# Effects of Insulin on Glucose Uptake and Leg Blood Flow in Patients With Sickle Cell Disease and Normal Subjects

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**The hemodynamic concept of insulin resistance assumes that vasodilatory effects of insulin determine glucose uptake. Sickle cell disease (SCD) is characterized by microangiopathy and microvascular occlusion. Therefore, we hypothesized that patients with SCD have a reduced insulin-mediated glucose uptake. In 8 patients with SCD and 8 matched normal controls, we studied the effects of a 4-hour insulin infusion (50 mU/kg/h) on glucose uptake and leg blood flow (LBF) using the euglycemic clamp technique and venous occlusion plethysmography. Time-control experiments were performed in the same subjects. Insulin-mediated glucose uptake (M value, mg/kg/min) did not differ between patients with SCD and control subjects during the second (6.3 ± 4.6 and 7.6 ± 2.6, $P = .5$), third (7.5 ± 4.6 and 9.3 ± 3.4, $P = .4$) and fourth hour (8.6 ± 4.7 and 11.0 ± 2.9, $P = .2$) of the clamp. At baseline, LBF was higher in the patients with SCD than in the controls (3.28 ± 1.68 and 1.37 ± 0.47 mL/min/dL, respectively; $P = .005$). Insulin-induced increases in LBF in patients with SCD and in normal subjects were not different ($P = .9$). Respectively, 56% and 24% of the changes in glucose uptake could be explained from changes in LBF in the course of the insulin infusion in the patients with SCD and controls. We suppose that the comparable insulin sensitivity between both groups is due to a compensatory hemodynamic state in SCD characterized by vasodilation and increased flow.**


A LINK BETWEEN the hemodynamic, ie, vasodilatory, and metabolic effects of insulin has been widely investigated, but not conclusively demonstrated. A direct relationship between insulin-mediated changes in leg blood flow (LBF) and glucose uptake has been shown during physiologic and supraphysiologic hyperinsulinemia in healthy volunteers.[1-4] Also, a colocalization of insulin-mediated muscle blood flow with regional glucose uptake using positron emission tomography has been shown.[5] However, it remains to be established whether insulin itself or its metabolic effects determine its vascular effects.

The hemodynamic concept of insulin resistance, originally propagated by Baron,[6] assumes that the hemodynamic effects of insulin determine glucose uptake. This concept is supported by previous observations that insulin itself rather than the rate of carbohydrate metabolism determines the magnitude of insulin-induced vasodilation.[7,8] In contrast, this hemodynamic concept has been questioned by the results of other studies that assessed the effects of diverse pharmacologic interventions on muscle blood flow and insulin-mediated glucose uptake.[9] These studies reported no or limited effects of changes in blood flow on glucose uptake. However, as an important drawback of these studies, it could not be excluded that the pharmacologic agents used had flow-independent effects on glucose uptake.

An alternative model to assess the role of insulin's hemodynamic effect on glucose uptake is provided by the presence of preexistent hemodynamic abnormalities, especially in the microcirculation. We have previously shown that microvascular function relates to insulin sensitivity in normal subjects.[10] Also, a correlation has been shown between skeletal muscle capillary density and insulin sensitivity in man.[11] These previous studies were not able to attribute causality to associations and, moreover, illustrated that insulin sensitivity relates to other factors as well, especially waist-to-hip ratio (WHR) as a measure of body fat distribution. The causality of microcirculatory abnormalities for insulin sensitivity could be studied further in two matched groups of individuals who differ only by the presence or absence of microvascular abnormalities.

In sickle cell disease (SCD), hemodynamic changes occur early in life. Microcirculatory flow disturbances and microvascular occlusion are hallmarks of SCD.[12-14] We, therefore, hypothesized that patients with SCD are insulin-resistant compared with matched normal subjects. Because the effect of insulin on muscle blood flow is time-dependent[15] and because we have previously found a correlation between insulin's effects on blood flow and glucose uptake during prolonged hyperinsulinemia,[2] we expected to find differences in insulin sensitivity between both groups during sustained hyperinsulinemia. Therefore, we measured insulin sensitivity and leg muscle blood flow during a 4-hour physiologic insulin infusion period in patients with SCD and matched normal subjects.

