Role of Basal Insulin in Maintenance of Intracellular Glucose Metabolic Pathways in Non–Insulin-Dependent Diabetes Mellitus

Kyo-Il Suh, Yuh-Min Song, Chisato Murata, Mary Joyce, Timothy M. Ditzler, and Robert R. Henry

Impairments of both basal and insulin-stimulated oxidative (G_{ox}) and nonoxidative (N_{ox}) glucose metabolism are documented to exist in non–insulin-dependent diabetes mellitus (NIDDM). Although these defects have been well characterized during insulin stimulation, little is known about the effects of basal insulin or its deficiency on intracellular glucose metabolism in NIDDM. To determine the physiological significance of basal insulin in the maintenance of glucose metabolism in NIDDM, we studied nine subjects with NIDDM in the basal and insulin-deficient state produced by 3 hours of somatostatin (SRIF) infusion (0.08 pmol/kg/min). Glucose turnover rates were quantified by [3-^{3}H]glucose turnover, and substrate oxidation was assessed by a combination of indirect calorimetry and urinary nitrogen measurements. Skeletal muscle glycogen synthase (GS) and pyruvate dehydrogenase (PDH) activities were also measured in the basal state and during SRIF infusion. Basal glucose levels were maintained during SRIF infusion by exogenous glucose infusion (12.5 ± 0.9 mmol/L in the basal state *v* 12.8 ± 0.8 during SRIF infusion, P = NS). During the last hour of SRIF infusion, plasma C-peptide levels declined by 88% from 0.73 ± 0.11 to 0.09 ± 0.02 nmol/L ($P < .001$), and serum insulin concentrations were undetectable (<14 pmol/L). During insulinopenic conditions, rates of glucose uptake (GU) were decreased by 12% from basal level of 2.26 ± 0.13 to 1.99 ± 0.12 mg/kg/min ($P < .05$), and were entirely accounted for by reduced rates of G_{ox} (1.01 ± 0.10 to 0.65 ± 0.14 mg/kg/min, $P < .01$). Corresponding measurements of active PDH (PDHa) activity in skeletal muscle were similarly decreased from 0.50 ± 0.14 to 0.31 ± 0.09 nmol/min/mg protein ($P < .05$) during insulinopenia, whereas rates of fat oxidation (F_{ox}) were increased from 0.87 ± 0.05 to 1.05 ± 0.07 mg/kg/min ($P < .01$). The reduction in energy expenditure (EE) from lower G_{ox} was completely offset by increased energy from enhanced F_{ox} and resulted in unchanged total EE (1.42 ± 0.10 *v* 1.41 ± 0.11 kcal/min, P = NS). Although skeletal muscle GS activity was significantly decreased (2.6% ± 0.7% to 1.8% ± 0.8%, $P < .05$), calculated rates of N_{ox} remained unchanged (1.24 ± 0.17 to 1.34 ± 0.21 mg/kg/min, P = NS) and were significantly correlated with non–insulin-mediated GU (NIMGU) during insulinopenia ($r = .76$, $P < .05$). In conclusion, approximately 35% of basal G_{ox} in NIDDM was dependent on basal insulin action. Basal N_{ox} was independent of basal insulin and was determined by the rate of NIMGU. Basal insulin regulates intracellular glucose oxidation by multiple mechanisms that include effects on glucose uptake, free fatty acid (FFA) substrate availability, and PDH enzyme activity.

ONCE GLUCOSE IS TAKEN UP by the cell, it is phosphorylated and routed into either oxidative (G_{ox}) or nonoxidative (N_{ox}) pathways of glucose metabolism. Each of these pathways has different dose-response characteristics to circulating insulin concentrations, and partitioning of intracellular glucose into each pathway is determined primarily by the prevailing insulin concentration.[1-3] In normal subjects, in vivo evaluations of the dose-response relationship between these pathways and insulin have demonstrated that the G_{ox} pathway is more sensitive to stimulation by insulin over the physiologic range than the N_{ox} pathway.[1-3] In agreement with this finding, Felber et al[4] have also demonstrated that basal levels of insulin stimulate G_{ox} but not N_{ox}, and that approximately 50% of basal G_{ox} is dependent on basal insulin action in normal subjects.

