

Metabolic Gene Expression in Fetal and Failing Human Heart

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Background—Previous studies suggest that the failing heart reactivates fetal genes and reverts to a fetal pattern of energy substrate metabolism. We tested this hypothesis by examining metabolic gene expression profiles in the fetal, nonfailing, and failing human heart.

Methods and Results—Human left ventricular tissue (apex) was obtained from 9 fetal, 10 nonfailing, and 10 failing adult hearts. Using quantitative reverse transcription–polymerase chain reaction, we measured transcript levels of atrial natriuretic factor, myosin heavy chain- α and - β , and 13 key regulators of energy substrate metabolism, of which 3 are considered “adult” isoforms (GLUT4, mGS, mCPT-I) and 3 are considered “fetal” isoforms (GLUT1, IGS, and ICPT-I), primarily through previous studies in rodent models. Compared with the nonfailing adult heart, steady-state mRNA levels of atrial natriuretic factor were increased in both the fetal and the failing heart. The 2 myosin heavy chain isoforms showed the highest expression level in the nonfailing heart. Transcript levels of most of the metabolic genes were higher in the nonfailing heart than the fetal heart. Adult isogenes predominated in all groups and always showed a greater induction than the fetal isogenes in the nonfailing heart compared with the fetal heart. In the failing heart, the expression of metabolic genes decreased to the same levels as in the fetal heart.

Conclusions—In the human heart, metabolic genes exist as constitutive and inducible forms. The failing adult heart reverts to a fetal metabolic gene profile by downregulating adult gene transcripts rather than by upregulating fetal genes. (*Circulation*. 2001;104:2923-2931.)

Key Words: heart failure ■ fetal heart ■ genes ■ polymerase chain reaction ■ metabolism

A switch in energy substrate preference from carbohydrates to fatty acids is a hallmark of the transition from fetal to adult cardiac metabolism.¹ This switch in energy substrate preference is accompanied by changes in gene expression.^{2,3} These genes encode for key regulators of energy production and energy utilization.⁴ Previous studies in animal models have identified several isogenes, which switch during ontogeny from a “fetal” to an “adult” isoform.^{2,3} A common feature in animal models of cardiac hypertrophy and failure is the induction of the fetal gene program.⁵ The profile of gene expression in the fetal and failing human heart, however, is largely unknown. In this study, we focus on the genes of energy substrate metabolism, because during the transition from normal to failing energy substrate, metabolism reverts to a fetal pattern.

Although transcript levels of relevant metabolic genes have never been quantified in the fetal human heart, we set out to test the hypothesis that the pattern of gene expression in the failing human heart reverts to that seen in the fetal heart. We

therefore characterized the metabolic gene expression profile of 13 key regulators of energy metabolism (including 3 pairs of isogenes) and the expression of the myosin heavy chain (MHC) isoforms α and β and atrial natriuretic factor (ANF) in normal fetal heart, nonfailing adult heart, and failing adult heart. The results show that the failing human heart reverts to a fetal metabolic gene profile by downregulating adult gene transcripts rather than by upregulating fetal gene transcripts.

Methods

Tissue Processing

We obtained myocardial samples from 3 groups of individuals: (1) Nine fetal heart tissues were harvested during elective abortions. The mean gestational age was 18 ± 1 weeks, and 6 of the 9 fetuses were male. Tissue from the left ventricular apex was used for gene analysis. (2) Nonfailing left ventricular tissue (left ventricular apex) was harvested from 10 donor hearts considered not suitable for transplantation for technical reasons. The mean age was 46 ± 4 years, and 5 of the 10 donors were male. The mean ejection fraction measured by echocardiography was $61 \pm 3\%$ (range 50% to 85%). (3) Tissue from 10 failing hearts (left ventricular apex) was obtained

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during the implantation of a left ventricular assist device. Causes of heart failure included idiopathic cardiomyopathy (n=6), ischemic cardiomyopathy (n=3), and peripartum cardiomyopathy (n=1). The mean age was 50 ± 5 years, and 7 of the 10 patients were male. The ejection fraction was $<20\%$. Informed consent was obtained from either the patient or next of kin, and all protocols were approved by the respective Institutional Review Board.

All heart samples were immediately frozen in liquid nitrogen for RNA extraction. RNA was extracted by standard methods⁶ and analyzed by reverse transcription followed by real-time quantitative polymerase chain reaction (PCR) for the transcripts of interest. The methodology of quantitative PCR has been described in detail previously.⁴ The nucleotide sequences for probes as well as forward and reverse primers of the human transcript assays are shown in the Table. As described previously, transcript levels were normalized to the amount of total RNA measured by UV spectrophotometry (Beckman DU 640B).⁷ Internal RNA standards were prepared by use of the T7 RNA polymerase method (Ambion).⁴ To exclude the possibility that changes in gene expression between the failing and nonfailing groups were due to changes in the myocyte/nonmyocyte ratio (eg, myocyte dropout, apoptosis, fibrosis), we measured a myocyte-specific marker (cardiac troponin I) in the nonfailing and failing groups. Cardiac troponin I gene expression was not significantly different between these 2 groups (data not shown).