## SUBJECTS AND METHODS

Eight patients with SCD participated in the studies. Five of them had sickle cell anemia, 2 were heterozygous for hemoglobin S and C, and 1 was heterozygous for hemoglobin S and $\beta$-thalassemia. All had microvascular disease, as illustrated by a defective concentrating capacity of urine: after 24 hours, thirsting maximal urine osmolality amounted to 435 + 51 mOsmol/kg water (mean ± SD). The healthy volunteers were matched to the patients with SCD for age, sex, race, and body mass index (Table 1). Blood pressure levels did not differ between both groups. The normal subjects had a normal hemoglobin A pattern as assessed by hemoglobin electrophoresis. All patients with SCD and normal subjects had a normal 75-g oral glucose tolerance test according to World Health Organization (WHO) criteria. Patients did not use medication other than folic acid supplementation. Informed

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*Submitted March 16, 2000; accepted October 24, 2000.*

*Supported by the Dutch Kidney Foundation (Nier Stichting Nederland, Grant No. C 94.1378).*

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*0026-0495/01/5004-0004$35.00/0*

*doi:10.1053/meta.2001.21681*

**Table 1. Characteristics of Healthy Volunteers (n = 8) and Patients With SCD (n = 8)**

| Characteristics | Healthy Volunteers | Patients With SCD | *P* Value |
|---|---|---|---|
| Age (yr) | 36 ± 9 | 36 ± 9 | .7 |
| Sex (male/female) | 5/3 | 5/3 | 1.0 |
| Height (cm) | 175 ± 13 | 170 ± 10 | .3 |
| Body weight (kg) | 73 ± 13 | 69 ± 15 | .3 |
| Body mass index (kg/m$^2$) | 23.9 ± 3.6 | 23.7 ± 4.5 | .8 |
| SBP (mm Hg) | 126 ± 13 | 122 ± 16 | .6 |
| DBP (mm Hg) | 70 ± 10 | 65 ± 12 | .4 |
| Hemoglobin (mmol/L) | 7.6 ± 1.0 | 5.4 ± 1.4 | .005 |
| Fasting glucose (mmol/L) | 4.2 ± 0.1 | 4.2 ± 0.3 | .8 |

NOTE. Data are reported as mean ± SD.

consent was obtained from all subjects. The protocol had been approved by the local ethical committee, and the study was performed in accordance with the Declaration of Helsinki.

### *Study Design*

Each subject underwent an insulin infusion and a time-control study in a randomized order on 2 separate days at least 7 days apart. After an overnight fast, all subjects came to the clinic at 8 AM. Two polytetrafluoroethylene cannulae (Venflon; Viggo, Helsinborg, Sweden) were inserted for intermittent blood sampling and infusions. Measurements were performed during the second hour of a 120-minute basal period and a subsequent 4-hour study period.

To achieve a similar degree of volume loading during both studies, all subjects were given 300 mL of water each hour. This volume was administered as tap water during the time-control studies. During insulin infusion, water was given as 20% D-glucose infusion added with tap water orally to 300 mL water each hour.

### *Insulin Infusion Study*

The hyperinsulinemic euglycemic clamp technique was used for insulin infusion and to assess sensitivity to insulin-mediated glucose uptake, as described previously.[16,17] Insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark), diluted to 50 mL with 45 mL of 0.9% saline and 5 mL of 20% human albumin, was infused in a primed, continuous manner at a rate of 50 mU/kg/h for 4 hours. Normoglycemia was maintained by adjusting the rate of a 20% D-glucose infusion based on frequent plasma glucose measurements with an automated glucose oxidase method (Yellow Springs Instruments, yellow Springs, OH). In normal subjects, it has been shown that hepatic glucose production is completely suppressed during the insulin infusion rate given.[18] Presumably, this also holds true for patients with SCD, because whole body glucose utilization and endogenous glucose production under steady state conditions are similar between patients with SCD and control subjects.[19] Therefore, whole body glucose uptake (M value) was calculated from the glucose infusion rate during the second, third, and fourth hour of insulin infusion.[20] Blood samples for measurement of plasma insulin were drawn 4 times during the second and fourth hour of the clamp. Plasma insulin concentrations were measured by radioimmunoassay (Immunoradiometric Assay; Medgenix Diagnostics, Fleurus, Belgium).