In patients with non–insulin-dependent diabetes mellitus (NIDDM), impaired sensitivity of both G_{ox} and N_{ox} to insulin stimulation is now well documented,[5-7] and the dose-response relationship between G_{ox} and insulin is demonstrated to be shifted rightward under hyperinsulinemic conditions.[6] However, the dependency of each pathway of glucose metabolism on basal insulin availability is presently unknown in NIDDM. Since the pathways of glucose metabolism are resistant to insulin action in NIDDM, the possibility exists that either of these pathways may not be directly influenced by basal insulin action in NIDDM. On the other hand, if a minimal level of insulinization is required for a pathway to be operative, basal insulin might be critically important to maintain some activity, even in NIDDM.

The mechanism by which basal insulin regulates the individual intracellular glucose pathways is also unknown. Current evidence indicates that during hyperinsulinemic conditions, activations of skeletal muscle pyruvate dehydrogenase (PDH) and glycogen synthase (GS) are the principal regulatory mechanisms leading to insulin stimulation of G_{ox} and N_{ox}, respectively, in NIDDM and normal subjects.[1,2,7] However, in the basal state of NIDDM, factors such as glucose and free fatty acid (FFA) substrate availability may emerge as primary determinants of intracellular glucose pathway activity when low insulin levels are combined with peripheral insulin resistance.

The current study was therefore undertaken in subjects with NIDDM to (1) determine the dependency of G_{ox} and N_{ox} pathways on basal insulin action, and (2) explore the mechanisms by which basal insulin modulates the pathways

From the Department of Medicine, University of California-San Diego, La Jolla; and the San Diego Veterans Administration Medical Center, San Diego, CA.

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Address reprint requests to Robert R. Henry, MD, Veterans Affairs Medical Center (V111G), 3350 La Jolla Village Dr, San Diego, CA 92161.

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Table 1. Characteristics of Study Subjects

Age (yr)	57.1 ± 6.6
Weight (kg)	95.6 ± 15.9
FFM (kg)	66.3 ± 12.3
BMI (kg/m^2)	31.7 ± 3.7
Fasting glucose (mmol/L)	12.5 ± 0.9
Fasting insulin (pmol/L)	192 ± 6
Duration of NIDDM (yr)	5.6 ± 1.2

NOTE. Values are the mean ± SE; n = 9.
Abbreviations: FFM, fat-free mass; BMI, body mass index.

of glucose and fat metabolism. To accomplish this goal, intracellular glucose and fat metabolism were assessed by open-circuit indirect calorimetry during measurement of glucose turnover in the presence of basal insulin (basal period) and in the absence of insulin (somatostatin [SRIF] infusion period). The activities of PDH and GS in skeletal muscle were also determined from needle biopsies of vastus lateralis muscle. Our results indicate that basal insulin primarily influences the oxidative pathways of both glucose and fat metabolism in NIDDM by altering substrate availability rather than enzyme activity.

SUBJECTS AND METHODS

Nine subjects with NIDDM (eight men and one woman) participated in this study. All were screened to ensure that except for diabetes they were healthy and without significant diabetic complications. Clinical and metabolic characteristics of the study subjects are listed in Table 1. All subjects were initially treated with sulfonylureas, and this medication was withdrawn at least 2 weeks before entry into the study. No subject was taking any other medication known to affect carbohydrate or lipid metabolism. The experimental protocol was approved by the Committee on Human Investigation of University of California-San Diego. After written informed consent was obtained, all subjects were admitted to the Special Diagnostic and Treatment Unit at the Veterans Affairs Medical Center, San Diego, for study.

For at least 24 hours before studies commenced and throughout the study period, subjects consumed a weight-maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein. A basal study and a SRIF infusion study were conducted in each subject on separate days.