Statistical Analysis

Data are expressed as mean \pm SEM. Isoform ratios are presented as percentage. Differences between the groups were calculated by 1-way ANOVA followed by the Bonferroni test. A value of $P < 0.05$ was considered significant.

Results

ANF and Myosin Heavy Chain Isoforms

Figure 1 shows ANF and MHC isoform expression in the fetal, nonfailing adult, and failing adult human heart. The mRNA levels of ANF were 10-fold increased in the fetal heart and 5-fold increased in the failing heart compared with the nonfailing adult heart (Figure 1A).

Transcript levels of MHC- α were 18- and 30-fold higher in the nonfailing hearts than in the fetal and failing hearts (Figure 1B). The major isoform MHC- β expression was also decreased in the failing and fetal hearts. This decrease was more pronounced in the fetal myocardium (Figure 1C).

Regulators of Glucose Transport and Utilization

Transcript levels of glucose transporter (GLUT) 1 and GLUT4 are shown in Figure 2A and 2B. Both GLUT1 and GLUT4 expression levels were increased in the nonfailing heart compared with the fetal heart and the failing heart.

Transcript levels of pyruvate dehydrogenase kinase (PDK) 2 and PDK4 increased from the fetal to the adult developmental stage, with a 3-fold increase in PDK2 (Figure 2C) and a 40-fold induction of PDK4 expression (Figure 2D). PDK2 transcript levels decreased in the failing heart, whereas PDK4 expression showed a trend toward decreasing but remained significantly higher than in the fetal heart.

Although previously never reported in human hearts, we detected liver and muscle glycogen synthase (IGS and mGS, respectively) transcripts in the fetal, nonfailing, and failing myocardium, with a predominance of the muscle isoform in all groups. IGS did not differ between any of the groups (Figure 2E), whereas the muscle isoform increased from fetal to adult, and it decreased in the failing heart to levels that were still higher than those in the fetal myocardium (Figure 2F).

Regulators of Long-Chain Fatty Acyl-CoA Transport and Oxidation

Figure 3 shows the transcript levels of carnitine palmitoyl transferase (ICPT)-I (liver isoform) and mCPT-I (muscle isoform). ICPT-I expression did not differ significantly between any of the groups (Figure 3A). mCPT-I mRNA levels increased in the adult heart and decreased in the failing heart to levels similar to those in the fetal heart (Figure 3B).

Long-chain acyl-CoA dehydrogenase (LCAD) transcript levels did not differ between the fetal and the nonfailing adult heart, whereas medium-chain acyl-CoA dehydrogenase (MCAD) expression increased by 10-fold. Compared with the fetal heart LCAD, mRNA levels were lower in the failing heart (Figure 3C). MCAD transcript levels decreased in the failing heart compared with the nonfailing heart (Figure 3D).

Citrate Synthase and Uncoupling Proteins

Citrate synthase expression was lower in the fetal heart than the nonfailing adult heart (Figure 4A). In the failing heart, citrate synthase expression decreased to levels similar to those in the fetal heart.

We also measured transcript levels of the uncoupling proteins UCP-2 and UCP-3. There were no significant differences in UCP-2 or UCP-3 mRNA levels between the fetal and the nonfailing adult heart (Figure 4B and 4C). UCP-2 but not UCP-3 decreased significantly in the failing heart (Figure 4C).

Isogene Profiles in the Fetal, Nonfailing, and Failing Adult Heart

In Figure 5, we compare the isoform profiles of 4 isogene pairs. When isoform ratios of molecule numbers are expressed as percentage of total amount of transcripts, an important observation comes to light: The adult isoforms are predominant in all groups. As shown in Figure 5, A through D, with the exception of MHC- α , relative levels of fetal genes become diluted because of the greater induction of the corresponding adult isogene. Thus, compared with the nonfailing heart, in the failing heart, total transcript levels of metabolic genes were repressed, mainly because of a decrease in adult isogenes.

Discussion

Our main findings and their interpretations are as follows. (1) In the human heart, metabolic genes exist as constitutive and inducible isogenes, rather than fetal and adult isoforms. (2) The human adult failing heart reverts to a fetal metabolic gene profile mainly by repressing genes, rather than reinducing fetal isogenes. Further studies are necessary to examine whether the repression of adult metabolic genes in the failing human heart is regulated by the induction of repressors or by a decrease in stimulation. These observations challenge prevailing notions that the hypertrophied and failing human heart reactivates the fetal gene program by upregulation of fetal genes.

