Mitochondrial-Encoded Gene Regulation in Rat Pancreatic Islets

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Mitochondrial adenosine triphosphate (ATP) generation plays a major role in insulin secretion in pancreatic islet β cells. The relationship between age and nutritional status of the islet and mitochondrial gene messenger RNA (mRNA) expression was investigated. Three animal groups were studied: infant (12-day-old) rats fed either mother's milk or a high carbohydrate (HC) diet; young (2 to 4-month-old) rats; and old (12 to 14-month-old) rats. The expression of mitochondrial cytochrome oxidase (CYO) (subunits I, II, and III), β-nicotinamide adenine dinucleotide, reduced form dehydrogenase subunit 4 (NADH-DH4), and ATP synthase (subunit 6) (ATP-SYN6) mRNAs was characterized by semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR). The mitochondrial gene mRNAs were identified in each of the groups of rat islets and in RINm5F cells. CYO-II mRNA expression in young and old rat pancreatic islets was 12.7- and 8.2-fold higher, respectively, compared with the level in infant rat islets. The expression of NADH-DH4 and ATP-SYN6 mRNAs was 47% and 40% lower, respectively, in young rat islets compared with the level in infant rat islets. CYO-I, CYO-III, and cytoplasmic glyceraldehyde-3-phosphate dehydrogenase (GPDH) mRNA expression did not differ between experimental groups. Artificial rearing of infant rat pups on a HC diet for 8 days lead to a 3.3-fold increase in islet CYO-II mRNA expression compared with mother-fed pups. However, glucose (11 mmol/L) stimulation of cultured isolated islets from young and old rats for 4 days failed to affect the expression level of mitochondrial gene mRNAs. Thus, aging affected the differential expression of CYO-II, NADH-DH4, and ATP-SYN6 mRNAs in rat islets. CYO-II mRNA expression was modulated only in infant rat islets after in vivo administration of carbohydrate.

MITOCHONDRIA CONTAIN closed circular, double-stranded DNA with a size of 14 to 39 kb depending on the animal species. Both strands of the mitochondrial DNA (mtDNA) are transcribed. The heavy strand encodes genes for 12 polypeptides, 12s and 16s mitochondrial ribosomal (r)RNA, and 14 transfer (t)RNAs, while the light strand encodes 1 polypeptide and 8 tRNAs. The mitochondrial polypeptides are cytochrome oxidase (CYO) (subunits I, II, and III), β-nicotinamide adenine dinucleotide, reduced form dehydrogenase (NADH-DH) (subunits 1, 2, 3, 4, 4L, 5, and 6), and adenosine triphosphate (ATP) synthase (ATP-SYN) (subunits 6 and 8).[1] Full-length mtDNA sequences of these genes have been reported for human, cow, mouse, and rat.[1,2] The polypeptides are translated by mitochondrial ribosomes, and their integrity is required to maintain cellular function. Mitochondrial polypeptides transport electrons from NADH to molecular oxygen and generate ATP through oxidative metabolism (Fig 1). ATP generation plays a major role in the regulation of glucose-stimulated insulin secretion in pancreatic islet β cells. Previous studies have shown that mtDNA is required for the regulation of glucose-stimulated insulin secretion, and that the expression of mitochondrial enzymes is regulated with long-term glucose stimulation and aging.[3-5] In addition, hormones, such as follicle-stimulating hormone (FSH) and estrogen, and vitamin D_3 regulate the expression of these genes.[6,7]

Glucose is the primary stimulus regulating insulin secretion in islet β cells, and intracellular Ca^{2+} levels play a major role in mediating insulin secretion.[8,9] This process requires glucose metabolism and mitochondrial oxidative phosphorylation leading to generation of ATP. ATP closes ATP-sensitive K^+ channels leading to depolarization of the β-cell membrane, opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influx, and insulin secretion.[10,11] Although 2 main processes, glycolysis and oxidative phosphorylation, are responsible for ATP synthesis from glucose metabolism, oxidative phosphorylation is the major pathway.[12] Insulin secretion induced by other secretagogues such as leucine and glyceraldehyde is also mediated by the production of ATP.[13,14] Thus, mitochondrial oxidative phosphorylation plays an important role in the insulin secretory process in β cells.

