lines were established and microdialysis probes properly positioned. A steady flow of 5 μL/min through the microdialysis probes was obtained. Once this was established, samples were collected in 5-minute aliquots. Pooled samples of 20-minute intervals at −40, −20, and 0 minutes before the start of insulin infusion were used for measurement of insulin concentration. During the hyperinsulinemic phase, 10-minute dialysate samples were pooled for ultrasensitive insulin assay. At the end of this period, insulin infusion was discontinued and samples were obtained for another hour, during which euglycemia was maintained. During this phase, again, 20-minute dialysate samples were pooled for ultrasensitive insulin assay.

Table 1. Subject Characteristics (mean ± SEM)

Characteristic	Obese (n = 7)	Non-obese (n = 8)
Age (yr)	28.8 ± 1.8	25.86 ± 1.7
Weight (kg)	95.75 ± 3.85	73.91 ± 1.52
Body mass index	30.44 ± 0.99	23.78 ± 0.78*
% Ideal body weight	135 ± 4.6	104 ± 3.076*
Waist to hip ratio	0.91 ± 0.01	0.84 ± 0.01
Fasting blood glucose (mmol/L)	5.06 ± 0.08	5.09 ± 0.15
Basal insulin (pmol/L)	82.26 ± 11.3	57.67 ± 4.47

*$P < .01$.

Determination of Interstitial Insulin Levels

Interstitial insulin levels were determined by microdialysis. A sterile 20-gauge needle was inserted approximately 1 cm into the subcutaneous fat of the lower quadrant of the abdomen and upper thigh on the same side. A sterile 22-gauge microdialysis probe was inserted through the needle and extended 1 cm beyond the cannula tip into the adipose tissue. Microdialysis probes (CMA/10) were purchased from Carnegie Medicin (Stockholm, Sweden). The probes consisted of a tubular membrane (polycarbonate/polyether copolymer, outer diameter 0.5 mm, length 16 mm, 10,000 molecular weight cutoff) glued to a double-lumen steel cannula. The needle and the probe were left in situ during the study. The inlet tubing of the probe was connected to a precision microinjection pump (CMA/100; Carnegie Medicin), and the probe was perfused with sterile buffer at a rate of 5 μL/min. The perfusate flowed between the membrane and the inner cannula of the probe, and dialysate was collected through the outer tubing (dead volume, <3 μL). Samples were collected continuously throughout each study at 5-minute intervals.

To check probe performance, the relative in vitro recovery of insulin was measured on each probe after every study. The probe was placed in a vial containing 1 mL perfusion medium with either ^{125}I-labeled insulin (100 pmol/L) or an equal quantity of buffer. The vials were maintained at 37°C in a water bath, and the probe was perfused with the same buffer and at the same rate used in sampling the subcutaneous tissue. The relative in vitro recovery was calculated as a ratio of the radioactive counts in the probe dialysate to the counts in the same volume of incubation medium. Probe performance was checked by varying the concentrations of labeled insulin. After the medium in the vial was quickly changed, dialysate radioactivity reached a new steady state in approximately 10 minutes. In vitro relative recovery for insulin was constant for each probe at each site.

Plasma and Dialysate Insulin Assays

Radioimmunoassay of plasma insulin was performed using an antibody from Linco Research (St Louis, MO). The primary antibody was raised against modified porcine insulin. The range of the assay was 7 to 1,085 pmol/L. The interassay coefficient of variation was approximately 5%. Cross-reactivity with human proinsulin was approximately 8%.

Radioimmunoassay of dialysate insulin was performed using an ultrasensitive human insulin assay (Linco). The primary antibody was raised against human insulin. The range of the assay was 0.7 to 70 pmol/L. The interassay coefficient of variation was approximately 5%. Cross-reactivity with human proinsulin was approximately 6%. Human insulin was used as a standard in both assay systems.

Statistical Analysis

All results are expressed as the mean ± SEM. Differences in basal measurements between groups were analyzed by Student's *t*

test. Differences in fold increases in interstitial insulin levels between and within sites were analyzed by ANOVA.