Fetal Gene Program During Development and Disease

The existence of a fetal gene program was first suggested by a study examining electrophoretic patterns of metabolic

Primer and Probe Sequence of Transcript Assays

Gene	Primer/ Probe	Sequence
ANF	Forward	5'-CCCATGTACAATGCCGTGT-3'
	Reverse	5'-TCTTCCAAATGGTCCAGCA-3'
	Probe	5'-FAM-CAACGCAGACCTGATGGATTTCAAGAAT-TAMRA-3'
MHC- α	Forward	5'-TCTCAGGGAGAGGTGTCCGT-3'
	Reverse	5'-GTTCCCGTAGTGCATGATGG-3'
	Probe	5'-FAM-CCACCGATAGTGCCTTTGACGTGCT-TAMRA-3'
MHC- β	Forward	5'-TGAGACTGTCGTGGGCTTGAT-3'
	Reverse	5'-TTGCCTTTGCCCTTCTCAA-3'
	Probe	5'-FAM-CATCAGCCCCAGCATAGTTGGCAAACA-TAMRA-3'
GLUT1	Forward	5'-CAGTTTGGCTACAACACTGGAGT-3'
	Reverse	5'-ATAGCGGTGGACCCATGTCT-3'
	Probe	5'-FAM-CCCCAGAAGGTGATCGAGGAGTTCT-TAMRA-3'
GLUT4	Forward	5'-GCTACCTCTACATCATCCAGAATCTC-3'
	Reverse	5'-CCAGAAACATCGGCCA-3'
	Probe	5'-FAM-CTGCCAGAAAGAGTCTGAAGCGCCT-TAMRA-3'
PDK2	Forward	5'-CAAACACATCGGCAGCATC-3'
	Reverse	5'-TTGTCACACAGGAGCTTAGCC-3'
	Probe	5'-FAM-AGGCATCTTTGACCACCTCAGAGACGTT-TAMRA-3'
PDK4	Forward	5'-TACTCCACTGCACCAACGC-3'
	Reverse	5'-AATTGGCAAGCCGTAACCA-3'
	Probe	5'-FAM-AACCAGCCAAAGGAGCATTCGG-TAMRA-3'
IGS	Forward	5'-TGTAATGATGCTGTGAGAAGAGC-3'
	Reverse	5'-TCAAAAAGTACCACATAAGGACTTCC-3'
	Probe	5'-FAM-TCCAAAATGCACCTGGCAGCCAT-TAMRA-3'
mGS	Forward	5'-GAGAACGCAGTGCTCTTCGA-3'
	Reverse	5'-TAGTTGTGCGCCCATTCGT-3'
	Probe	5'-FAM-AGGTGGCIAACAAGGTGGGTGGCAT-TAMRA-3'
ICPT-1	Forward	5'-GTCCCGGCTGTCAAAGACA-3'
	Reverse	5'-CCGACAGCAAAATCTTGAGCA-3'
	Probe	5'-FAM-TGTGAACAGGTATCTACAGTCGGTGAGGCC-TAMRA-3'
mCPT-1	Forward	5'-CAGGCGAGAACACGATCTTC-3'
	Reverse	5'-GCGGATGTGGTTTCCAAAG-3'
	Probe	5'-FAM-CTGGGCGTTCGTCTCTGAGCTTGA-TAMRA-3'
LCAD	Forward	5'-CTTACAGGAATAAAAACAAATGCTAA-3'
	Reverse	5'-CGCAACTACAATCACAACATCAC-3'
	Probe	5'-FAM-CCATTAITGATGAACACCTTGCTTCCATTGA-TAMRA-3'
MCAD	Forward	5'-AGAATTGGCTTATGGATGTACAGG-3'
	Reverse	5'-TTTGTGATCATTTCCAGCAATAAT-3'
	Probe	5'-FAM-AGGCATTTGCCCCAAGAATTTGCTTC-TAMRA-3'
Citrate synthase	Forward	5'-AGTTGGCAAAGATGTGTGATG-3'
	Reverse	5'-CGCGGATCAGTCTTCCCTTAGTA-3'
	Probe	5'-FAM-AAGTTACGAGACTACATCTGGAACACACTCAACTCA-TAMRA-3'
UCP-2	Forward	5'-CTACAAGACCATTGCCCGAGA-3'
	Reverse	5'-ACAATGGCATTACGAGCAACA-3'
	Probe	5'-FAM-TGGGAGAGGTCCCTTTCCAGAGGCC-TAMRA-3'
UCP-3	Forward	5'-GTGACCTACGACATCCTCAAGG-3'
	Reverse	5'-GCTCCAAAGGCAGAGACAAAG-3'
	Probe	5'-FAM-CTGGACTATCACCTGCTCACTGACAACCTCC-TAMRA-3'

I indicates inosine; FAM, 6-carboxyfluorescein; and TAMRA, 6-carboxy-tetramethyl-rhodamine.