In contrast to acute glucose-stimulated insulin secretory responses, the prolonged exposure of islets to elevated glucose concentrations both in vivo and in vitro has been shown to impair glucose-stimulated insulin secretion.[15-17] In an in vitro model of glucose desensitization of insulin secretion, which uses isolated islets cultured for up to 7 days at 11 mmol/L glucose (desensitized islets), β-cell sensitivity to glucose is reduced.[16,18] In addition, feeding a high carbohydrate (HC) diet to young rats during the sucking period results in an elevated basal level of insulin secretion and a reduced glucose sensitivity.[19,20] Thus, studying the mechanism(s) underlying changes in glucose sensitivity in these models is important to our understanding of type 2 diabetes mellitus associated with long-term glucose stimulation and aging.

In this study, age-related and carbohydrate-stimulated changes in the expression of mitochondrial gene (CYO I, II, and III, NADH-DH4, and ATP-SYN6) mRNAs were characterized in isolated islets from infant (12-day-old), young (2- to 4-month-old), and old (12- to 14-month-old) rats. Carbohydrate effects were evaluated using cultured islets or islets isolated from infant rats fed a HC diet. The hypothesis to be tested using these models was that age and early dietary intervention regu-

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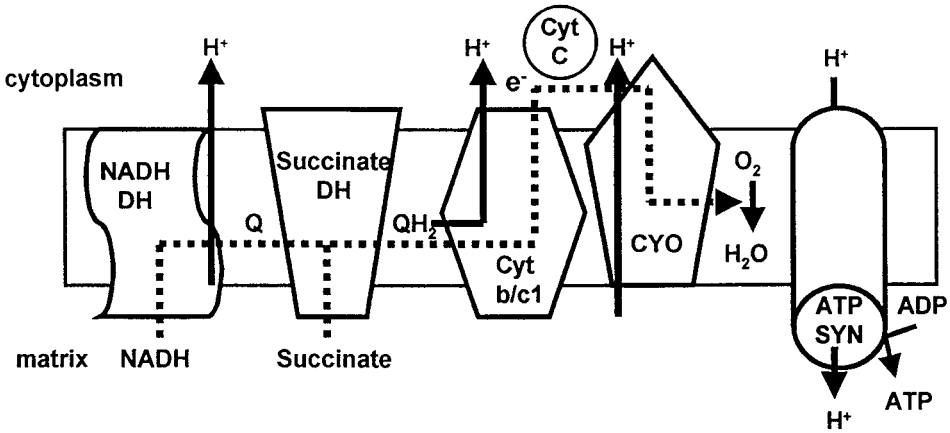

Fig 1. Enzymes in the mitochondrial inner membrane involved in oxidative phosphorylation. NADH-DH; succinate DH; Cyt b, c, and c1; and CYO constitute the electron transfer chain to O_2. The proton gradient promotes ATP generation by ATP synthase (SYN). Coenzyme Q (Q).

late mitochondrial gene mRNA expression in pancreatic islets. Because both age and dietary changes affected specific mitochondrial enzyme mRNA expression levels, it is likely that changes in oxidative metabolism enzyme expression play a role in the insulin secretory response.

MATERIALS AND METHODS

Materials

Trypsin (249 U/mg) was from Worthington Biochemical (Freehold, NJ). Bovine serum albumin (BSA) fatty acid free, fraction V, and RPMI-1640 medium were from Sigma Chemical (St Louis, MO). Collagenase (type P) was from Boehringer Mannheim (Indianapolis, IN). Fetal bovine serum was from Atlanta Biologicals (Norcross, GA). Random hexamer, SuperScript II ribonuclease H^- reverse transcriptase (RT), TRIzol, and CMRL-1066 medium were from GIBCO/Life Technologies (Grand Island, NY).