RESULTS

During the euglycemic-hyperinsulinemic clamp, dialysate insulin level reached steady state less than 20 minutes after beginning the insulin infusion (Figs 1 to 4). Since equilibration of the microdialysis probe took 10 minutes, interstitial insulin levels must have attained steady state within 10 minutes after the start of insulin infusion. The decrease in dialysate insulin levels was also equally rapid (~10 minutes) after discontinuing insulin infusion. Steady-state interstitial insulin levels were reached in about 10 minutes in both obese and non-obese subjects. There was rapid (~10 minutes) change in dialysate insulin concentration in subcutaneous tissue of the abdomen and thigh after the start and end of insulin infusion during the euglycemic-hyperinsulinemic clamp. Body mass index and waist to hip ratio did not affect the time required to reach steady-state levels.

The relative change in dialysate insulin during the euglycemic-hyperinsulinemic clamp in the abdomen in obese subjects (3.26 ± 0.508) was not significantly different from that in the thigh in the same group (3.04 ± 0.58, $P > .05$). Similarly, the relative change in dialysate insulin in non-obese subjects between the abdomen (2.62 ± 0.18) and thigh (2.86 ± 0.19) was not significantly different ($P > .05$). Furthermore, the relative increase in dialysate insulin was not significantly different at the sites studied or between obese and non-obese individuals ($P > .05$). There was no correlation between the waist to hip ratio and the relative change in dialysate insulin at either the abdomen or the thigh.

DISCUSSION

In the present study, there was a rapid increase in dialysate insulin concentration during conditions of physiologic hyperinsulinemia in both obese and non-obese men. Following a change in circulating insulin concentration, the rapidity of change in dialysate insulin concentration was unaffected by obesity or the pattern of fat distribution. Our results indicate a more rapid transendothelial transport of insulin across the capillary wall than previously suggested by lymph studies.[10-12]

Studies of lymph insulin and differences between insulin action in perfused adipose tissue and cultured adipocytes have suggested that the transport of insulin across the capillary endothelium is restricted, which may account for the reported delay in the achievement of steady-state insulin concentration between arterial blood and samples obtained from lymph vessels.[5-10,22] The importance of this barrier to the dynamics of insulin action has been emphasized by studies of lymph insulin during euglycemic clamps. Insulin transport across the capillary endothelium is probably a receptor-mediated process. Insulin receptors have been demonstrated in the cell membrane of vascular endothelial cells.[23] Receptor-mediated transport of insulin across cultured vascular endothelial cells with little degradation of insulin has been demonstrated.[24-26] The importance

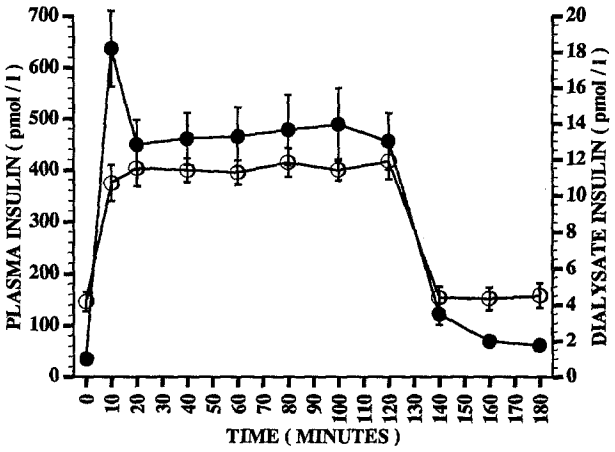

Fig 2. Plasma (●) and dialysate (○) insulin concentrations during a euglycemic-hyperinsulinemic clamp in non-obese subjects with the microdialysis probe in the thigh.

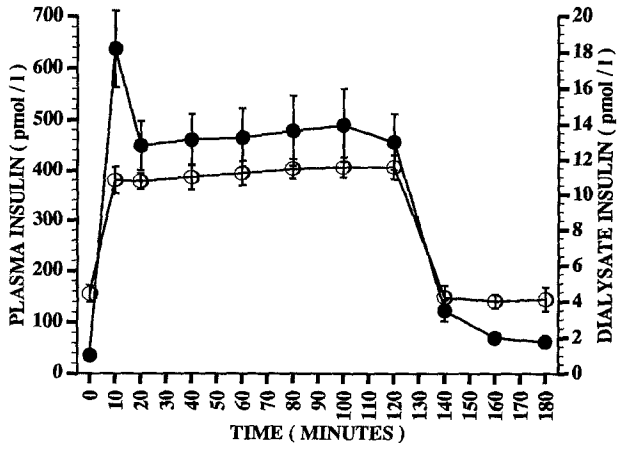

Fig 1. Plasma (●) and dialysate (○) insulin concentrations during a euglycemic-hyperinsulinemic clamp in non-obese subjects with the microdialysis probe in the abdomen.

