Coordinate Reduction of Rat Pancreatic Islet Glucokinase and Proinsulin mRNA by Exercise Training

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Exercise training results not only in enhanced insulin sensitivity but also in a reduction in insulin secretion. In this study, we examined the effects of exercise training on the expression of genes potentially related to insulin synthesis and glucose-stimulated insulin release by measuring pancreatic islet proinsulin, glucose-transporter (GLUT2), and glucokinase mRNAs. Female Wistar rats were subjected to 100 min of running at 25 m · min^{-1} up a 15% incline for 90 min/day for 6 days/wk for 3 wk. Pancreatic mRNA was evaluated by Northern- and dot-blot analysis with [^{32}P]cRNA probes. We found no change in the pancreatic content of GLUT2 mRNA but found marked decreases in the content of proinsulin mRNA (78%, $P < 0.005$) and glucokinase mRNA (65%, $P < 0.001$). These results suggest that exercise modulates both islet glucose metabolism and insulin synthesis at the level of gene expression. Furthermore, there was a significant correlation between the decreases in glucokinase and proinsulin mRNA concentrations (r = 0.95, $P < 0.001$), suggesting that expression of these genes is regulated in parallel. *Diabetes* 40:401–404, 1991

Exercise training in humans is associated with improved insulin action, as assessed by glucose-clamp techniques (1–4), and diminished glucose-stimulated insulin response (4–6). Little is known of the mechanism for the diminished insulin response, but islets isolated from trained rats exhibit decreased glucose-stimulated insulin secretion (7,8). Glucose metabolism by islet β-cells is directly proportional to plasma glucose concentration. Glucose phosphorylation by the high-K_m glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2) appears to be a rate-limiting step for glucose metabolism of islet β-cells, and glucokinase has been suggested as the glucose sensor and mediator of glucose-stimulated insulin secretion (9). If true, then a decrease in β-cell response to glucose should be associated with modulation of glucokinase. In fact, decreases in both islet glucokinase activity and insulin secretion have been observed under conditions of fasting (10) and insulin-induced hypoglycemia (11). Because the effects of exercise training on the glucose-stimulated insulin response are prolonged and last at least 48 h after the last bout of exercise (12), we studied the possibility that this effect of training is exerted at the level of islet β-cell gene transcription. We found that exercise training of rats is associated with a marked coordinate decrease in islet glucokinase and proinsulin mRNA and no change in islet glucose-transporter (GLUT2) mRNA.

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Received for publication 18 October 1990 and accepted in revised form 30 November 1990.

RESEARCH DESIGN AND METHODS

Female Wistar rats (SASCO, Omaha, NE) were trained on a treadmill at 25 m · min^{-1} and 15% incline for 90 min/day for 6 days/wk for 3 wk. The control group consisted of sedentary rats. The rats had free access to rat chow (Ralston-Purina, St. Louis, MO). At the end of the treatment period, rats were fasted overnight and anesthetized with pentobarbital sodium (50 mg · kg^{-1} body wt i.p.). The pancreas was excised, weighed, and frozen in liquid N_2.

Frozen samples of pancreas were homogenized in acid-ethanol, and peptide hormones were extracted as previously described (13). Insulin and glucagon concentrations in diluted samples were determined by double-antibody radioimmunoassay with purified rat insulin or beef glucagon as standards (Novo, Copenhagen).

Total tissue RNA was extracted with a guanidine thiocyanate water-saturated phenol-extraction method (14). Samples were homogenized in 4 M guanidine thiocyanate containing octylphenol–ethylene oxide detergent (Nonidet P-40, Sigma, St. Louis, MO), and multiple phenol-chloroform–isoamyl alcohol extractions were performed. The quan-

tity and quality of RNA samples were determined by absorbance at 260 and 280 nm. The $A_{260/280}$ ratios were >2 for all samples. Isolation of poly(A)$^+$ RNA by oligo-dT for Northern-blot analysis was performed by a standard procedure (15).