Protocol

Both studies were performed after a 12- to 14-hour overnight fast. A schema of the study design is shown in Fig 1. In each study, a [3-^{3}H]glucose infusion was started at least 4 hours before glucose turnover measurements were begun. During the SRIF infusion study, SRIF was infused at a constant rate of 0.08 pmol/kg/min for 3 hours. This period of infusion was chosen to eliminate any residual effect of insulin during measurements, since the half-life for deactivation of insulin action on in vivo glucose disposal has been reported to be 30 to 50 minutes.[8] To avoid the confounding influence of changes in glycemia during SRIF-induced insulinopenia, serum glucose was maintained at the preinfusion level (12 to 13 mmol/L) using a variable infusion of 20% glucose. The specific activity of [3-^{3}H]glucose, as well as concentrations of insulin and C-peptide, were measured at 10-minute intervals during the last 30 minutes of the basal state and SRIF infusion period to determine isotopically the rates of glucose appearance (R_a) and disappearance (R_d) and to confirm complete suppression of endogenous insulin secretion. Respiratory-exchange measurements of O_2 consumption and CO_2 production were determined by open-circuit indirect calorimetry as described in detail previously.[6] During the last 30 minutes of the basal and SRIF infusion periods, steady-state measurements of glucose, fat, and protein oxidation were performed. Blood samples for measuring concentrations of FFAs, glucagon, and lactate were also obtained on at least two occasions during the last half-hour of the basal and SRIF infusion studies. Finally, a needle biopsy of vastus lateralis muscle, as previously

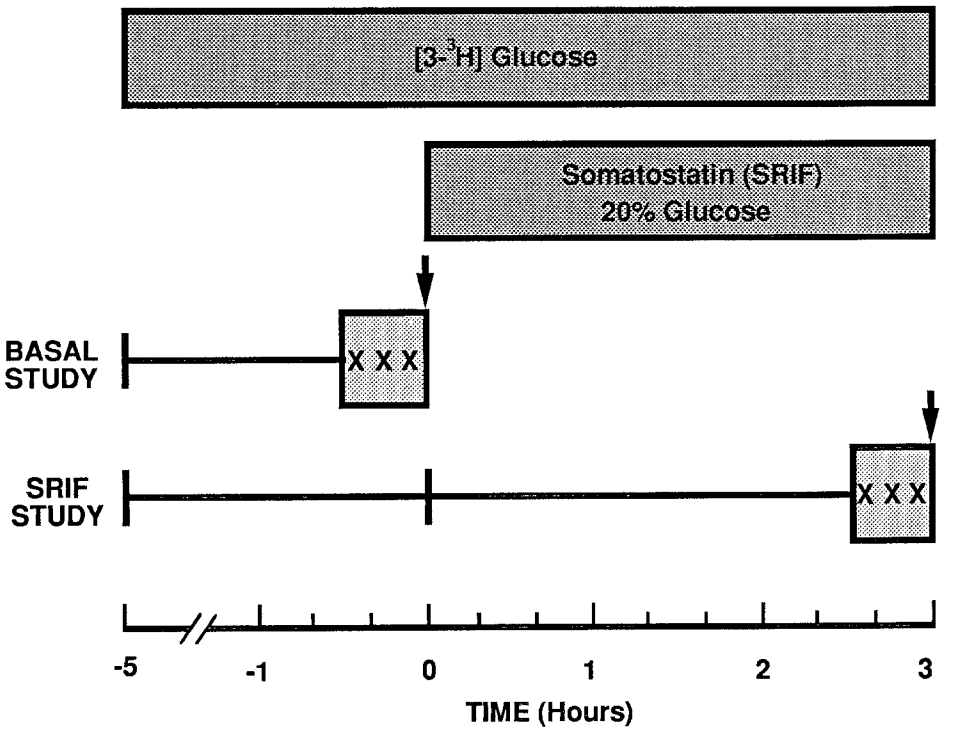

Fig 1. Experimental study design. Indirect calorimetry and steady-state measurements were made during the last 30 minutes of each study period. (X) Glucose turnover measurements; (↓) needle biopsies of skeletal muscle.

described,[5] was performed at completion of the basal and SRIF infusion periods to determine skeletal muscle GS and PDH activity.