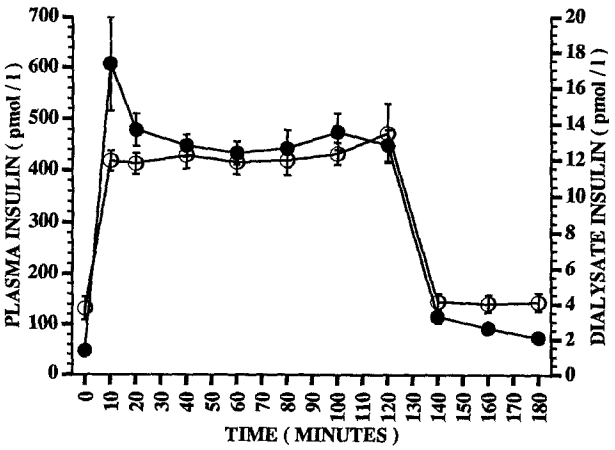

Fig 3. Plasma (●) and dialysate (○) insulin concentrations during a euglycemic-hyperinsulinemic clamp in obese subjects with the microdialysis probe in the abdomen.

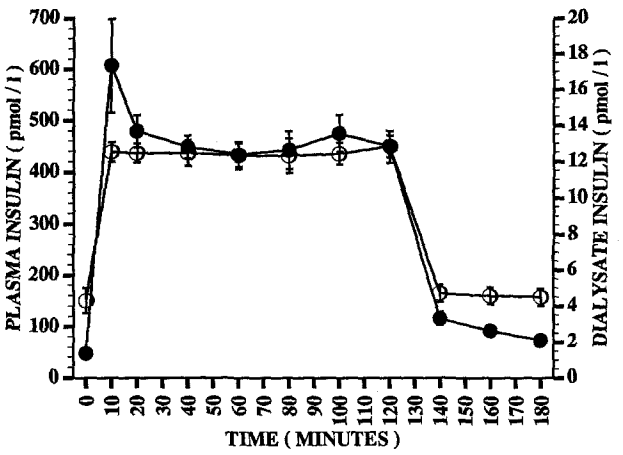

Fig 4. Plasma (●) and dialysate (○) insulin concentrations during a euglycemic-hyperinsulinemic clamp in obese subjects with the microdialysis probe in the thigh.

of receptor-mediated transcapillary insulin transport in skeletal muscle has been recently reported.[27]

Vascular receptor-mediated transport has been demonstrated in vitro in different tissues and in vivo in animal models.[24,28,29] Dernovsek et al[24] reported that in cultured endothelial cells, 95% of cellular-bound insulin dissociated intact within 5 to 15 minutes. Our results are consistent with receptor-mediated transport of insulin across the capillary wall. Since lymph insulin is a less direct measure of interstitial insulin, it is possible that the delay in appearance of insulin in the lymph is partly due to the delay in transport into the lymphatic capillaries after it has reached the interstitial space. Studies of lymph insulin have emphasized the delay in the increase of insulin in lymph samples and in achieving a steady-state concentration as compared with insulin in the plasma. The close correlation between the dynamics of glucose uptake and the dynamics of lymphatic insulin levels has been interpreted as proof that transendothelial insulin transport is the rate-limiting factor for insulin action. Our studies would indicate that a delay in transendothelial insulin transport is only partly responsible for the reported delay in achieving steady-state glucose uptake during euglycemic clamp studies.

The only other study in which microdialysis was used to measure interstitial insulin levels in humans showed a delay in the appearance of insulin slightly greater than that observed in the present study.[15] However, there are some important methodological differences between the two studies. Hyperinsulinemia was achieved by infusion of insulin at 120 mU/m^2/min, and after a period of equilibrium this was increased to 240 mU/m^2/min. These rates are severalfold greater than the rate used in the present study, and the plasma insulin concentration reached was at or above the levels at which maximal effects of insulin have been reported.[30,31] Furthermore, the glucose utilization rate did not change when the insulin infusion rate was increased from 120 to 240 mU/m^2/min. It is important to note that Jansson et al[15] did not measure interstitial insulin levels in the basal state. It is possible that there is a greater delay in transport when insulin levels are increased from already unphysiologically high levels, when transport mechanisms could be near saturation. In a recent study, Holmang et al[27] measured muscle insulin concentration by microdialysis in the rat during a euglycemic-hyperinsulinemic clamp, and found that transcapillary transport of insulin is saturable. At the plasma insulin levels achieved in the study reported by Janssen et al, transendothelial insulin transport was near saturation. The present study used insulin levels in the physiologic range and measured the rate of change from basal arterial and interstitial insulin concentrations. This was made possible by the use of an ultrasensitive insulin assay that allows determination of low levels of insulin present in the dialysate during both the basal period and the insulin infusion.