DNA concentrations of tissue homogenates were measured with a TKO 100 DNA fluorometer (Hoefer Scientific, San Francisco, CA) with the Hoechst 33258 dye (Poly-Sciences, Warrington, PA) and calf thymus DNA (Sigma) as a standard.

The probes used for study were *1*) rat proinsulin I cDNA fragment, 304 base pairs of coding information (16); 2) rat islet glucokinase cDNA (17); and 3) rat GLUT2 cDNA isolated from a rat liver cDNA library with a human GLUT2 clone (18). Sequence analysis indicated that the 2.2-kilobase (kb) rat GLUT2 cDNA was identical to that of the published rat GLUT2 clone (19). The cDNA fragments were subcloned in a pGEM (Promega, Madison, WI) or Bluescript SK$^+$ (Stratagene, La Jolla, CA) plasmid. Transcription by SP6, T3, or T7 RNA polymerase enzymes of uniformly labeled [^{32}P]cRNA probes and synthetic insulin and glucokinase mRNA standards was performed according to protocols provided by the suppliers and as previously described (13).

Proinsulin, GLUT2, and glucokinase mRNA concentrations were quantitated by dot-blot hybridization. Aliquots of pancreatic RNA (10, 5, and 2.5 μg) for determination of proinsulin mRNA or poly(A)$^+$ RNA for determination of GLUT2 and glucokinase mRNA were dissolved in 15% formaldehyde in 10× sodium chloride and sodium citrate and blotted onto Nytran membranes (Schleicher & Schuell, Keene, NH). Membranes were incubated with appropriate probes for 18 h at 60°C in 50% formamide and 5× SSPE (0.75 M NaCl, 50 mM $NaH_2PO_4 \cdot H_2O$, and 5 mM EDTA-Na_2, pH 7.4) and then washed at 60°C in 0.1% sodium dodecyl sulfate. Blots were exposed to Kodak XAR-5 film (Rochester, NY) at −70°C with intensifying screens (Cronex Lightning Plus, Du Pont, Wilmington, DE) for various periods, so that the intensity of the unknown samples was within the linear range of the standards. The amount of mRNA in each sample, determined in duplicate, was measured by densitometric analysis comparing the intensity of the dots. The amounts of proinsulin and glucokinase mRNA were quantitated against dilutions of a known amount of synthetic proinsulin and glucokinase mRNA as described (13).

Differences between groups were analyzed by an unpaired Student's *t* test. Correlation was analyzed by univariate linear regression with the CLINFO program on a VAX computer.

RESULTS

After 3 wk of training, there was no significant difference in body weight (192 ± 8 vs. 185 ± 14 g) between sedentary and trained rats. Pancreatic hormone and nucleic acid contents are presented in Table 1. The tendency for a decrease in insulin and an increase in glucagon resulted in a decrease in the insulin-glucagon ratio with training. There was no significant effect of exercise training on pancreas weight (mean ± SE 723 ± 184 mg for 7 sedentary rats vs. 681 ± 133 mg for 6 trained rats). RNA content tended to decrease, and with the 40% increase in DNA, the RNA-DNA ratio decreased 52% with training.

TABLE 1
Rat pancreatic hormone and nucleic acid content

	Sedentary (n = 7)	Trained (n = 6)
Insulin (ng · mg^{-1} tissue)	199 ± 65	96 ± 17
Glucagon (ng · mg^{-1} tissue)	3.3 ± 0.6	4.8 ± 0.6
Insulin-glucagon ratio	51 ± 10	22 ± 4*
RNA (μg · mg^{-1} tissue)	4.1 ± 0.5	2.9 ± 0.3
DNA (μg · mg^{-1} tissue)	1.5 ± 0.2	2.1 ± 0.2*
RNA-DNA ratio	2.9 ± 0.2	1.4 ± 0.1*

Values are means ± SE.
*$P < 0.05$.