Calculations

Glucose R_a and R_d were quantified from the [3-^{3}H]glucose specific activity in blood using the modified equations of Steele.[9] Hepatic glucose output was determined from isotopically measured R_a in the basal state. During the SRIF study, rates of glucose infusion were subtracted from R_a to calculate hepatic glucose output. Glucose uptake (GU) was determined from R_d with correction for changes in glucose concentration within its distribution pool and urinary glucose loss under both study conditions. Substrate oxidation rates were calculated according to previously described methods.[10]

Determination of GS

The GS level was determined as described in detail previously[5] by measuring the incorporation of [^{14}C]glucose from uridine 5′-diphosphate glucose (UDPG) into glycogen. GS activity was assayed at concentrations of 0.1 and 10 mmol/L glucose-6-phosphate (G6P) and physiologic levels of substrate (0.3 mmol/L UDPG). Protein was assayed in muscle extract, and units of GS activity were expressed per nanogram protein. Results are expressed as the fractional velocity of GS at 0.1 mmol/L G6P, ie, the activity assayed at 0.1 mmol/L G6P divided by the activity at 10 mmol/L G6P. In addition, GS activity was measured in each biopsy specimen at saturating concentrations of UDPG (5 mmol/L) and G6P (10 mmol/L) to assess the total activity of the enzyme.

Determination of PDH

The PDH level was determined by the modified method of Mandarino et al[11] by measuring production rates of $^{14}CO_2$ from [^{14}C]pyruvate. Active PDH (PDH_a) activity was measured in the presence of fluoride with 12.5 μmol/L Ca^{2+} and 12.5 μmol/L Mg^{2+}, and total PDH (PDH_t) activity was measured in the absence of fluoride with 250 μmol/L Ca^{2+} and 20 mmol/L Mg^{2+}. Results are expressed for PDH_a in nanomoles per minute per milligram protein and for PDH fractional velocity, ie, PDH_a/PDH_t.

Analytic Methods

Blood drawn for glucose assay was immediately separated by an Eppendorf microcentrifuge (Brinkman, Westbury, NY), and serum glucose was determined using the glucose oxidase method (model 23A, YSI, Yellow Springs, OH). Blood for serum insulin assay was collected in untreated tubes, and blood for glucagon assay was collected in tubes containing 150 μL Trasylol (500 KIU/mL; FBA, New York, NY). Both were allowed to clot at room temperature before the supernatant was removed. Blood for measurement of lactate and glucose specific activity was collected in tubes containing potassium oxalate plus sodium fluoride. Blood for analysis of FFA was collected in tubes containing 100 μL EDTA, and C-peptide was collected in tubes containing 150 μL Trasylol and 100 μL EDTA/1.5 mg/mL. These samples were immediately placed on ice until centrifugation. All specimens were stored at −20°C until assayed.

The insulin level was measured by a specific double-antibody radioimmunoassay.[12] Serum glucagon was assayed by the charcoal-separation method of Faloona and Unger.[13] Plasma FFA levels were measured by the colorimetric method of Itaya and Vi.[14] Plasma lactate was determined by the lactate oxidase method using a Model 23A Lactate Analyzer (YSI). Plasma C-peptide measurement was kindly performed in the laboratory of K.S. Polonsky at the University of Chicago by a nonequilibrium ethanol-precipitation radioimmunoassay.[15] Urea nitrogen, creatinine, and uric acid were assessed in serum and urine using routine semiautomated methods (Hitachi 737, Boehringer Mannheim, Indianapolis, IN).

Statistics

Data calculations and statistical analyses were performed using the StatView program (Abacus Concepts, Berkeley, CA). All data are expressed as the mean ± SE. Statistical significance was tested by Student's two-tailed paired *t* test. Correlation coefficients were calculated using the method of least squares.

RESULTS

Serum Hormone and Glucose Concentrations

Mean basal fasting serum glucose and insulin were 12.5 ± 0.9 mmol/L and 192 ± 6 pmol/L, respectively. During SRIF infusion, serum insulin decreased rapidly within the first hour and was undetectable (<14 pmol/L) thereafter. C-peptide also declined, decreasing 88% from basal values of 0.73 ± 0.11 to 0.09 ± 0.02 nmol/L during the last 30 minutes of SRIF infusion. Following SRIF, serum glucagon decreased by 48% from basal values of 210 ± 30 to 110 ± 10 ng/L. Throughout the SRIF infusion period, serum glucose was maintained at basal levels of 12.8 ± 0.8 mmol/L (Table 2).