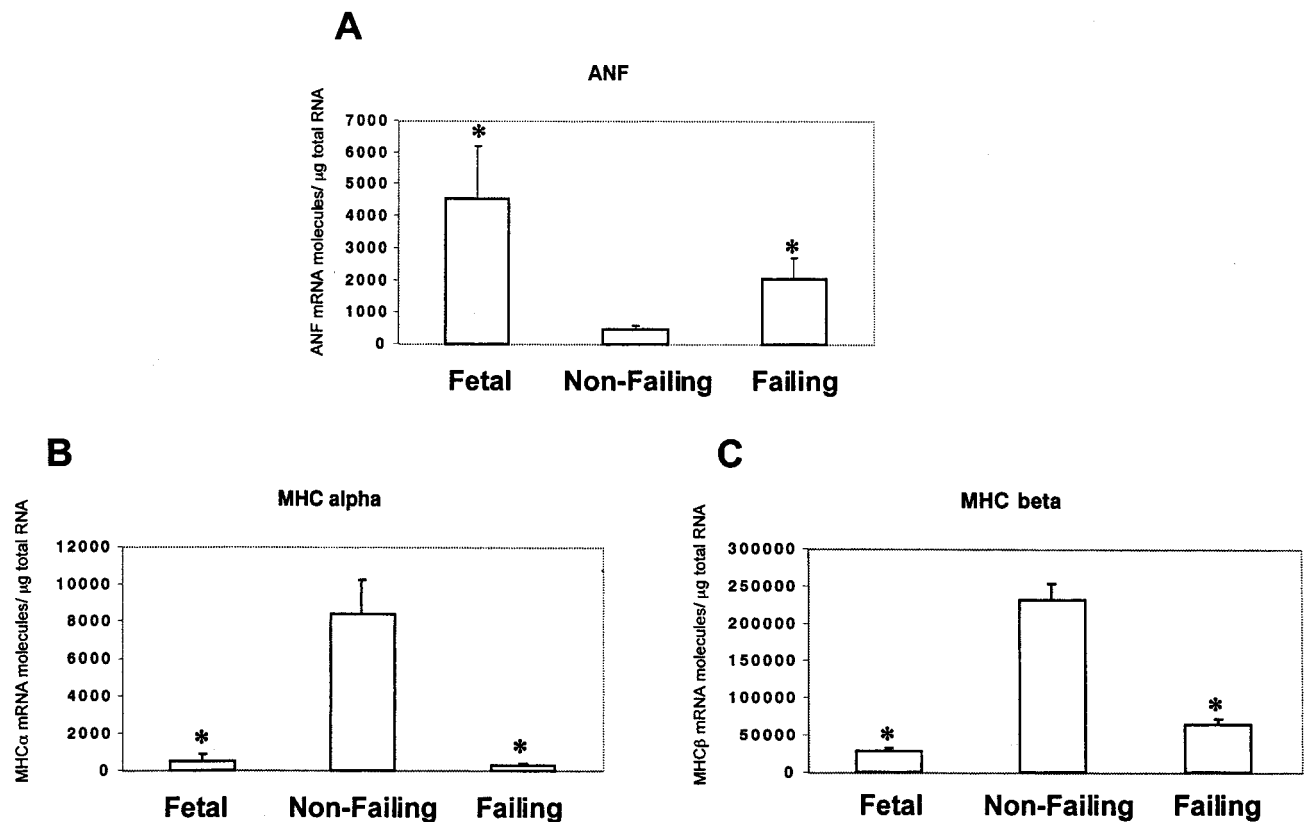


Figure 1. ANF and MHC isoform gene expression in fetal, nonfailing, and failing human heart. Transcript levels of ANF are increased in fetal and failing heart (A), whereas both MHC isoforms show highest levels in nonfailing hearts (B, C; * $P < 0.05$ vs nonfailing).

enzymes in erythrocytes, which showed different isozyme profiles in fetal compared with adult red blood cells.⁸ Since then, numerous studies have focused on the regulation of fetal specific isogenes. The fetal gene program is regulated in response to developmental, hormonal, and hemodynamic stimuli.⁵ We have previously shown that diabetes, pressure-induced hypertrophy, and unloading of the heterotopically transplanted rat heart all reactivate the fetal gene program.^{4,9} In these studies, we proposed that the reactivation of the fetal gene program reflects an adaptive response toward an energy-sparing metabolic profile. We were therefore interested to know whether the findings in a rodent model of cardiac hypertrophy and failure also apply to the human heart.

