Skeletal Muscle Content of Membrane Glycoprotein PC-1 in Obesity

Relationship to Muscle Glucose Transport

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Membrane glycoprotein PC-1, an inhibitor of insulin signaling, produces insulin resistance when overexpressed in cells transfected with PC-1 cDNA. In the present study, we determined whether PC-1 plays a role in the insulin resistance of skeletal muscle in obesity. Rectus abdominus muscle biopsies were taken from patients undergoing elective surgery. Subjects included both NIDDM patients (n = 14) and nondiabetic patients (n = 34) across a wide range of BMI values (19.5–90.1). Insulin-stimulated glucose transport was measured in incubated muscle strips, and PC-1 content, enzymatic activity, and insulin receptor content were measured in solubilized muscle extracts. Increasing BMI correlated with both an increase in the content of PC-1 in muscle (r = 0.55, P < 0.001) and a decrease in insulin stimulation of muscle glucose transport (r = –0.58, P = 0.008). NIDDM had no effect on either PC-1 content or glucose transport for any given level of obesity. Insulin stimulation of muscle glucose transport was negatively related to muscle PC-1 content (r = –0.68, P = 0.001) and positively related to insulin receptor content (r = 0.60, P = 0.005). Multivariate analysis indicated that both skeletal muscle PC-1 content and insulin receptor content, but not BMI, were independent predictors of insulin-stimulated glucose transport. Muscle PC-1 content accounted for 42% and insulin receptor content for 17% of the variance in glucose transport values. These studies raise the possibility that increased expression of PC-1 and a decreased insulin receptor content in skeletal muscle may be involved in the insulin resistance of obesity. *Diabetes* 45:1324–1328, 1996

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Received for publication 29 January 1996 and accepted in revised form 23 May 1996.

BSA, bovine serum albumin; PNTP, thymidine 5′ monophosphate p-nitrophenyl ester.

Insulin resistance is a common feature of patients with NIDDM (1,2). The appearance of insulin resistance precedes the onset of the disease (3,4), and is independently associated with numerous health risks even in the absence of NIDDM (5). While insulin resistance is an inheritable trait (6), resistance to insulin develops secondarily to obesity (7,8), which is also an independent risk factor for NIDDM (9). The negative impact of obesity on peripheral insulin action is considerable, as ~75–80% of NIDDM patients are obese (10).

Skeletal muscle is the principal site for insulin-mediated glucose disposal (8). Obesity is associated with an impaired capacity for insulin stimulation of glucose uptake into muscle both in vivo and in vitro (8,11,12). Activation of the tyrosine kinase activity of the insulin receptor is requisite for increasing the rate of glucose uptake (2). An impairment in insulin receptor tyrosine kinase activation by insulin has been reported in muscle from obese nondiabetic subjects (13–15). The cause of this reduced activity, however, is unknown.

We have recently identified membrane glycoprotein PC-1 as an inhibitor of insulin receptor tyrosine kinase activity in fibroblasts from an insulin-resistant NIDDM patient (16,17). We have subsequently shown that PC-1 inhibits insulin action in vivo and is elevated in fibroblasts from other insulin-resistant individuals (17,18). These studies suggest that PC-1 overexpression may play a role in states of insulin resistance.

In the present investigation, we have studied PC-1 content in rectus abdominus muscle biopsies from patients with a wide range of BMI values. There was an inverse correlation between PC-1 content and in vitro insulin-stimulated glucose uptake, and PC-1 content was positively correlated with BMI across all subjects. These findings suggest, therefore, that skeletal muscle PC-1 content may be related to the insulin resistance of obesity.

RESEARCH DESIGN AND METHODS

Human subjects. A total of 48 subjects (5 men, 43 women), 29 to 59 years of age (42 ± 1, mean ± SE), admitted to the East Carolina University Hospital were studied. The ethnic mix of the study group roughly reflected that of the surrounding community; 26 of the subjects were Caucasian and 20 were African-American. One subject was of mixed race. The BMI values of the subjects ranged from 19 to 90 (38 ± 2). The experimental protocol was approved by the East Carolina University Policy and Review Committee on

Human Research, and informed consent was obtained from all patients. Of the patients, 14 were classified as having NIDDM according to the National Diabetes Data Group criteria (19). All NIDDM patients were obese with BMI values <30. Muscle biopsies were obtained from patients undergoing various elective abdominal surgeries, including gastric bypass and hysterectomies. None of the subjects had any diseases or had taken any medications known to alter carbohydrate metabolism for at least 1 week before surgery.