Substrate Metabolism

Glucose turnover rates during both studies are shown in Table 3. Basal hepatic glucose output decreased from 2.29 ± 0.17 to 1.72 ± 0.21 mg/kg/min ($P < .005$) during the SRIF infusion. To maintain plasma glucose at basal levels during the SRIF study, exogenous glucose was infused at 0.63 ± 0.21 mg/kg/min. Urinary glucose loss was 0.13 ± 0.05 during the basal period and 0.22 ± 0.12 mg/kg/min during SRIF infusion (P = NS). Figure 2 illustrates the changes in GU and glucose metabolism before and after SRIF infusion. In the basal state, G_{ox} and N_{ox} contributed 45% and 55% of GU, respectively. Following SRIF infusion, GU decreased by 12% from 2.26 ± 0.13 to 1.99 ± 0.12 mg/kg/min ($P < .005$). Thus, approximately 88% of basal GU was accounted for by non–insulin-mediated GU (NIMGU) in these patients. G_{ox} also decreased during SRIF infusion by approximately 35% from 1.01 ± 0.10 to 0.65 ± 0.14 mg/kg/min ($P < .01$). However, N_{ox} remained essentially unchanged (1.24 ± 0.17 to 1.34 ± 0.21 mg/kg/min, P = NS). Thus, the SRIF-induced decrease in GU was entirely accounted for by the reduction in G_{ox}.

Circulating FFA levels and fat oxidation (F_{ox}) increased during SRIF infusion by 62% (0.86 ± 0.08 *v* 1.39 ± 0.07 mmol/L, $P < .05$) and 21% (0.87 ± 0.05 *v* 1.05 ± 0.07 mg/kg/min, $P < .01$), respectively. During SRIF-induced insulinopenia, total energy expenditure (EE) remained

Table 2. Fasting Glucose and Hormone Concentrations

	Glucose (mmol/L)	Insulin (pmmol/L)	C-peptide (nmol/L)	Glucagon (ng/L)
Basal	12.5 ± 0.9	192 ± 6	0.73 ± 0.11	210 ± 30
SRIF	12.8 ± 0.8	<14	0.09 ± 0.02	110 ± 10
P	NS	<.001	<.001	<.01

NOTE. Values are the mean ± SE; n = 9.

Table 3. Glucose Turnover Measurements

	HGO (mg/kg/min)	Ginf (mg/kg/min)	GU (mg/kg/min)	Uglu (mg/kg/min)
Basal	2.29 ± 0.17	—	2.26 ± 0.13	0.13 ± 0.05
SRIF	1.72 ± 0.21	0.63 ± 0.21	1.99 ± 0.12	0.22 ± 0.12
P	<.005	<.001	<.005	NS

NOTE. Values are the mean ± SE; n = 9.

Abbreviations: HGO, hepatic glucose output; Ginf, glucose infusion rate; Uglu, urinary glucose loss.

unchanged from basal (1.41 ± 0.10 *v* 1.42 ± 0.11 kcal/min, P = NS) as the reduced EE from decreased G_{ox} (0.35 ± 0.03 to 0.21 ± 0.04 kcal/min, $P < .005$) was completely offset by a corresponding increase in EE due to accelerated F_{ox} (0.82 ± 0.09 to 0.97 ± 0.11 kcal/min, $P < .01$). Plasma lactate concentrations (1.21 ± 0.17 *v* 1.26 ± 0.17 mmol/L, P = NS) and rates of protein oxidation (0.53 ± 0.05 *v* 0.47 ± 0.04 mg/kg/min, P = NS) were unchanged by SRIF infusion.

Muscle PDH and GS Activity

PDH_a was significantly reduced during SRIF-induced insulinopenia from 0.50 ± .014 to 0.31 ± 0.09 nmol/min/mg ($P < .05$). When expressed as fractional velocity, PDH activity was unchanged by SRIF infusion (22.2% ± 3.6% *v* 21.7% ± 4.9%, P = NS). Total GS activity measured at saturating concentrations of UDPG and G6P was similarly unchanged following SRIF infusion (0.43 ± 0.14 *v* 0.40 ± 0.20 nmol/min/mg protein, P = NS). The fractional velocity of GS, measured at more physiologic levels of UDPG and G6P, was decreased by 31% from 2.6% ± 0.7% to 1.8% ± 0.6% ($P < .05$).