Feeding a HC Formula During Suckling Period

Newborn rat pups were pooled and assigned to each nursing mother (11 pups/dam) and were left with the mothers until postnatal day 4. On postnatal day 4, pups were assigned randomly to control and experimental groups. In the milk-fed (MF) control group, pups were reared by their nursing mothers. Pups in the experimental group were reared artificially on a HC formula by intragastric cannula, as described previously.[20] On postnatal day 12, the rats were killed by decapitation, and the pancreas was processed for the isolation of islets. All animal procedures were approved by the Institutional Animal Care and Use Committee.

Isolation and Culture of Rat Islets and RINm5F Cells

Pancreatic islets from infant (12-day-old), young (2 to 4-month-old) male, and old (12 to 14-month-old) male Sprague-Dawley rats were isolated using the collagenase method, as described previously.[16,20] Isolated islets were either used immediately as freshly isolated (fresh) islets, or they were cultured at 35°C for 4 days in CMRL-1066 culture medium containing 5.5 or 11 mmol/L glucose, as described previously.[21] Islets cultured for RNA extraction also included the mitotic inhibitor, cytosine arabinoside, which was reported previously to reduce nonspecific changes in mRNA expression during long-term islet culture.[22] Cells of the rat insulinoma cell line, RINm5F, were maintained in RPMI-1066 medium at 35°C, as described previously.[23]

Total RNA Isolation and cDNA Synthesis

Total RNA was extracted from rat pancreatic islets and RINm5F cells using TRIzol. First strand cDNA was synthesized from 0.5 μg of total RNA by using random hexamer and SuperScript II ribonuclease H^- RT.[22,23]

Polymerase Chain Reaction Amplification and Quantitation of Mitochondrial Gene Transcript Levels

Polymerase chain reactions (PCRs) were performed as described previously.[24] The sequences of primer pairs for mitochondrial genes, GPDH, and β-actin used in this study are shown in Table 1. Amplification primers for mitochondrial gene analysis were selected to be distinct for each subunit based on the reported DNA sequences of the rat mitochondrial CYO-I, -II, and -III, NADH-DH4, and ATP-SYN6[1] (Table 1). The PCR products corresponded with the expected base pair product size (Table 1). PCR was performed for 23 cycles under the

Table 1. Oligonucleotide Primers for Mitochondrial DNA, GPDH, and β-Actin

Genes	Sequences	Products (bp)
CYO-I		
Forward	5′-CATCTTCTCTCACTGCCA-3′	
Reverse	5′-GTAGTGTAGCGAGTCAGCTG-3′	407
CYO-II		
Forward	5′-CTCATCAGCTCCCTAGTAC-3′	
Reverse	5′-GACCTGGTCGGTTTGATGTG-3′	475
CYO-III		
Forward	5′-GAACATACCAAGGCCACCAC-3′	
Reverse	5′-CGTGGAGGCCATGAAATC-3′	429
NADH-DH		
Forward	5′-GAGGCAACCAAACAGAACGC-3′	
Reverse	5′-CGTAGGCAGATTGAGCTAG-3′	426
ATP-SYN6		
Forward	5′-CAGCAACCGACTACACTC-3′	
Reverse	5′-GTCGTACTGCTAGTGCTATCG-3′	355
GPDH		
Forward	5′-GTTGCCATCAACGACCCCTTC-3′	
Reverse	5′-GGATGCAGGGATGATGTTCTG-3′	592
β-actin		
Forward	5′-CTACAGATCATGTTTGAGACC-3′	
Reverse	5′-GAAGGAAGGCTGGAAGAGAGC-3′	441