Muscle tissue and blood sampling. Muscle tissue was clamped in situ between two pairs of hemostatic forceps that were welded together so that the jaws were parallel and 3.0 cm apart. Tissue was excised by sharp dissection or with an electrocautery by cutting outside the clamp assembly. Muscle was quickly removed from the hemostats and frozen between dry ice–cooled tongs. Tissue that was to be incubated for glucose transport was placed in Krebs-Henseleit buffer gassed with O_2:CO_2 (95%:5%). Venous blood was drawn at the time that the muscle samples were taken.

Plasma insulin and glucose determination. Insulin levels were determined by either manual radioimmunoassay (Linco Research Inc., St. Louis, MO) or by automated MEIA on an Abbott IMx analyzer (Abbott Laboratories Diagnostics Division, Abbott Park, IL) with equivalent results. Glucose measurements were made polarographically using a Beckman Glucose 2 glucose analyzer (Beckman Instruments, Diagnostic Systems Group, Brea, CA).

Glucose transport measurements. Glucose transport measurements were made essentially as described previously (11). Rectus abdominus muscle obtained during abdominal surgical procedures was teased into 10–40 mg muscle strips that were mounted in 14-mm-wide acrylic clips. The muscle strips were preincubated for 60 min at 29°C in a medium consisting of Krebs-Henseleit bicarbonate saline in which the calcium concentration was reduced to 0.5 mmol/l (20), 1% bovine serum albumin (BSA), and 1 mmol/l pyruvate. The preparation was continuously gassed with 95% O_2:5% CO_2. After 50 min of preincubation, 100 nmol/l porcine zinc insulin was added to those bundles in which insulin-stimulated transport would be measured. After this preincubation, the bundles were transferred to fresh medium containing 5 mmol/l [^{14}C] 2-deoxyglucose (NEN Research Products, Boston, MA) and 20 mmol/l [^{3}H] sorbitol (Sigma, St. Louis, MO) (0.02 and 0.1 μCi/μmol, respectively) with or without insulin. After 60 min of incubation, the muscle bundles were rinsed twice in Krebs-Henseleit buffer at 4°C, blotted, cut out of the clip, and weighed. The tissue was dissolved in 11.7% (wt/vol) hexadecyltrimethylammonium bromide and 1.65% (wt/vol) potassium hydroxide in 50% methanol. Radioactivity was determined by liquid scintillation counting (Beckman Instruments, Fullerton, CA).

Muscle extract preparation. Soluble extracts were prepared from frozen muscle tissue to measure both PC-1 activity by hydrolysis of thymidine 5′ monophosphate p-nitrophenyl ester (PNTP) and PC-1 content by radioimmunoassay. Approximately 250 mg frozen tissue were pulverized under liquid nitrogen, and the resultant powder homogenized in 2 ml buffer (20 mmol/l Tris, 5 mmol/l $MgCl_2$, 1 mmol/l PMSF, 2 μmol/l leupeptin, and 2 μmol/l pepstatin, pH 8.7) at 4°C using a polytron homogenizer (Kinematica, Lucerne, Switzerland) for 10 s at a setting of 9. Triton X-100 was added to a final concentration of 1%, and the homogenates were solubilized for 60 min at 4°C. The material was then centrifuged at 100,000*g* for 60 min at 4°C. The supernatants were collected and stored at –70°C.

Enzyme assays. The phosphodiesterase activity of PC-1 was measured by the hydrolysis of PNTP. The reaction was carried out at saturating concentrations of substrate by incubating 125 μg of muscle protein with 1 μmol PNTP in 250 μl buffer (0.1 mol/l 2-amino-2-methyl-propanol, 7.5 mmol/l $Mg[OAc]_2$, pH 9.4) for 90 min at 37°C. The reaction was stopped by the addition of 2 ml 0.1 N NaOH. Liberated p-nitrophenol was quantified spectrophotometrically by reading at 401 nm (Beckman Scientific Instruments Division, Irvine, CA). Protein content of muscle extracts was determined by the Bradford method (21).