### *Time-Control Study*

Control experiments were performed in an identical fashion with administration of the same amount of fluids and with sampling of the same amount of blood. Control experiments enabled us to correct for nonspecific changes in the variables under evaluation.

### *Hemodynamic Measurement*

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured every 10 minutes with a semicontinuous blood pressure measuring device (Nippon Colin BP 103 N Sphygmomanometer; Hayashi, Komaki-City, Japan). Six measurements were performed during each 1-hour clearance period. LBF was measured in the calf at baseline and during the second and fourth hour of the clamp. Measurements were performed by venous occlusion plethysmography using mercury-in-silastic strain gauges.[2] Each result of measurement of resting LBF (mL/min/dL) represents the average of 7 to 10 separate recordings. In our hands, this technique has a coefficient of variation (CV) of 14%.[2]

### *Power Calculation*

Using data from previous studies in normal subjects,[21,22] we have calculated a CV for measurements of insulin-mediated glucose uptake of 22%. Using these data, the present study had a power .80 at a significance level of .05 to measure a difference in M value of 2.1 mg/kg/min between patients with SCD and control subjects.

Likewise, the present study had a power of .80 at a significance level of .05 to measure an increase in LBF of 0.43 mL/min/dL in normal subjects.

### *Statistics*

Between-group differences were analyzed with the Student's *t* test. To detect differences over time and between the studies and between the groups, the variables were analyzed by 2-way analyses of variance (ANOVA) for repeated measurements. To aid interpretation of the relative magnitude of changes in LBF in the figures, the percentage change from baseline during the time-control studies was subtracted from the percentage change during insulin infusion. Correlation analyses were used to investigate associations between insulin's metabolic and vasodilatory effects and were performed by standard methods. A value of $P < .05$ was considered to be significant. Data are expressed as mean ± SD unless stated otherwise.

## RESULTS

Baseline values of blood pressure, heart rate, and LBF did not differ between the insulin infusion and the time-control studies in both groups. Comparison of the averaged mean baseline values of both groups showed that LBF was higher in the patients with SCD than in the normal subjects (3.28 ± 1.68 and 1.37 ± 0.47 mL/min/dL, respectively; $P = .005$). Except for the expected differences in hemoglobin levels, other characteristics did not differ between both groups (Table 1).

### *Euglycemic Clamp Data*

During the euglycemic clamp, glucose levels averaged 4.2 ± 0.3 mmol/L in patients with SCD and 4.2 ± 0.1 mmol/L in control subjects. The results of insulin-mediated glucose uptake (M value, mg/kg/min) in patients with SCD and controls are shown in Fig 1.

Insulin-mediated glucose uptake increased during the course of the insulin infusion in both groups. However, M values did not differ between both groups during the second ($P = .5$), third ($P = .4$), and fourth hour ($P = .2$) of the clamp. Values at the third hour are not further discussed because they were always intermediate between the second and fourth hour. The CV of the blood glucose level during insulin infusion was 7.4 ± 2.2 in the patients with SCD and 9.4% ± 2.7% in the controls. Measurement of plasma insulin levels in patients with

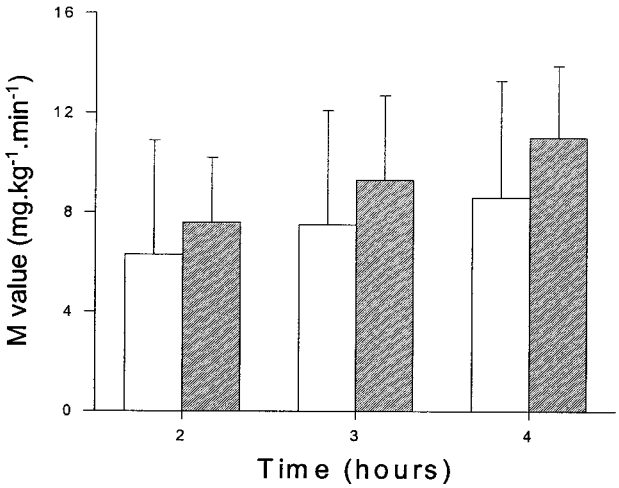


**Fig 1. Insulin-mediated glucose uptake (M value, mg/kg/min) in patients with SCD (□, n = 8) and matched normal subjects (▩, n = 8) during hyperinsulinemic euglycemic clamp studies.**

SCD was hampered by chronic low-grade hemolysis. Plasma insulin levels averaged 513 ± 103 and 521 ± 100 pmol/L during the second and fourth hour of insulin infusion in the normal subjects.