following conditions: denaturation for 1 minute at 94°C, annealing for 1 minute at 60°C, and extension for 1 minute at 72°C with the final extension for 7 minutes. Preliminary studies established the linearity of amplification rates under the conditions used for these experiments. Polymerization reactions were performed in a Sprint thermalcycler (Hybaid, Franklin, MA) using 10 μL 1:1 dilution cDNAs (for β-actin), 1:2 dilution cDNAs (for CYO-I and GPDH), 1:4 dilution cDNAs (for CYO-II), 1:16 dilution cDNAs (for CYO-III), or 1:32 dilution cDNAs (for NADH-DH4 and ATP-SYN6) as templates in a 25-μL reaction volume for which amplification was in the exponential phase. The amplimers were separated by electrophoresis in a 1.5% agarose gel. The gel was stained by ethidium bromide and viewed by Gel Doc 1000 (Bio-Rad, Hercules, CA). The density of each PCR fragment was analyzed by Molecular Analyst software (Bio-Rad, Hercules, CA). The image densities of PCR products for mitochondrial-encoded genes and GPDH were compared with the density of coamplified β-actin to determine the ratio of expression. Values are expressed as relative levels of mitochondrial gene/β-actin mRNA or GPDH/β-actin mRNA after correcting for the dilution factors.

Statistical Analysis

Values are means ± SE. Significant differences between treatment groups were determined by Student's *t* test (2-tailed) or 1-way analysis of variance (ANOVA) with post hoc analysis using Student/Newman-Keuls multiple comparison test. Values of $P < .05$ were considered significant.

RESULTS

Age-Related Changes of Mitochondrial Gene mRNA Levels in Rat Pancreatic Islets

RT-PCR amplification using islet cDNA showed the expression of mitochondrial gene mRNAs for CYO-I, -II, and -III, NADH-DH4, and ATP-SYN6 mRNA (Fig 2). In contrast to the heterogeneous population of cells in the islet, RINm5F cells, representative of a homogeneous population of β cells, also expressed each of the mitochondrial gene mRNAs (data not shown). Age-related changes in the levels of mitochondrial gene mRNAs (CYO-I, -II, and -III, NADH-DH4, and ATP-SYN6) were determined in infant, young, and old rat pancreatic islets. The relative abundance of each mitochondrial gene subunit mRNA was determined by comparing the amount of amplification product for each gene with amplification of β-actin mRNA expressed in the same sample. Although the relative levels of CYO-I and CYO-III mRNAs were not different among the experimental age groups (Fig 3A and B), the levels

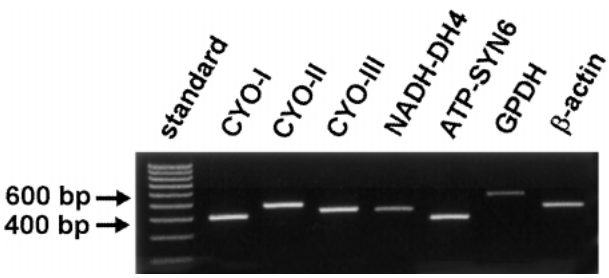

Fig 2. PCR amplification of mitochondrial genes, GPDH, and β-actin mRNAs from rat pancreatic islets. cDNA was amplified with primers specific for CYO-I, -II, and -III, NADH-DH4, ATP-SYN6, GPDH, and β-actin; the sizes of the products are listed in Table 1.