An example of Northern-blot analysis of islet glucokinase mRNA is presented in Fig. 1. Glucokinase mRNA could not be detected in whole pancreatic RNA. In poly(A)$^+$ RNA, glucokinase mRNA was observed as a single 2.8-kb band as previously described (20) and was markedly decreased in trained versus sedentary rats. Therefore, glucokinase and GLUT2 mRNA were quantitated by dot-blot analysis of poly(A)$^+$ RNA. Proinsulin mRNA was determined by analysis of total RNA. The quantitation of pancreatic mRNA species is presented in Table 2. Whether expressed as a concentration of total RNA or based on the content of DNA, there was no significant change in GLUT2 mRNA with training. However, there were significant declines in proinsulin mRNA concentration (54%) and content (78%) with training, which agree with previously observed suppression of the glucose-stimulated insulin response by training (7,8,12). There was no correlation between pancreatic insulin content and proinsulin mRNA content (r = −0.016, P = 0.96). Glucokinase

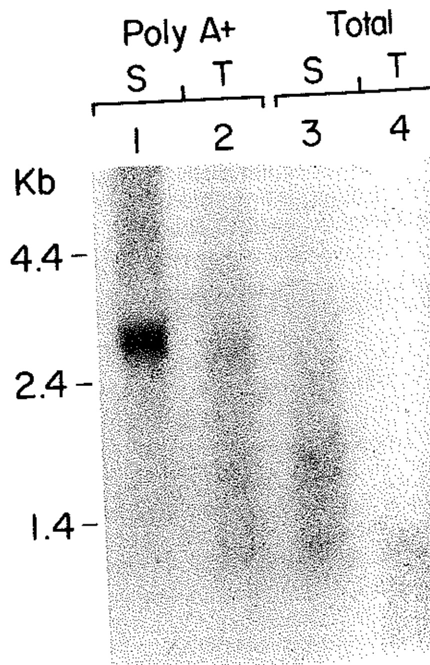

FIG. 1. Northern-blot analysis of pancreatic RNA from sedentary (S; n = 7) and trained (T; n = 6) rats. Total pooled RNA (30 μg; *lanes 3 and 4*) or pooled poly(A)$^+$ RNA (10 μg; *lanes 1 and 2*) was electrophoresed under denaturing conditions, blotted, and hybridized to [^{32}P]glucokinase cDNA as described in METHODS.

TABLE 2
Rat pancreatic mRNA species

	Sedentary (n = 7)	Trained (n = 6)
Proinsulin mRNA		
pg · μg^{-1} RNA	7.72 ± 1.10	3.55 ± 0.92*
pg · μg^{-1} DNA	22.20 ± 3.70	4.99 ± 1.34†
Glucokinase mRNA		
pg · μg^{-1} RNA	0.95 ± 0.09	0.72 ± 0.10
pg · μg^{-1} DNA	2.85 ± 0.33	1.01 ± 0.16†
GLUT2 mRNA		
pg · μg^{-1} RNA	0.370 ± 0.031	0.580 ± 0.140
pg · μg^{-1} DNA	1.01 ± 0.12	0.86 ± 0.26

Values are means ± SE. GLUT2, pancreatic islet glucose transporter.
* $P < 0.02$, † $P < 0.005$.

mRNA decreased 65% when calculated on the basis of DNA content and correlated closely with proinsulin mRNA concentration (Fig. 2).

DISCUSSION

An increase in islet β-cell glucose metabolism appears to be a necessary step in glucose-stimulated insulin secretion (9). The predominant glucose transporter of hepatocytes and islet β-cells (GLUT2) has been cloned (18,19). Within the pancreatic islets, it appears to be expressed only in β-cells (21,22). Glucose transport has been presumed to not be rate limiting for β-cell glucose metabolism or insulin secretion (9). Consistent with the hypothesized lack of a regulatory role for this glucose transporter, we found no change in GLUT2 mRNA with exercise training.