*Effect of Insulin on Systemic Hemodynamics*

The hemodynamic data obtained in the normal subjects and in the patients with SCD are listed in Table 2. SBP and DBP did not change during the experiments. Heart rate increased significantly during insulin infusion in the normal subjects, but not in the patients with SCD. Comparison of changes in heart rate between both groups showed that changes in heart rate in the normal subjects exceeded the changes in the patients with SCD (ANOVA, $P = .001$). The changes in LBF are shown in Fig 2. Insulin-induced increases in LBF reached significance in normal subjects ($P = .004$), but not in patients with SCD ($P = .14$). The increases in LBF did not differ between both groups ($P = .9$).

*Correlation Analysis*

Because basal LBF was higher in the patients with SCD, while insulin sensitivity tended to increase more in the normal subjects, we performed additional regression analyses looking for explanations of the changes observed.

First, we examined the correlation between LBF at baseline and the changes in LBF during insulin infusion (Fig 3). We found a significant positive correlation ($r = .75$, $P = .03$) in the normal subjects as opposed to the patients with SCD ($r = -.38$, $P = .3$). These results suggested that the relationship between changes in LBF and basal LBF was modified by the the presence or absence of SCD. Indeed, effect-modification by group was confirmed by additional regression analysis with changes in LBF as dependent variable, and basal LBF, group and product-term of basal LBF and group as independent variables. The product-term appeared to be a significant ($P = .04$) determinant of changes in LBF in this model.

In addition, we investigated the correlation between changes in glucose uptake and LBF during sustained insulin infusion, ie, the changes that occurred from the second until the fourth hour of the insulin infusion. This analysis showed similar regression coefficients in the group of SCD patients and controls (Fig 4).

**Table 2. Changes in Systemic Hemodynamics During Insulin Infusion and Time-Control Studies in Patients With SCD and Normal Subjects**

| | Baseline | 2 Hour | *P*2 | 4 Hour | *P*4 |
|---|---|---|---|---|---|
| SBP (mm Hg) | | | | | |
| Sickle cell | | | | | |
| Insulin | 123.9 ± 22.9 | 122.4 ± 16.3 | NS | 128.6 ± 19.0 | NS |
| Control | 119.3 ± 9.8 | 122.0 ± 13.7 | | 121.9 ± 13.6 | |
| Normals | | | | | |
| Insulin | 123.2 ± 12.2 | 126.7 ± 12.0 | NS | 126.2 ± 11.7 | NS |
| Control | 129.2 ± 14.0 | 129.4 ± 12.8 | | 129.4 ± 14.8 | |
| DBP (mm Hg) | | | | | |
| Sickle cell | | | | | |
| Insulin | 65.7 ± 13.0 | 64.8 ± 11.0 | NS | 66.5 ± 13.6 | NS |
| Control | 63.7 ± 12.1 | 65.7 ± 11.4 | | 65.4 ± 11.0 | |
| Normals | | | | | |
| Insulin | 67.9 ± 9.0 | 67.8 ± 10.4 | NS | 68.3 ± 8.1 | NS |
| Control | 72.9 ± 12.0 | 72.4 ± 13.6 | | 72.6 ± 11.4 | |
| Heart rate (beats/min) | | | | | |
| Sickle cell | | | | | |
| Insulin | 65.4 ± 10.0 | 66.3 ± 11.1 | NS | 65.8 ± 10.9 | NS |
| Control | 60.1 ± 11.4 | 61.6 ± 12.1 | | 62.7 ± 11.5 | |
| Normals | | | | | |
| Insulin | 61.1 ± 5.5 | 65.1 ± 6.4 | .002 | 66.6 ± 6.8 | .01 |
| Control | 63.0 ± 8.3 | 62.3 ± 6.0 | | 62.7 ± 9.4 | |

NOTE. Data are expressed as mean ± SD. The *P*2 and *P*4 columns denote significant differences between the insulin infusion and time-control studies at 2 hours and 4 hours, respectively.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; sickle cell, patients with sickle cell disease; normals, normal subjects; insulin, insulin infusion study; control, time-control study.