ANF and MHC Isoforms

Transcript levels of ANF are higher in the human fetal and failing hearts.¹⁰ Possible mechanisms in the failing heart are humoral factors, cardiac nerve activity, stretch, and heart rate.¹¹ Although ANF is considered to be part of the fetal gene program, *in situ* hybridization studies have shown that ANF and skeletal α -actin are not colocalized in the failing myocardium, suggesting differential regulation of ANF and fetal isogenes.¹² This concept is supported by a study showing that in the mouse heart, ANF and MHC- β (fetal isoform) are differentially regulated.¹³ Our results in the human heart confirm increased ANF expression in the fetal and failing heart.

The present study shows that MHC- β expression is the predominant isoform in the heart regardless of the stage of

development or disease. This is consistent with a previous study showing that MHC- β predominated throughout all developmental stages in the human heart.¹⁴ Both MHC isogenes are downregulated in the fetal and failing hearts, consistent with the observation of decreased myofibril contents in the fetal and failing hearts.^{15,16} Others have already reported a decrease in MHC- α expression and content in the failing human left ventricle.^{14,17} We also found a 30-fold reduction of MHC- α in the failing human heart. Although MHC- β expression was also decreased in the failing heart compared with the nonfailing heart, the relative proportion increased because of the larger repression of MHC- α . Other investigators have already shown a relative decrease of MHC- α and a relative increase of MHC- β expression in the left ventricle of patients with cardiomyopathy.¹⁷

Energy Metabolism of the Developing and the Diseased Heart

The fetal heart functions in a relatively hypoxic environment, and the developing heart undergoes a metabolic transformation.¹ Whereas the fetal heart relies predominantly on carbohydrate substrates for ATP synthesis, with increased supply of fatty acid and oxygen after birth, β -oxidation of fatty acids becomes the main energy source for the heart.¹ The hypertrophied and failing adult heart reverts to a fetal metabolic profile.¹⁸ In hypertension, this switch can occur before the onset of hypertrophy.¹⁹ Tracer studies in humans with dilated