We found a rapid increase in dialysate insulin concentration after starting an insulin infusion in both obese and non-obese individuals, at both abdominal and thigh sites, irrespective of the pattern of fat distribution. It has been postulated that defects in insulin transport could be present in various states of insulin resistance. Defects in insulin receptor structure and function have been demonstrated in endothelial cell cultures from diabetic rats and in endothelial cells cultured with a high glucose concentration, suggesting that changes in insulin transport across the capillary endothelium could have pathophysiologic significance.[32,33] No differences between obese and non-obese individuals could be detected in transcapillary insulin transport in this study, but this does not preclude the possibility that small differences may exist in interstitial fluid insulin concentration or in the degree of endothelial receptor availability between the two groups. Obtaining the actual interstitial insulin concentration at each site was not possible in this study for technical reasons, requiring the titration of insulin levels by calculating the rate of loss or gain of insulin from the probes in the tissues. Titration requires an additional 8 to 10 hours during which the subject is immobile and fasting. Since dialysate insulin concentrations are low and the ultrasensitive insulin assay requires a volume of at least 50 μL, at perfusion flow rates of 5 μL/min, only samples taken at 10-minute intervals can be accurately analyzed. Furthermore, the time required to reach a new steady-state dialysate insulin concentration after a rapid change in medium insulin concentration during in vitro calibration precludes the determination of small differences in the rapidity of transendothelial insulin transport. It could also be due to the fact that obese individuals in this group were only mildly obese and had normal glucose tolerance. Our findings are in agreement with recent observations in lymph studies in humans. Castillo et al[12] also failed to demonstrate any relation between lymph insulin and insulin resistance, suggesting that transendothelial insulin transport may not be a factor in insulin resistance. It is possible to estimate the rate of change of insulin concentration in the interstitial fluid by our technique, but absolute levels cannot be precisely validated. This would require a lengthy in vivo

calibration, which we did not perform out of consideration for the comfort of the subjects.

During euglycemic-hyperinsulinemic clamp conditions, the muscle is the major site of glucose disposal; adipose tissue plays a relatively minor role. It is possible that changes in muscle interstitial fluid transport are different from those in adipose tissue. It has been shown that there is a greater correlation of glucose utilization with hindlimb lymph insulin, which is derived from the muscle, than with thoracic duct lymph insulin, which is predominantly derived from the abdominal viscera. It has also been demonstrated that transendothelial transport is much more rapid in the liver than in the muscle.[34] Scintigraphic studies have shown hepatic localization of insulin analogs within minutes of systemic injection.[35] It is possible that transendothelial transport in the metabolically active adipose tissue is more akin to that in the liver. Measurement of interstitial fluid insulin levels in the muscle by microdialysis could potentially demonstrate differences in transcapillary insulin transport between adipose tissue and muscle. The failure to demonstrate significant differences in the changes in interstitial insulin between abdomenal and thigh adipose tissue would indicate that differences in transendothelial insulin transport are unlikely to be an important determinant of the insulin resistance associated with abdominal fat distribution in normal individuals. Thus, it appears that factors at or within the cells are responsible for the insulin resistance. Determination of interstitial insulin, particularly in the muscle, by microdialysis during oral or intravenous glucose tolerance tests and in diabetic individuals could further clarify the role of the endothelial barrier in physiologic and pathologic states. The rapid transport of insulin across the endothelium could be expected to be more efficient in transmitting the peripheral insulin pulses to insulin-responsive cells. These pulses are considered important determinants of insulin action.[36] In conclusion, changes in adipose tissue interstitial insulin levels during euglycemic-hyperinsulinemic clamping, as measured by microdialysis, are more rapid than the changes in lymph insulin that have been previously reported. Transendothelial insulin transport is not a major determinant of the insulin resistance associated with obesity and abdominal fat distribution in the absence of abnormal glucose tolerance.

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