DISCUSSION

Glucose disposal into peripheral tissues occurs through either insulin-mediated glucose uptake (IMGU) or NIMGU.[16,17] IMGU, by definition, occurs only in insulin-sensitive tissues, and NIMGU occurs in both insulin-sensitive and non–insulin-sensitive tissues.[16,17] While IMGU plays a major role in the disposal of a glucose load in the postprandial state, NIMGU is reported to be the major mechanism of glucose disposal in the basal state in both normal subjects and those with NIDDM.[16] In the present study, we have demonstrated that approximately 88% of total GU in the basal state occurs through NIMGU in NIDDM. This value, although similar, is slightly higher than that reported in previous studies.[4,17,18] Part of this discrepancy could be related to the fact that the subjects of previous studies were lean, whereas the majority of our subjects were obese. Since obese NIDDM subjects are quantitatively more insulin-resistant than lean NIDDM subjects in response to an insulin infusion,[19] our more-obese subjects might be expected to have greater resistance to the effect of basal insulin.

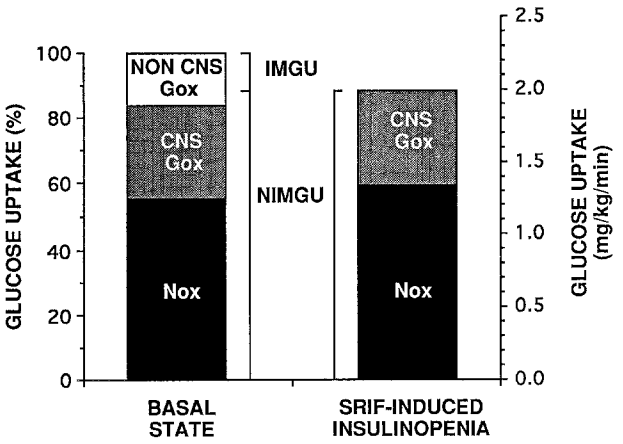

Fig 2. Distribution of CNS and non-CNS G_{ox} and N_{ox} in the basal state and during SRIF-induced insulinopenia. Estimated value of 0.65 mg/kg/min is used for CNS G_{ox}. Total height of each bar represents GU in the basal state and during SRIF-induced insulinopenia.

In this study, the decrease in GU during SRIF-induced insulinopenia, ie, basal IMGU, was entirely accounted for by decreased G_{ox}, indicating that the glucose transported into body tissues through IMGU in the basal state is almost entirely routed into the G_{ox} pathway. This finding is consistent with previous reports[1,3] in normal subjects, which demonstrate that G_{ox} is more sensitive to insulin than N_{ox}, based on half-maximally effective insulin concentrations. In fact, in normal subjects Mandarino et al[1] have observed that the entire enhancement of GU caused by an increase in insulin from a basal value of 7 to 22 μU/mL is accounted for by enhanced G_{ox}. Furthermore, Felber et al,[4] using an experimental design similar to that of the present study, also reported that SRIF-induced insulinopenia resulted in decreased GU and G_{ox} but not N_{ox} in normal subjects. The present study has now demonstrated that G_{ox} in NIDDM also retains its sensitivity and is dependent on basal insulin despite the well-documented impaired response of this pathway to exogenous insulin stimulation.[6]

If central nervous system (CNS) GU is assumed to be insulin-independent and completely oxidized at a rate of 0.65 mg/kg/min,[20,21] which was the level of G_{ox} obtained during SRIF-induced insulinopenia in this study, then an estimate of glucose metabolic pathway activity in non-CNS tissue can be made. After correcting total and oxidative glucose disposal for CNS glucose metabolism, 22% of GU in non-CNS tissue is metabolized by oxidation and 78% is metabolized nonoxidatively in the basal state. However, during SRIF infusion 100% of GU is metabolized by N_{ox} in non-CNS tissue. In support of this notion, a significant relationship, shown in Fig 3, was found between N_{ox} and GU measured during SRIF infusion ($r = .76$, $P < .05$). This formulation also indicates that basal G_{ox} in non-CNS tissue is totally insulin-dependent.