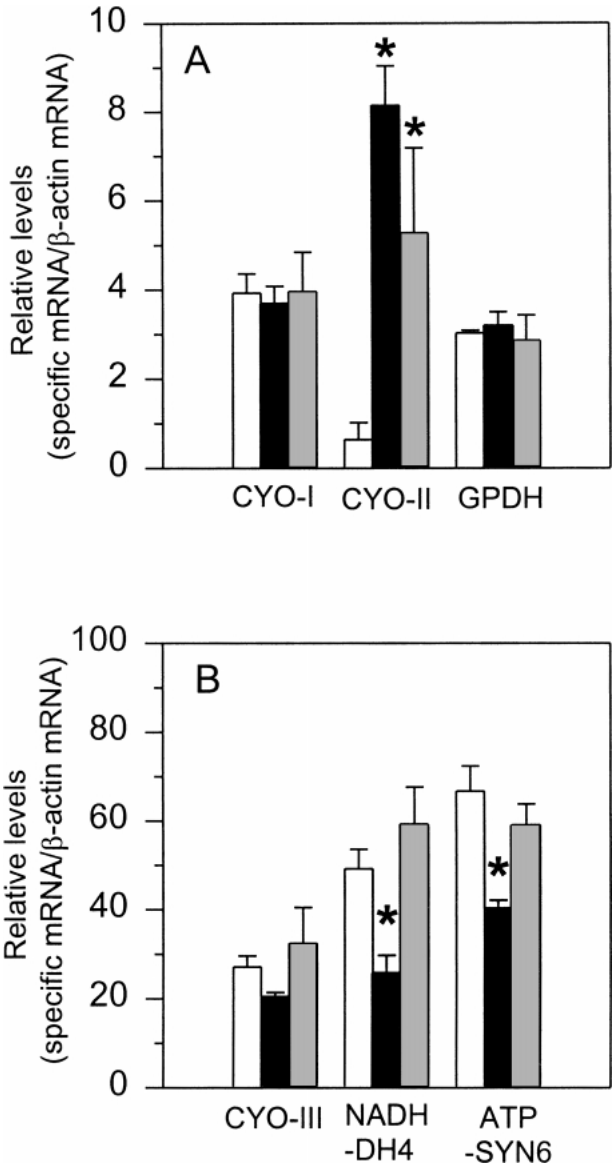

Fig 3. Age-dependent mitochondrial gene and GPDH mRNA expression in rat pancreatic islets. cDNAs from isolated infant (□), young adult (■), and old adult (▨) rat islets were amplified by PCR for mitochondrial and cytoplasmic mRNAs as indicated. Values are means ± SE of mitochondrial-encoded gene and GPDH mRNAs relative to β-actin mRNA expression, for 3 to 10 independent determinations. Significant differences between groups were determined by 1-way ANOVA followed by Student-Newman-Keuls multiple-comparison test. *$P < .01$ *v* infant rat islets.

of CYO-II, NADH-DH4, and ATP-SYN6 mRNA levels were significantly different (Fig 3A and B). The levels of CYO-II mRNA in infant rat islets was 13 ± 1-fold and 8 ± 3-fold lower than in young rat and old rat islets, respectively (Fig 3A). The level of ATP-SYN6 mRNA in infant rat islets was approximately 47% higher than the level in young rat islets, but was not significantly different from the level in old rat islets (Fig 3B). The level of NADH-DH4 mRNA in infant rat islets was approximately 40% higher than the level in young rat islets, but

was not significantly different from that of old rat islets (Fig 3B). Cytoplasmic GPDH mRNA was quantitated for comparison with the mitochondrial gene mRNAs. The relative level of GPDH mRNA was not different among the 3 different age groups of rat islets (Fig 3A).

Effects of Dietary Carbohydrate Feeding on Mitochondrial Gene mRNA Expression in Infant Rat Islets

After feeding 4-day-old infant rats a HC formula for 8 days, the mitochondrial gene mRNA levels were compared with the age-matched MF rats. The relative level of CYO-II mRNA in HC rat islets was increased to 3.3-fold of the level in MF rat islets (Fig 4). However, the expression levels of CYO-I and CYO-III mRNAs in HC rat islets remained unchanged at 92% ± 7% and 96% ± 7%, respectively, of the levels in MF rat islets (Fig 4). The expression of NADH-DH4 and ATP-SYN6 mRNAs in HC rat islets was 101% ± 12% and 98% ± 9%, respectively, of the levels in MF rat islets. Relative GPDH mRNA levels were also unchanged between HC rat and MF rat islets (Fig 4).

Glucose-Stimulated Changes of Mitochondrial Gene mRNA Expression in Rat Pancreatic Islets

In previous studies, the culture of islets in vitro for 4 days or longer at a physiologically relevant hyperglycemic concentration of glucose (11 mmol/L) showed that chronic glucose stimulation alters glucose-stimulated insulin secretion, as well as the mRNA levels for specific genes.[16,22] The 4-day culture of isolated islets from young rats and old rats in the presence of basal glucose (5.5 mmol/L) or a stimulatory glucose concentration (11 mmol/L) was performed to determine the effect of chronic glucose stimulation on mitochondrial gene mRNA levels. Culture of young rat islets with 11 mmol/L glucose did not evoke any significant change in the relative levels of CYO-I (99% ± 4% of control), -II (95% ± 2% of control), and -III (103% ± 10% of control), NADH-DH4 (110% ± 6% of control), ATP-SYN6 (111% ± 2% of control), and GPDH (118% ± 12% of control) mRNA. Relative basal levels of mitochondrial gene mRNA and GPDH mRNA after islet culture at 5.5 mmol/L glucose for 4 days were similar to the levels in fresh islets (Figs 3A-B and 4).