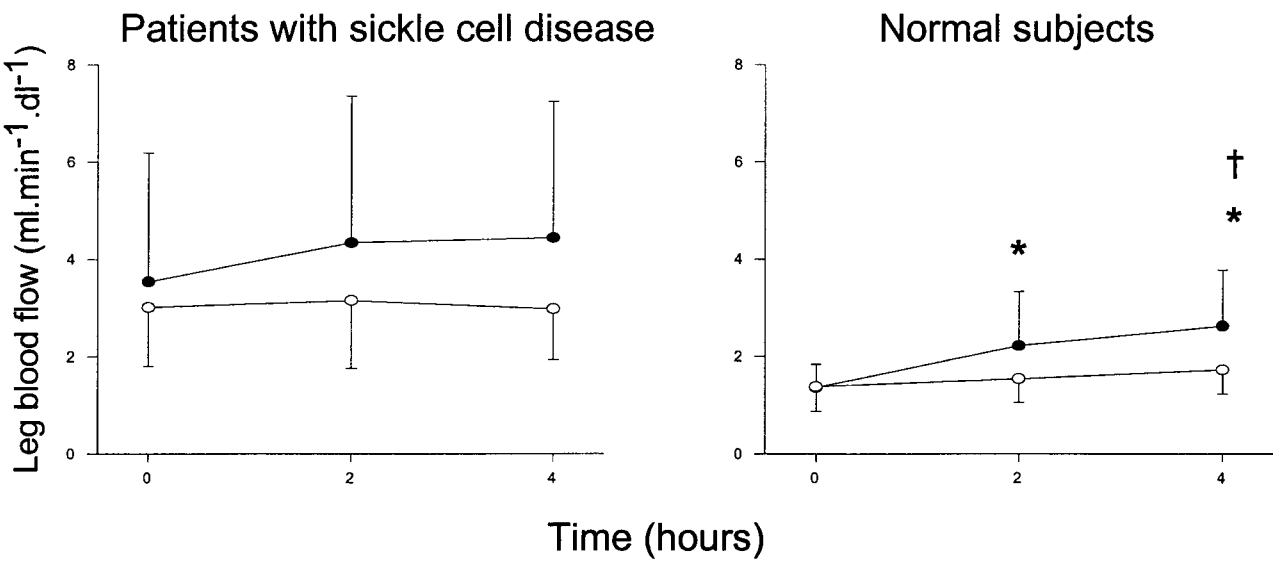


**Fig 2. LBF in patients with SCD and matched normal subjects during hyperinsulinemic euglycemic clamp (●) and time-control studies (○). * $P < .05$ for the changes during insulin infusion compared with baseline. † $P < .05$ for the changes during insulin infusion compared with time-control studies.**

Using the latter data in a regression analysis, we found that 56% and 24% (adjusted $R^2 = .56$ and .24) of the changes in glucose uptake from the second until the fourth hour of insulin infusion could be explained by concomitant changes in LBF in patients with SCD and controls, respectively.

## DISCUSSION

The hypothesis that patients with SCD are insulin-resistant compared with matched control subjects could not be confirmed by the results of our study. This observation does not yet reject the concept that insulin's hemodynamic effects determine glucose uptake, because basal LBF was higher in patients with SCD. Moreover, 56% and 24% of the changes in glucose uptake from the second until the fourth hour of insulin infusion could be explained from changes in LBF in patients with SCD and controls, respectively.

The outcome of this study rests on the assumption that patients with SCD differ from their matched controls by microcirculatory abnormalities, but not by other factors known to affect insulin-mediated glucose uptake. In general, capillary blood flow in SCD is reduced due to increased blood viscosity related to red blood cell deformity and increased adhesiveness between sickle erythrocytes and endothelium.[12-14] However, little is known about microcirculatory blood flow in patients