Radioimmunoassay procedures

Insulin receptor. The preparation of ^{125}I-labeled insulin receptor and the radioimmunoassay for the insulin receptor content of muscle extracts were performed as described previously (22).

PC-1. The radioimmunoassay procedure for measuring the insulin receptor was adapted for the quantification of PC-1 in muscle. PC-1, purified from human placenta (17), was labeled with ^{125}I by the Bolton-Hunter reagent. The following were added to 12 × 75 mm borosilicate tubes: 200 μl of Tris-based radioimmunoassay buffer (20 mmol/l Tris, 150 mmol/l NaCl, 0.1% Triton-X 100, 0.2% BSA, 0.2 mmol/l PMSF, pH 8.7) containing 0.5–30 ng of unlabeled PC-1, or multiple dilutions of unknown sample, and 100 μl of radioimmunoassay buffer containing 5 ng of specific rabbit anti-PC-1 antiserum (provided by Dr. I. Yamashina, Kyoto University, Kyoto, Japan) plus nonimmune rabbit serum (final dilution 1:400). After 24 h at 4°C, ^{125}I-PC-1 (5,000–10,000 counts/min) was added in 100 μl of buffer. After a 24-h incubation at 4°C, 100 μl of goat antirabbit γ-globulin serum were added (final dilution 1:125). After 18 h at 4°C, the tubes were centrifuged at 3,000*g* for 20 min. The supernatants were aspirated and the pellets counted.

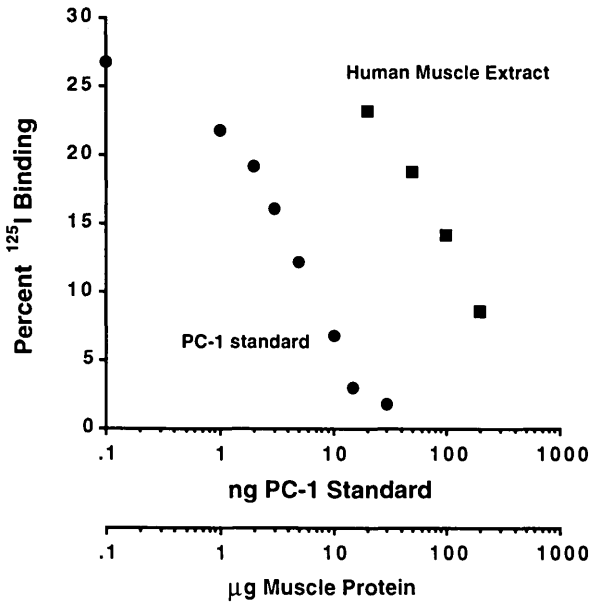

FIG. 1. Competition-inhibition plots of purified placental PC-1 and human muscle extracts on the binding of labeled ^{125}I-PC-1 to anti-PC-1 antiserum.

Data analysis. Statistical analysis was performed using SPSS/PC+ Version 5 (SPSS Inc., Chicago, IL) using the Regression procedure (23). Relationships between the various patient characteristics (subject age, BMI, plasma insulin and glucose, muscle content of PC-1 and insulin receptor, and muscle glucose transport) were analyzed with Pearson correlations. A stepwise multiple regression was employed to identify predictors among these variables, which best explained insulin-stimulated glucose transport in the patients for which data on all parameters were available.

RESULTS

BMI and muscle PC-1 content. To measure muscle PC-1 content, we used a radioimmunoassay for the PC-1 protein (Fig. 1). Using a purified placental PC-1 standard, half-maximal inhibition of ^{125}I-PC-1 binding was observed at 5 ng/tube. Human muscle extracts produced a dilution slope that paralleled the PC-1 standard.

Muscle PC-1 content correlated with BMI across all subjects (Fig. 2) (r = 0.55, P < 0.001). Since PC-1 hydrolyzes phosphodiester bonds, its enzymatic activity can be measured by the degradation of the substrate, PNTP (24). We measured the PNTP-hydrolyzing activity of these muscle extracts and found that this parameter significantly correlated with the PC-1 content (r = 0.9, P < 0.001) and with BMI (r = 0.35, P = 0.02).