Culture of old rat islets in the presence of 11 mmol/L glucose did not induce any significant changes in the relative levels of CYO-I (124% ± 24% of control), -II (126% ± 22% of control), and -III (132% ± 42% of control), NADH-DH4 (125% ± 16% of control), and ATP-SYN6 (139% ± 28% of control) mRNAs. However, the expression of GPDH mRNA was significantly increased to 197% ± 21% of control ($P < .01$) in islets cultured with 11 mmol/L glucose compared with basal 5.5 mmol/L glucose (3.2 ± 0.3 GPDH/β-actin mRNA).

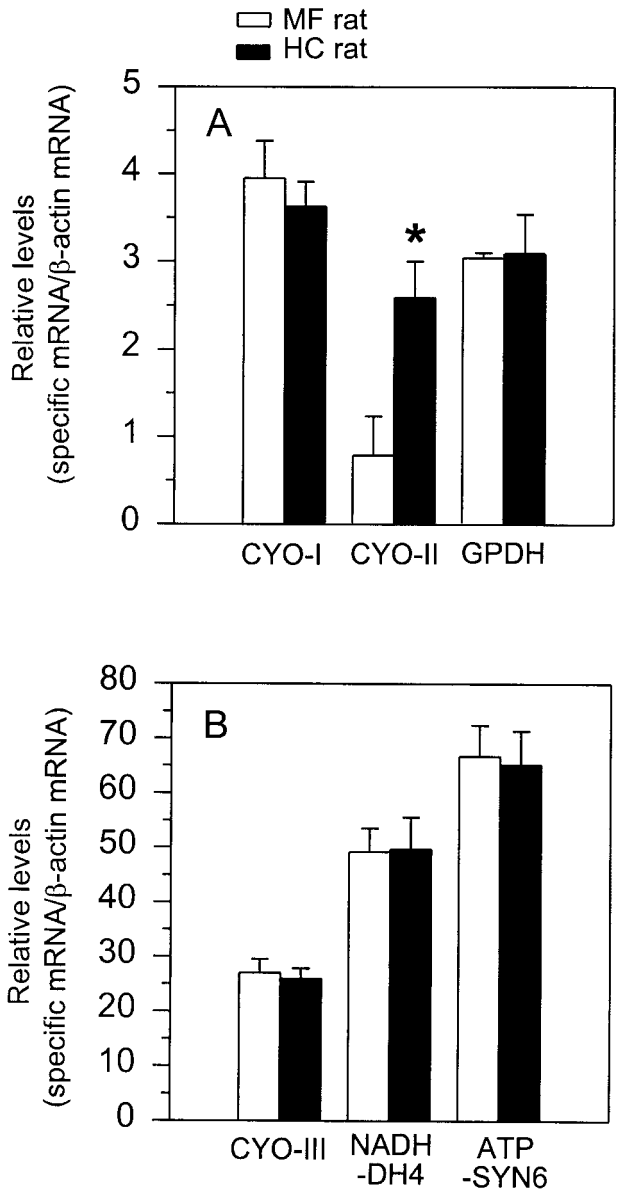

Fig 4. Mitochondrial and cytoplasmic mRNA expression in infant rat islets. Four-day-old rats were either reared for 8 days on mother's milk (MF, □) or fed a HC, formula. mRNA expression was determined by RT-PCR. Values are means ± SE of mitochondrial or cytoplasmic mRNAs, relative to β-actin mRNA, for 3 to 4 independent determinations. Significant differences between groups were determined by unpaired Student's *t* test. *$P < .05$ *v* infant MF rat islets.