Following SRIF infusion, the marked decrease in G_{ox} was associated with corresponding decreases in GU and skeletal muscle PDH activity. This change in PDH activity occurred in the setting of increased FFA levels and rates of F_{ox}, consistent with the proposal by Randle et al[22] that the activity of this enzyme is reduced in the presence of increased F_{ox} rates. These findings can be interpreted to indicate that basal insulin may regulate intracellular G_{ox} in

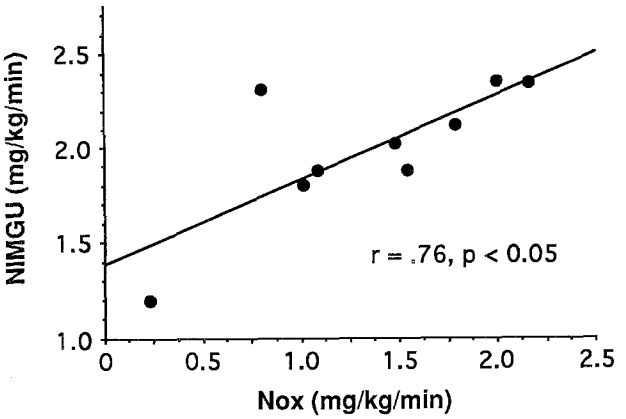

Fig 3. Relationship between NIMGU and N_{ox} during SRIF-induced insulinopenia. Regression equation is y = 1.39 + 0.42x; r = 0.76, P < .05.

NIDDM by multiple mechanisms including changes in GU, PDH enzyme activity, FFA availability, and rates of F_{ox}.

Interestingly, the decrease in total EE resulting from decreased G_{ox} was completely compensated for by a gain in EE from increased F_{ox}, such that EE was unaffected by the SRIF infusion. Endogenous glucose production decreased substantially by 0.57 ± 0.14 mg/kg/min during the third hour of SRIF infusion. This decrease in endogenous glucose production could be due to reductions in glycogenolysis, gluconeogenesis, or both pathways. Since gluconeogenesis is a more costly energy-requiring process than glycogenolysis, the fact that we found no change in EE is consistent with one or more of the following explanations: First, glycogenolysis may have been the principal pathway affected during SRIF infusion, so that changes in EE would be negligible. Alternately, any reduction in gluconeogenesis and its energy cost could be equally offset by increased EE in other metabolic processes. Finally, the majority of energy generated in the basal state results from substrate oxidation. Thus, even if the decrease in endogenous glucose production was solely due to decreased gluconeogenesis, the contribution would be minor and probably difficult to detect by the indirect calorimetry technique.

Basal GS activity in skeletal muscle was also shown to be dependent on basal insulin availability in this study. However, N_{ox}, which includes glycogen synthesis, was not changed following SRIF infusion. Consistent with these results, it would be unlikely for glycogen synthesis to be a major component of N_{ox} under the experimental conditions of this study. Rather, net glycogenolysis would be expected to occur under these study conditions, so that the major component of N_{ox} would be other pathways such as the release of 3-carbon fragments as lactate. From the present study, it is not possible to determine definitely which pathway(s) represents N_{ox} or whether there were any changes in the flux through different N_{ox} pathways. However, considering the significant relationship between N_{ox} and GU (NIMGU) during SRIF infusion, the current study does indicate that the pathway(s) contributing to N_{ox} appears to be almost entirely independent of insulin availability and regulated principally by glucose supply.

In summary, short-term deprivation of basal insulin results in decreased G_{ox} but not N_{ox} in subjects with NIDDM. The activities of both PDH and GS in skeletal muscle were decreased while circulating FFA levels and F_{ox} were increased in the insulinopenic state. Based on these studies, we conclude that approximately 35% of basal G_{ox} but not N_{ox} was dependent on basal insulin action in NIDDM. Furthermore, regulation of basal glucose oxidation by insulin appeared to be mediated by changes in GU, FFA availability, and PDH enzyme activity.

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