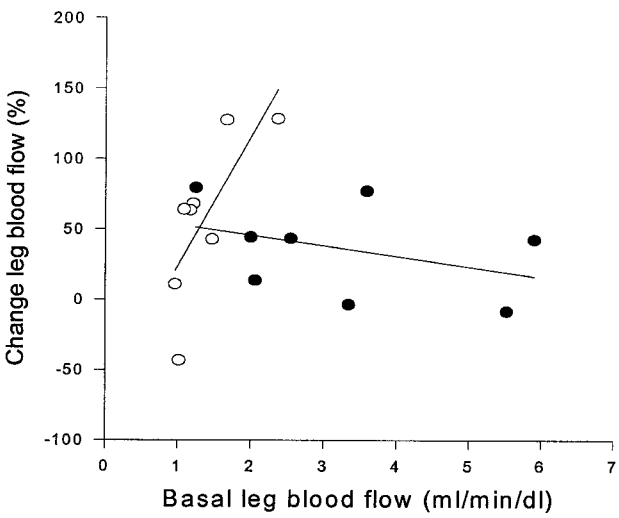


**Fig 3. Correlation between basal LBF and percentage changes in LBF during hyperinsulinemic euglycemic clamp studies in patients with SCD (●, $r = -.38$, $P = .3$) and normal subjects (○, $r = .75$, $P = .03$).**

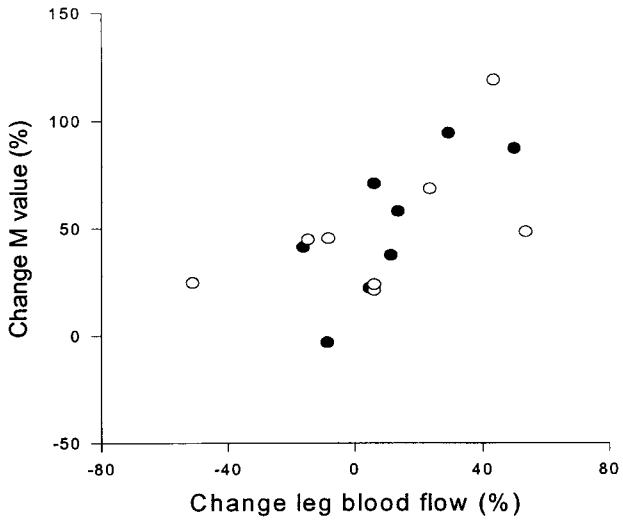


**Fig 4. Correlation between percentage changes in LBF and glucose uptake during the fourth *v* second hour of insulin infusion in patients with SCD (●, $r = .75$, $P = .03$) and normal subjects (○, $r = .59$, $P = .13$).**

with SCD. Perfusion of animal muscle with human sickle red blood cells resulted in reduced microvascular flow and an increase in microvascular resistance and compensatory vasodilation with shunting of blood through pathways parallel to capillaries occluded by red blood cells.[23] In patients with SCD, microvascular blood flow has been studied in skin and nailfold capillaries.[24-26] These studies showed large oscillations in local blood flow consistent with periodic flow at the arteriolar level.[24,25] These observations have been attributed as compensatory to intermittent vascular occlusion.[25,26]

Considering these abnormalities, we originally hypothesized the following causes for a decreased insulin-mediated glucose uptake in patients with SCD: (1) inhomogenity of muscle blood flow and increased diffusion distance from capillary to tissue due to intermittent capillary occlusion, (2) decreased time for diffusion due to enhanced blood flow in the open capillaries, and (3) lack of additional vasodilating effect of insulin.

Contrary to our expectations, insulin-mediated glucose uptake did not differ between patients with SCD and matched control subjects. Both groups were individually matched for possible confounding factors relating to insulin sensitivity such as age, sex, race, and body mass index, as well as physical fitness. We did not not measure muscularity,[15] but 24-hour urinary excretion of creatinine did not differ between both groups (data not shown). By matching for these factors, we expected to have a specific and reliable model to study the role of hemodynamics as a determinant of insulin sensitivity.

However, baseline values of LBF differed significantly between both groups. We had taken this possibility into account, considering previous observations of an increased overall skeletal muscle blood flow in SCD,[27] which as been attributed to the presence of anemia.[28] Nevertheless, we hypothesized that the microcirculatory defects would decrease insulin sensitivity despite an elevated basal muscle blood flow in SCD due to arteriovenous shunting.