PC-1 content and in vitro muscle glucose transport. In a subset of 20 individuals for which adequate samples were available, we measured glucose transport using the nonmetabolized glucose analog, [^{14}C] 2-deoxyglucose. Muscle strips were preincubated in the presence and absence of 100 nmol/l insulin. Exposure to insulin increased the rate of glucose transport on average 1.6 ± 0.1 fold. Insulin stimulation of glucose transport was significantly and negatively correlated with BMI (r = –0.58, P < 0.01) (Fig. 3*A*). Also, muscle PC-1 content was significantly and negatively correlated with insulin-stimulated glucose transport (r = –0.68, P < 0.001) (Fig. 3*B*).

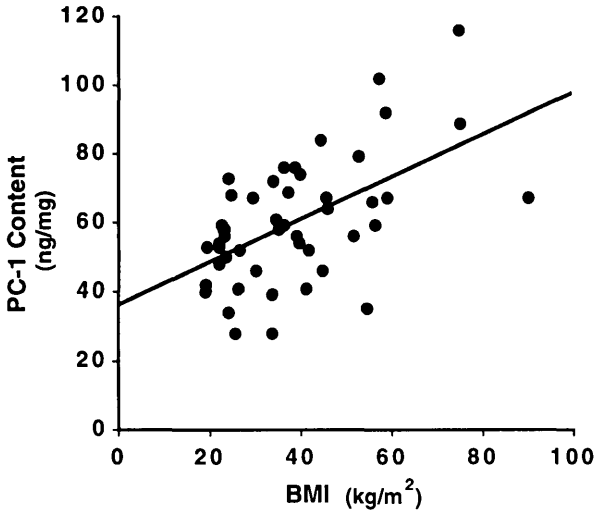

FIG. 2. The relationship between muscle PC-1 content and subject BMI (r = 0.55). The PC-1 content of soluble muscle extracts was measured by radioimmunoassay as described.

Insulin receptor content. Muscle insulin receptor content, measured by radioimmunoassay, was significantly correlated with insulin stimulation of glucose transport (r = 0.60, P < 0.01) (Fig. 4*A*). Insulin receptor content, however, did not correlate with PC-1 content (r = –0.03) (Fig. 4*B*).

Impact of NIDDM. Consistent with our previous findings, the presence of NIDDM in the obese subjects (all diabetic subjects had BMI values >30) did not result in a further impairment of insulin stimulation of glucose transport beyond that seen for nondiabetic subjects of similar BMI values. Likewise, the skeletal muscle PC-1 and insulin receptor content was not impacted by NIDDM beyond the effect of the degree of obesity.

Statistical analysis. To determine which subset of the predictor variables best explained insulin stimulation of muscle glucose transport, a stepwise multiple regression analysis was performed (Table 1). While univariate analyses revealed that glucose transport was significantly and inversely related to BMI and PC-1 content and positively related to insulin receptor content, the stepwise regression indicated that only PC-1 and insulin receptor content were significantly related to the variability in glucose transport. Although BMI was strongly correlated to glucose transport, it was not selected in the regression analysis because while both PC-1 and insulin receptor content were strongly correlated to glucose transport and BMI, PC-1 and insulin receptor content were only slightly correlated with each other. PC-1 content accounted for 42% of the variance in glucose transport, while insulin receptor content accounted for 17% of the variance.