DISCUSSION

The results of this study indicate that mitochondrial-encoded gene mRNAs (CYO-I, -II, and -III, NADH-DH4, and ATP-SYN6) are expressed in rat pancreatic islets and a β-cell line, and the expression of specific mRNAs is regulated by aging and carbohydrate stimulation. The proteins encoded by mitochondrial DNA are the subunits of mitochondrial respiratory chain complexes (Fig 1), with the CYO mitochondrial-encoded subunits forming the catalytic core of that enzyme, and ATP-SYN being the final step in the generation of ATP. Normal mitochondrial function mediates glucose-induced signaling in β cells by increasing the cytosolic ATP:ADP ratio, which is followed by depolarization of the plasma membrane and Ca^{2+} influx.[10] The subsequent increase in cytosolic Ca^{2+} is the main trigger of insulin exocytosis.[4,10,24] Increases in cytosolic Ca^{2+} also lead to increased mitochondrial Ca^{2+} levels that stimulate

mitochondrial metabolism through the activation of Ca^{2+}-sensitive NADH-DH.[25-27] It is likely that mitochondrial gene regulation and protein synthesis regulate ATP synthesis and hence insulin secretion in pancreatic β cells.[4,24] Changes in mitochondrial gene expression have been reported for skeletal muscle from patients with type 2 diabetes mellitus that showed increased expression of CYO-I, CYO-III and NADH-DH4 mRNAs.[28]

This study shows for the first time that the levels of specific mitochondrial gene mRNAs in islets are sensitive to aging and carbohydrate availability. The stimulatory effect of a HC diet on infant rat β-cell CYO-II mRNA suggests that carbohydrate metabolism influences mitochondrial gene transcription in the developing pancreas. CYO is a complex enzyme composed of 13 subunits, 3 of which (I, II, and III) are coded by the mitochondrial genome and the other 10 by the nuclear genome.[29] Mitochondria contain all of the elements allowing transcription and translation responsible for mitochondrial DNA replication,[30] and mitochondrial DNA replicate independently and at a much higher rate than the corresponding events in the nucleus.[31] Little is known about the coordinate synthesis of the various CYO subunits. However, it has been postulated that the mitochondrial CYO-I, -II, and -III mRNA levels could be affected by increased levels of the nuclear-encoded subunits.[7,32,33] Proteins potentially involved in initiation of mitochondrial transcription are mitochondrial transcription factor A and RNA polymerases.[34-37] Differences in mitochondrial steady state mRNA levels can also result from differences in mRNA stability.

The differentiation of pancreatic islets during the late intrauterine and suckling periods modulates the insulin secretory responses to glucose.[38] During growth, increased glucose-sensitive insulin secretion is paralleled by a large increase in mitochondrial oxidation without a significant alteration in the rates of glycolysis.[39] CYO-II plays an important role in the oxidative pathway because it carries the metal center, which is the initial electron acceptor from cytochrome c. In the present study, an increase in CYO-II mRNA was observed in young and old rat islets compared with infant rat islets, which correlates with the increased oxidative activity reported during growth. Interestingly, when infant rats were fed a HC diet their CYO-II mRNA increased significantly within 8 days. The increase in CYO-II mRNA in HC rat islets was not, however, at levels comparable to adult rat islets. In the HC rat model, enzymes associated with glycolysis are increased in activity,[20] however, mitochondrial-encoded genome enzyme activity has not been determined. The elevated levels of CYO-II mRNA in infant HC rat and adult rat islets raise the possibility that increased translation of certain enzymes may be associated with the state of differentiation and functional activity. In fact, CYO activity in mouse brain increases progressively up to 5 months after birth and is followed by a gradual decrease with aging.[40] An increased CYO-III mRNA level correlates with increased CYO activity in the hippocampus.[7] Thus, it is possible that an increased level of CYO-II mRNA in this model mediates the increase in CYO levels and activity during differentiation of the pancreas, and that feeding the HC formula during the suckling period hastens this process. This possibility is supported by the finding that HC rat islets show a leftward shift in the insulin secretory response to glucose.[20] During differentiation, the rapidly replicating β cells require high levels of cytosolic nicotinamide adenine dinucleotide phosphate (NADPH) and ATP for the synthesis of nucleic acids. This demand is supplied by constitutively enhanced glucose phosphorylation rates by hexokinase and increased mitochondrial oxidative phosphorylation. The infant HC rat islet also shows an elevation in hexokinase activity[20] that complements the increased CYO-II mRNA expression, and possibly increased CYO activity, in the present study.