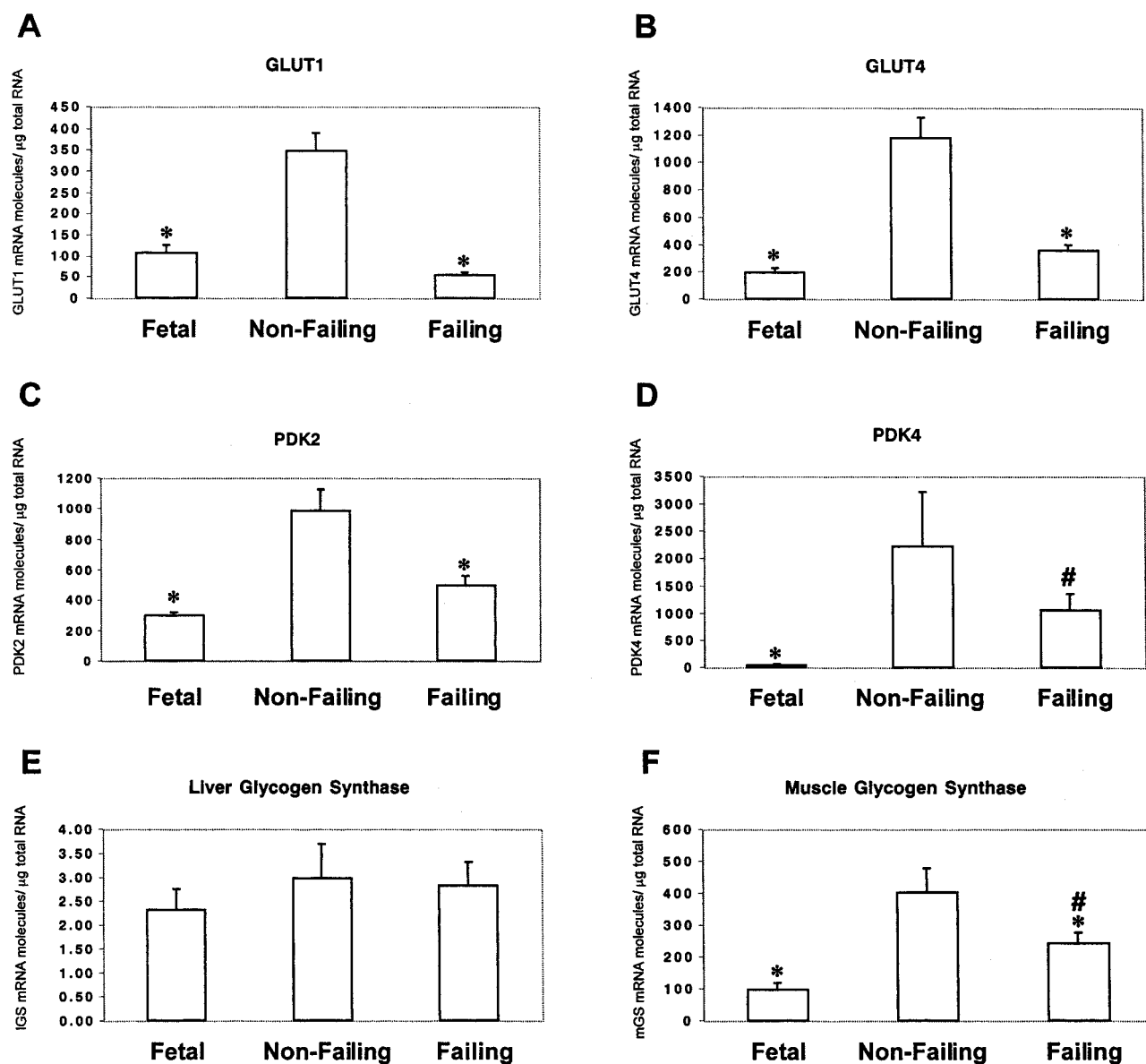


Figure 2. Gene expression of regulators of glucose transport and utilization. Except for IGS (E), all transcripts increased in nonfailing vs fetal heart. GLUT1, GLUT4, PDK2, and mGS all decrease in failing vs nonfailing heart (A through D and F; * $P < 0.05$ vs nonfailing, # $P < 0.05$ vs fetal).

cardiomyopathy have suggested that fatty acid utilization decreases, whereas glucose utilization increases.²⁰ Furthermore, in patients with dilated cardiomyopathy, a downregulation of genes encoding for enzymes involved in β -oxidation has been observed.²¹ In the fetal rat heart, low expression levels of fatty acid-oxidizing enzymes corresponded with decreased fatty acid oxidation during the in utero period.²² Here, we have now shown that this change in energy substrate metabolism is accompanied by changes in metabolic gene expression. These findings suggest that changes in energy substrate metabolism during development and during the transition to failure are in part due to changes in metabolic gene expression.

Regulators of Glucose Metabolism

The fetal and the failing human hearts show similar expression profiles in transcripts of key regulators of glucose

transport and utilization. We have now found an increase of GLUT1 and GLUT4 mRNA from the fetal to the adult stage and a decrease of both isoforms in the failing heart. Studies in rat heart have demonstrated that GLUT4 protein levels are increased ≈ 3 -fold during the transition from the fetal to the adult period.² We found approximately the same fold induction of GLUT4 expression in the adult human heart. A possible mechanism for the increase in GLUT4 levels in the adult heart is the postnatal increase of such hormones as insulin or thyroid hormone.²³ Studies in the rat heart have also shown that GLUT1 protein levels decrease after birth.²⁴ In contrast, the present study shows an increase of GLUT1 mRNA levels during the transition from the fetal to the adult stage. This difference may be due to either a differential regulation among species or more specific posttranscriptional mechanisms. Indeed, GLUT1 contains a 3'-untranslated re-