At first sight, the lack of difference in insulin sensitivity between both groups argues against a causal role of insulin's hemodynamic effects for determining insulin sensitivity. At a closer look, our results may even support the hemodynamic concept of insulin resistance taking into account the elevated LBF at baseline in patients with SCD. This elevated basal LBF presumably reflects a compensatory state characterized by vasodilation and increased microcirculatory flow to compensate for intermittent capillary occlusion in SCD and attendant anemia. As a result of this concept, muscle blood flow reserve should be limited in SCD, especially in the patients with the highest basal levels of LBF. Indeed, a limited muscle blood flow reserve in SCD was suggested by the outcome of correlation analysis between basal LBF and insulin-induced changes therein. Insulin-mediated changes in LBF showed a positive correlation with baseline values in normal subjects, whereas we did not find such a correlation in the patients with SCD. This apparent difference between the 2 groups showed to be statistically significant on testing for effect-modification of the associations by group ($P = .04$ for the product-term of basal LBF and group).

Thus, the elevated basal LBF in SCD presumably prevented the demonstration of a difference in insulin sensitivity between both groups. Alternatively, the possibility of a type 2 error cannot be excluded, because mean values of insulin-mediated glucose uptake in the normal subjects exceeded mean values in patients with SCD at all points of measurement, albeit not significantly. According to our power calculation, the size of our study population was sufficient to detect significant differences. We calculated that our study had a power of 80% to detect a difference in M value of 2.1 mg/kg/min between patients with SCD and controls. Differences in M value amounted to 1.30, 1.79, and 2.45 mg/kg/min during the second, third, and fourth hour of the insulin infusion, respectively. In addition, we calculated that our study had a power of 80% to detect an increase in LBF of 0.43 mL/min/dL. As expected, we observed a significant increase in LBF in the normal subjects. In patients with SCD, increases in LBF were similar, but varied widely, not reaching statistical significance.

We assumed that differences in insulin-mediated glucose uptake between both groups would become especially apparent during sustained hyperinsulinemia considering a time-dependent effect of insulin on muscle blood flow and glucose uptake.[2,15] As expected, insulin-mediated glucose uptake increased in the normal subjects, but also in the SCD patients. Insulin sensitivity tended to dissociate between both groups in the course of the insulin infusion, albeit not significantly. By correlation analysis, remarkably similar relationships were observed between changes in glucose uptake and changes in LBF during prolonged hyperinsulinemia in both groups. Regression analysis showed that 56% and 24% of the changes in glucose uptake could be explained by changes in LBF in patients with SCD and controls, respectively. However, causality cannot be attributed to associations. Thus, as an alternative possibility, insulin-induced glucose metabolism could explain 56% and 24% of changes in LBF in SCD patients and controls.

A primary role for insulin's metabolic effects in determining blood flow gains support from a recent in vitro observation. In a study of endothelial cell cultures, it was shown that wortmannin, an inhibitor of phosphatidylinositol 3-kinase, inhibited insulin-stimulated nitric oxide production.[29] Because phosphatidylinositol 3-kinase is necessary for insulin-stimulated glucose transport, these results indicate that nitric oxide as an effector of insulin signalling pathways that are also involved with glucose metabolism.[29]

Finally, heart rate increased only in the normal subjects. This increase in heart rate is possibly secondary to insulin-induced vasodilation. Alternatively, the lack of increase in heart rate in SCD patients could reflect an impairment of sympathetic nervous system activation, in analogy to previous observations that cold-induced sympathetic stimulation did not affect forearm vascular resistance in SCD.[27]

To conclude, insulin-mediated glucose uptake did not differ between normal subjects and patients with SCD presumed to be characterized by intermittent microvascular occlusion. We suppose that the lack of difference in insulin sensitivity between both groups has been confounded by a compensatory hemodynamic state in SCD displaying vasodilation and increased microvascular flow. Therefore, our observations do not refute the hemodynamic concept of insulin resistance. Rather, during sustained hyperinsulinemia, respectively, 56% and 24% of the changes in glucose uptake could be explained from changes in LBF in patients with SCD and controls.