The mitochondrial genome of β cells and other cell types can be regulated by different physiologic modulators such as glucose, estrogen, and FSH.[3,6,7] The regulation of mitochondrial gene transcription also has a potential role in the development of diabetes mellitus.[41,42] In the present study, the levels of CYO-I, -II, and -III, NADH-DH4, and ATP-SYN6 mRNAs in young adult and old rat islets were not modified by an elevated concentration of glucose in long-term culture, suggesting that the transcriptional activity of these genes in adult rat β-cell mitochondria is most likely not glucose sensitive. In islets cultured over the long-term at an elevated glucose concentration (11 mmol/L) physiologically relevant to the hyperglycemia associated with diabetes mellitus, the ability of the cells to generate ATP in response to glucose is compromised.[16,43] How glucose desensitization affects metabolism to reduce ATP generation is not entirely known, but it does not appear that changes in β-cell mitochondrial mRNA for the genes investigated in this study accounts for the desensitization. Similarly, the levels of GPDH mRNA were neither different among infant, young, and old rat islets, nor in infant HC versus MF rat islets, confirming similar findings reported previously that the level of GPDH mRNA is not altered in glucose-stimulated young adult rat islets.[3] However, chronic glucose stimulation in vitro did increase GPDH mRNA levels in aged rat islets in the present study. In the rat β-cell line INS-1, glucose stimulation also increased GPDH mRNA levels.[44] In comparison to mRNA levels, GPDH activity was reported previously to be increased in islets from young rats chronically stimulated by glucose in vitro[45] and in islets from infant HC rats.[20] These results suggest that transcriptional and translational regulation of GPDH are independent, and that enzyme activity is related to posttranscriptional events. One explanation for the results is that cytoplasmic GPDH gene transcription is not modulated by carbohydrate-stimulation except in aged rat islets. Alternatively, islet cytoplasmic and mitochondrial mRNA stability may differ among these age-group models and account for the relative mRNA levels in this study.

Our data indicate that biphasic changes in NADH-DH4 and ATP-SYN6 mRNA expression occurred during cell differentiation and aging in pancreatic islets. The expression of NADH-DH4 and ATP-SYN6 mRNA levels were lower in young adult rat islets compared with the levels in infant and old rat islets. An elevated NADH-DH4 and ATP-SYN6 mRNA expression in infant rat islets may be important for glucose oxidation and differentiation in the early stage of development because the activity of CYO may be low as related to the expression of CYO-II mRNA during this period. Insulin secretion is greater in aged rats than in young adult rats,[46] suggesting that the elevated NADH-DH4 and ATP-SYN6 mRNA expression in

aged rat islets may contribute to the activity of glucose oxidation, ATP production (Fig 1), and insulin secretion. However, there is an inconsistent report that glucose oxidation is lower in aged rat islets.[47] In fact, regulation of the oxidative enzyme pathway is complex, with posttranslational proteolytic processing of CYO-II, and perhaps other enzyme proteins, playing a role.[48] Thus, the functional implication of the mRNA levels in the present study remains to be determined.

In summary, islets and insulinoma cells from the rat express mitochondrial gene mRNA. The level of CYO-II, NADH-DH4, and ATP-SYN6 mRNA are regulated by aging and at least CYO-II appears to be regulated by aging and the metabolic responses in the infant rat β cell. Changes in the mRNA levels of this gene during the suckling period suggest that CYO-II mRNA expression may mediate changes in β-cell responsivity to certain secretagogues and has the potential to be affected by hyperglycemic concentrations during pancreatic development. The changes in CYO-II mRNA levels could be a response of pancreatic β cells to a demand for ATP during islet development.

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