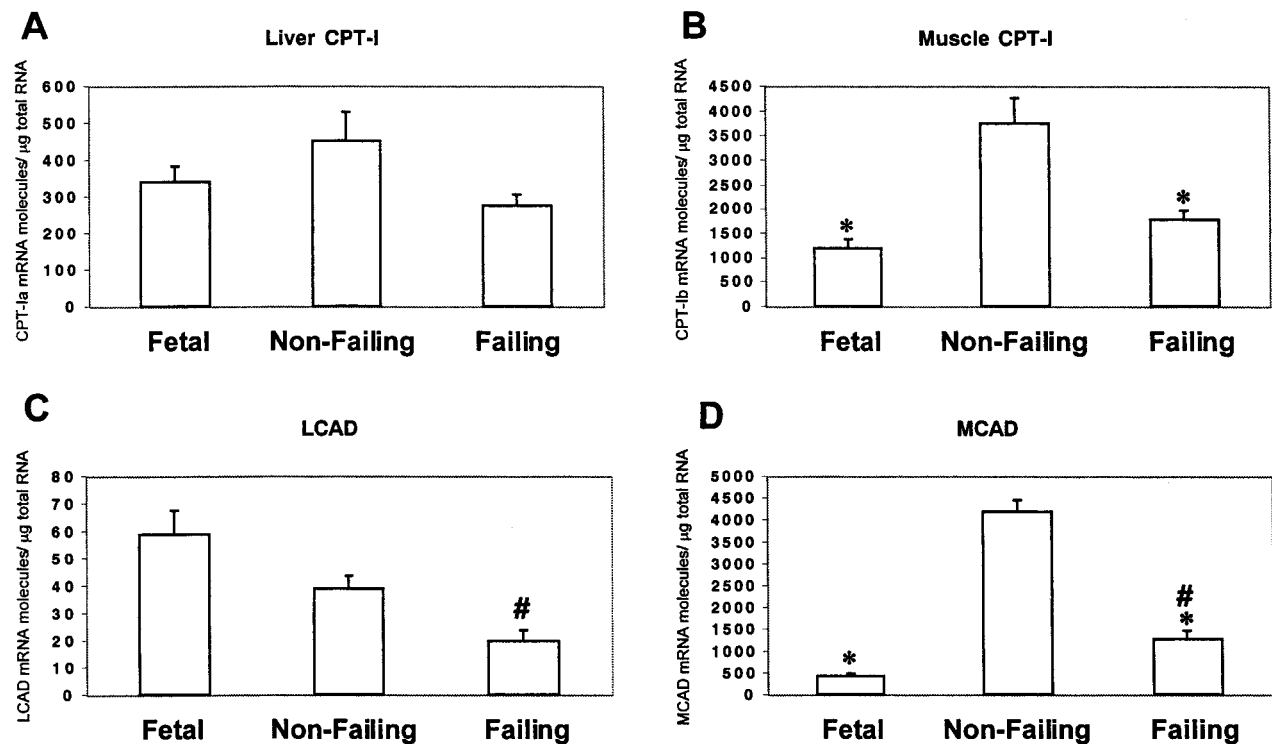


Figure 3. Gene expression of regulators of long-chain fatty acyl-CoA transport and oxidation. Transcript levels of mCPT-I and MCAD increased in nonfailing vs fetal heart and decreased in failing vs nonfailing heart (B, D). In contrast, ICPT-I and LCAD expression did not differ between fetal and nonfailing or between nonfailing and failing groups (A, C; * $P < 0.05$ vs nonfailing, # $P < 0.05$ vs fetal).

gion, which is known to play an important role in posttranscriptional regulation of GLUT1 transcript levels.²⁵ Comparing the isogene profiles of the glucose transporter, we found that relative levels of GLUT1 are decreased in the adult heart compared with the fetal heart because of dilution by the relatively higher induction of GLUT4. Thus, in the human heart, the decrease in GLUT1 transcript expression during development seems to occur only in relative amounts, rather than absolute amounts.

In addition to the glucose transporter isoforms, we measured the expression of PDK2 and PDK4. PDK is a key regulator of glucose and lactate oxidation through inhibitory phosphorylation of the PDK complex.²⁴ We show that transcript levels of both isoforms are lower in the fetal heart than the nonfailing adult heart. This is consistent with a study examining protein levels of PDK2 and PDK4 in the fetal and adult rat heart, which showed an increase in both isoforms during postnatal development.²⁴ Low levels of PDK decrease inhibition of PDK complex and therefore increase glucose and lactate oxidation.²⁴ Studies in the working heart have demonstrated that the fetal pig heart exhibits high rates of lactate oxidation, which might be an adaptive response to the high lactate levels in the fetal circulation.²⁶ Comparing the fetal and the failing hearts, we found a similar expression profile for PDK. The failing human heart shows a significant decrease of PDK2 and a trend to decrease for PDK4. This finding is consistent with the observation that rates of glucose oxidation are increased in the failing heart.²⁰

To the best of our knowledge, there are no published data on the expression of GS isoforms in the human heart. We

were able to detect transcripts of both the lGS and the mGS isoforms in all 3 groups. The muscle isoform was predominant irrespective of the stage of development or of the heart function. Although myocardial glycogen content is high during the fetal period, we found that the fetal heart expressed lower levels of the mGS (and similar levels of the lGS) isoforms of GS compared with the nonfailing and failing adult hearts.¹ This discrepancy in GS expression and glycogen content is probably due to the altered rate of glycogenolysis and/or altered glucose influx into the fetal heart, because a previous study has shown that glycogen phosphorylase activity, a key regulator of glycogenolysis, is low in the fetal heart.²⁷

In summary, the expression profiles of key regulators of glucose utilization are very similar in the human fetal and failing hearts. The highly inducible isoforms GLUT4, PDK4, and mGS show the largest increase during the transition from the fetal to the adult stage, suggesting a postnatal exposure to activators of these isoforms.