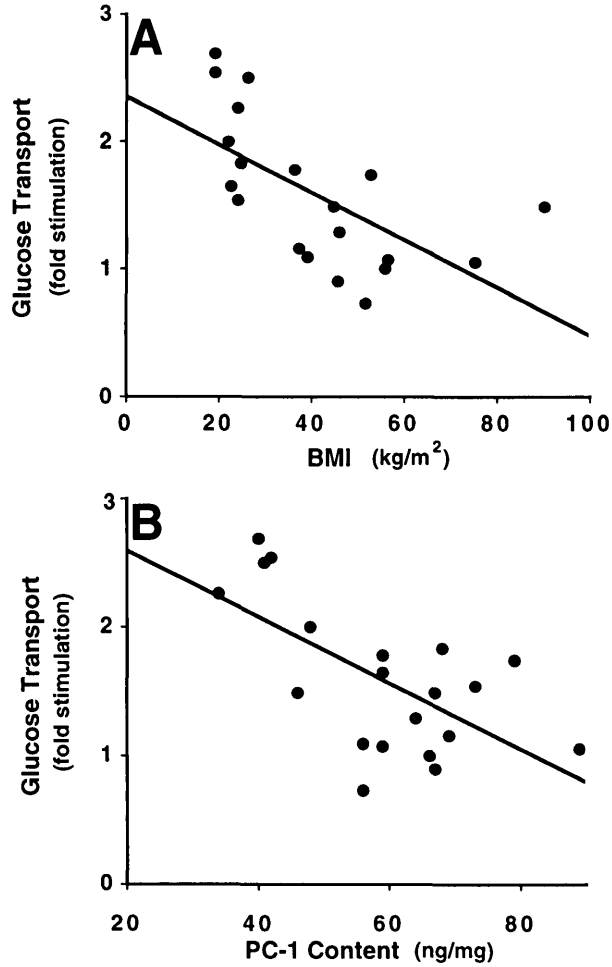

FIG. 3. *A:* the relationship between insulin stimulation of muscle glucose transport and subject BMI (r = –0.58). *B:* the relationship between insulin stimulation of muscle glucose transport and muscle PC-1 content (r = 0.55). Glucose transport was measured in muscle strips incubated in the presence and absence of 100 nmol/l insulin. Insulin stimulation of transport was calculated as the insulin-induced fold increase in [^{14}C] 2-deoxyglucose uptake over basal values. The PC-1 content of soluble muscle extracts was measured by radioimmunoassay.

DISCUSSION

It has been well established that insulin resistance is an inheritable trait in humans (3,6,25,26). In our previous study of dermal fibroblasts, we investigated the possibility that overproduction of membrane glycoprotein PC-1 was involved in this intrinsic resistance to insulin. Elevated levels of PC-1 were found in cells of seven of nine lean insulin-resistant NIDDM patients and three of seven lean insulin-resistant nondiabetic subjects (17). When PC-1 cDNA was transfected into several lines of cultured cells, overexpression of PC-1 protein was associated with impairment in the biological effects of insulin and decreased insulin receptor tyrosine kinase activity (17,18). Together, these observations suggested that PC-1 may be involved in the intrinsic insulin resistance of certain individuals.

The present study was undertaken to determine whether PC-1 is related to obesity, a common cause of acquired insulin resistance (7,8). We now have observed, by both enzymatic and immunological analyses, that PC-1 is present in skeletal muscle and that PC-1 content was elevated in muscle from obese subjects. Moreover, there was a correlation between PC-1 content and BMI across all subjects. Further, PC-1 content and BMI both correlated negatively with the ability of insulin to stimulate glucose transport in isolated muscle strips. Multivariate analysis of the data indicated, however, that BMI was not independently related to glucose transport, while PC-1 was an independent predictor of insulin-stimulated mus-

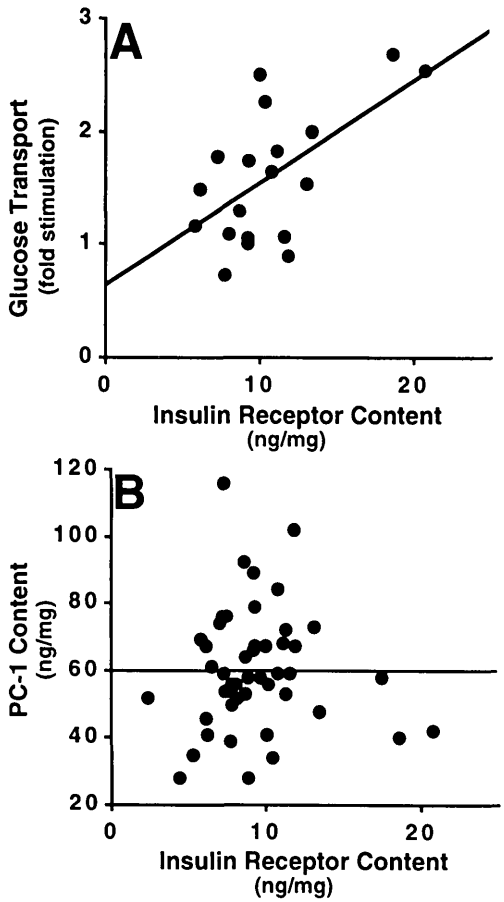

FIG. 4. *A:* **the relationship between insulin stimulation of glucose transport and muscle insulin receptor content (r = 0.60).** *B:* **the lack of a correlation between PC-1 content and insulin receptor content in muscle (r = 0.0). Glucose transport was measured in muscle strips incubated in the presence and absence of 100 nmol/l insulin. Insulin stimulation of transport was calculated as the insulin-induced fold increase in [^{14}C] 2-deoxyglucose uptake over basal values. Both PC-1 and insulin receptor content of soluble muscle extracts were measured by radioimmunoassay.**

cle glucose transport. These observations suggest that PC-1 is related to, and may play a role in, the insulin resistance of certain obese patients.