Presence of pancreatic glucokinase, on the other hand, appears to be rate limiting for β-cell glucose metabolism (9,10), and changes in activity with fasting/refeeding (10) or insulin-induced hypoglycemia (11) have correlated with glucose-stimulated insulin secretion. A previous attempt to correlate changes in glucokinase activity during fasting with changes in glucokinase mRNA led to the conclusion that there was no change in mRNA (20). However, this conclusion was based on quantitation of Northern blots, and mRNA quantity was therefore expressed in terms of total RNA concentration. This analysis failed to take into account a decline in total RNA content of islets of up to 50% with fasting (23) and may have missed a decrease in the islet content of glucokinase mRNA. In our study, for example, there was a 50% decline in RNA-DNA ratio with training.

The results of this study demonstrate that adaptation to exercise results in decreases in pancreatic glucokinase mRNA and proinsulin mRNA contents and suggest that at least part of the effect of exercise on glucose-stimulated insulin secretion is mediated at the level of gene transcription. There was no suggestion of correlation between pancreatic insulin content and proinsulin mRNA (r = −0.16, P = 0.96). The suggestion that pancreatic proinsulin mRNA might better correlate with the glucose-stimulated insulin response than insulin content is compatible with studies of partially pancreatectomized rats that showed that proinsulin mRNA and glucose tolerance were unchanged in the face of a 50% reduction in pancreatic insulin content (24). In our study, there was also no apparent relationship between pan-

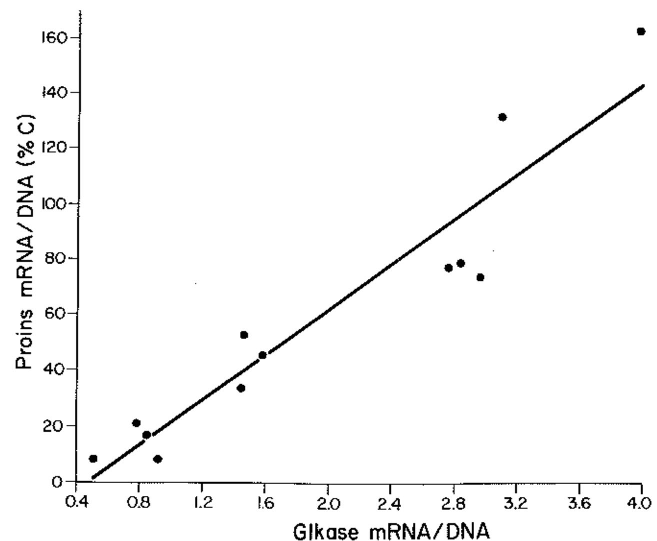

FIG. 2. Correlation between proinsulin (proins) mRNA (pg · μg^{-1} DNA) as percentage of that in control (C) rats and glucokinase (glkase) mRNA (pg · μg^{-1} DNA) in sedentary and trained rats (r = 0.95, P < 0.001).

creatic glucagon content or insulin-glucagon ratio and proinsulin mRNA.

The excellent correlation between glucokinase mRNA and proinsulin mRNA contents suggests that these gene transcription products may be regulated in parallel (Fig. 2). The factors responsible for regulation of these genes have not yet been identified. The findings that fasting and insulin-induced hypoglycemia decrease both glucokinase activity (10,11) and proinsulin mRNA (20) imply that some aspect of glucose metabolism may play a key role in this regard. A possible role for catecholamines has been suggested by the finding that phentolamine (25) or adrenalectomy (26) can block the effects of physical training on insulin secretion. The relationship between the factors responsible for the regulation of β-cell glucokinase and proinsulin genes and their roles in physiological and pathological modulation of insulin secretion need to be further characterized.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grants DK-16746 and AG-00425, a Juvenile Diabetes Foundation Fellowship (L.K.), and National Research Service Awards AG-00078 (C.A.S.) and DK-07120 (R.E.B.).

We thank Dr. Mark Magnuson (Vanderbilt Univ., Nashville, TN) for providing the rat islet glucokinase cDNA. The laboratory assistance of Cris Welling and Rachel Janssen and manuscript preparation by Jeannie Wokurka are also gratefully acknowledged.

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