## REFERENCES


1. Baron AD, Brechtel-Hook G, Johnson A, et al: Skeletal muscle blood flow: A possible link between insulin resistance and blood pressure. Hypertension 21:129-135, 1993
2. Ter Maaten JC, Voorburg A, Heine RJ, et al: Relationship between insulin's haemodynamic effects and insulin-mediated glucose uptake. Eur J Clin Invest 28:279-284, 1998
3. Bonadonna RC, Saccomani MP, Del Prato S, et al: Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. Circulation 98:234-241, 1998
4. Cleland SJ, Petrie JR, Ueda S, et al: Insulin-mediated vasodilation and glucose uptake are functionally linked in humans. Hypertension 33:554-558, 1999
5. Utriainen T, Nuutila P, Takala T, et al: Intact insulin stimulation of skeletal muscle blood flow, its heterogeneity and redistribution, but not of glucose uptake in non-insulin dependent diabetes mellitus. J Clin Invest 100:777-785, 1997
6. Baron AD: Hemodynamic actions of insulin. Am J Physiol 267: E187-E202, 1994
7. Vollenweider P, Tappy L, Randin D, et al: Differential effects of hyperinsulinemia on carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest 92:147-154, 1993
8. Tack CJJ, Schefman AEP, Willems JL, et al: Direct vasodilator effects of physiological hyperinsulinemia in human skeletal muscle. Eur J Clin Invest 26:772-778, 1996
9. Yki-Järvinen H, Utriainen T: Insulin-induced vasodilation: Physiology or pharmacology? Diabetologia 41:369-379, 1998
10. Serné EH, Stehouwer CDA, ter Maaten JC, et al: Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. Circulation 99:896-902, 1999
11. Lillioja S, Young AL, Culter CL, et al: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. J Clin Invest 80:415-424, 1987
12. Francis RB, Johnson CS: Vascular occlusion in sickle cell disease: Current concepts and unanswered questions. Blood 77:1405-1414, 1991
13. Hebbel RP: Adhesive interactions of sickle erythrocytes with endothelium. J Clin Invest 99:2561-2564, 1997
14. Bunn HF: Pathogenesis and treatment of sickle cell disease. N Engl J Med 337:762-769, 1997
15. Utriainen T, Malmström R, Mäkimattila S, et al: Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. Diabetologia 38:555-564, 1995
16. Gans ROB, van der Toorn L, Bilo HJG, et al: Renal and cardiovascular effects of exogenous insulin in healthy volunteers. Clin Sci 80:219-225, 1991
17. Ter Maaten JC, Voorburg A, Heine RJ, et al: Renal handling of urate and sodium during acute physiological hyperinsulinaemia in healthy subjects. Clin Sci 92:51-58, 1997
18. Ponchner M, Heine RJ, Pernet A, et al: A comparison of the artificial pancreas (glucose-controlled insulin infusion system) and a manual technique for assessing insulin sensitivity during euglycaemic clamping. Diabetologia 26:420-425, 1984
19. Borel MJ, Buchowski MS, Turner EA, et al: Alterations on basal nutrient metabolism increase resting energy expenditure in sickle cell disease. Am J Physiol 274:E357-E364, 1998
20. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1978
21. Ter Maaten JC, Voordouw JJ, Bakker SJL, et al: Salt sensitivity correlates positively with insulin sensitivity in healthy volunteers. Eur J Clin Invest 29:189-195, 1999
22. Ter Maaten JC, Bakker SJL, Serné EH, et al: Insulin's acute effects on glomerular filtration rate correlate with insulin sensitivity whereas insulin's acute effects on proximal tubular sodium reabsorption correlate with salt sensitivity in normal subjects. Nephrol Dial Transplant 14:2357-2363, 1999
23. Lipowsky HH, Usami S, Chien S: Human SS red cell rheological behaviour in the microcirculation of cremaster muscle. Blood Cells 8:113-126, 1982
24. Lipowsky HH, Sheikh NU, Katz DM: Intravital microscopy of capillary hemodynamics in sickle cell disease. J Clin Invest 80:117-127, 1987
25. Rodgers GP, Schechter AN, Noguchi CT, et al: Periodic microcirculatory flow in patients with sickle-cell disease. N Engl J Med 311:1534-1538, 1984
26. Kennedy AP, Williams B, Meydrech EF, et al: Regional and temporal variation in oscillatory blood flow in sickle cell disease. Am J Hematol 28:92-94, 1988
27. Hatch FE, Crowe LR, Miles DE, et al: Altered vascular reactivity in sickle hemoglobinopathy. A possible protective factor from hypertension. Am J Hypertens 2:2-8, 1989
28. Varat MA, Adolph RJ, Fowler NO: Cardiovascular effects of anemia. Am Heart J 83:415-426, 1972
29. Zeng G, Quon MJ: Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. J Clin Invest 98:894-898, 1996