In various hyperinsulinemic insulin-resistant states, insulin receptor content is decreased (7,27). Since insulin downregulates its receptor in vitro (28), it is likely that, in vivo, hyperinsulinemia plays a major role in receptor downregulation. In the present study, as in our previous studies (14,15), insulin receptor content was decreased in muscle with increasing obesity. Decreased insulin receptor content correlated with decreased insulin-stimulated glucose transport in muscle strips but was a less potent predictor of this variable than was muscle PC-1 content. In obesity, the potential effects of the increased PC-1 may be exacerbated by downregulation of the insulin receptor.

In obese subjects, we previously found no differences between in vitro insulin-stimulated muscle glucose transport values in NIDDM patients and nondiabetic subjects of similar degrees of obesity (11). Thus, the present findings of similarly decreased glucose transport and elevated PC-1 content in muscle from both diabetic and nondiabetic subjects are consistent with our earlier results. However, a further loss of insulin stimulation of whole-body glucose disposal in obese NIDDM versus obese nondiabetic subjects has been reported with the hyperinsulinemic–euglycemic clamp (29). Thus, it is possible that factors other than skeletal muscle glucose transport (such as movement of insulin into the interstitial space and glucose delivery through vascular beds) can contribute to the diminished effect of insulin in that model.

Although we have demonstrated the ability of PC-1 to inhibit insulin receptor in vivo and in vitro, the mechanisms of this inhibition remain unknown. PC-1 is a class II (cytoplasmic amino terminus) membrane glycoprotein, and is the same protein as liver nucleotide pyrophosphatase/alkaline phosphodiesterase I (30). PC-1 has been reported to be expressed in plasma and intracellular membranes of plasma cells, placenta, the distal convoluted tubule of the kidney, ducts of the salivary gland, epididymis, proximal part of the vas deferens, chondrocytes, and dermal fibroblasts (17,31). PC-1 exists as a homodimer of 230–260 kDa; the reduced form of the protein has a molecular weight of 115–135 kDa, depending on the cell type. Human PC-1 is predicted to have 925 amino acids (24), but the transcription start site has been controversial (24,32). The PC-1 gene maps to chromosome 6q22-6q23 (30). While the extracellular domain has enzymatic activity that hydrolyzes phosphosulfate, pyrophosphate, and phosphodiesterase bonds (24), we have recently shown that elimination of this enzymatic activity by mutagenesis does not interfere with the ability of PC-1 to inhibit insulin receptor tyrosine kinase activity.

In summary, in the present study we find that muscle PC-1 content is significantly and negatively related to the insulin-stimulated glucose transport capacity of skeletal muscle across subjects with a wide range of BMI values. In these subjects, muscle PC-1 content is significantly

TABLE 1
Univariate and multivariate analysis to identify variables contributing to the variance in insulin stimulation of glucose transport

Variable	Univariate r value	Multivariate P value	Contribution to variance
Patient age	–0.25	0.28	
Plasma insulin	–0.46	0.70	
Plasma glucose	–0.39	0.74	
Patient BMI	–0.58*	0.86	
PC-1 content	–0.68*	0.01*	0.42
Insulin receptor content	0.60*	0.03†	0.17

n = 18; *P < 0.01; †P < 0.05.

related to BMI. Additionally, multivariate analysis indicates that PC-1 content accounts for a high percentage of the variance in muscle glucose transport values. In view of these correlations, we hypothesize that elevations in the level of PC-1 in skeletal muscle may play a significant role in the insulin resistance of obese individuals.

ACKNOWLEDGMENTS

This study was funded in part by NIH grant DK-38416. J.F.Y. was supported by NHRSA T32AG00212.

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