Regulators of Fatty Acid Metabolism

Gene expression of key regulators in fatty acid transport and oxidation is similar in the human fetal and failing hearts. Fatty acid transport across the mitochondrial membrane is the rate-limiting step in fatty acid oxidation.³ The heart is unique in that it expresses both the liver and the muscle isoforms of the long-chain fatty acyl-CoA transporter CPT-I.³ We found that mCPT-I predominates in the fetal and the adult heart and that only this isoform is increased during development from the fetal to the adult heart, whereas the liver isoform does not

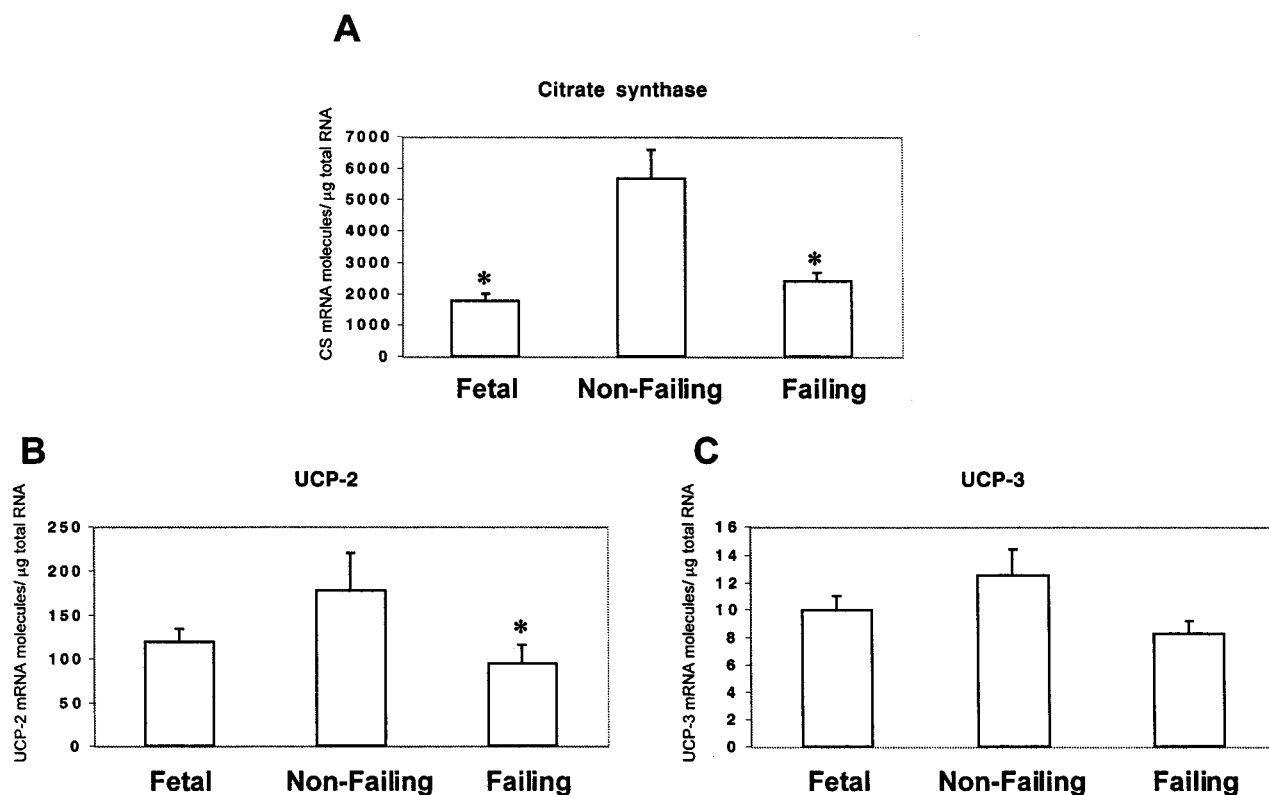


Figure 4. Gene expression of markers of mitochondrial function. mRNA levels of citrate synthase were highest in nonfailing heart (A). There were no differences between UCP-2 and UCP-3 expression between fetal and nonfailing hearts (B, C). UCP-2, however, decreased in failing heart (C; * $P < 0.05$ vs nonfailing).

change significantly during development. Recent studies in the rat heart have shown that ICPT-I activity declines and mCPT-I increases after birth.²⁸ By use of a selective inhibitor for ICPT-I, it has also been demonstrated that the liver isoform contributes $\approx 25\%$ to total CPT-I activity in the newborn rat heart, whereas this value decreases to levels of 2% to 3% with age.²⁹ Comparing the nonfailing and the fetal hearts, we found a similar relative decrease of ICPT-I expression. This change in isoform ratio during cardiac development is the result of differential regulation of ICPT-I and mCPT-I. Characterization of the promoters of these 2 isoforms revealed that mCPT-I, but not ICPT-I, is regulated by peroxisome proliferator-activated receptor- α .³⁰

Although studies in the rat heart have shown that LCAD and MCAD change in a similar way during development,³¹ we did not find a uniform increase in these components of β -oxidation in the nonfailing adult heart. LCAD levels did not differ between fetal and nonfailing hearts, whereas MCAD levels were lower in the fetal heart. The rise of MCAD transcript levels during development is regulated in part by the increase of thyroid hormone after birth. The lack of increase in LCAD expression might be due to species-specific differences in transcriptional regulation.

In contrast to the fetal heart, transcript levels of the mCPT-I isoform are decreased in the failing heart. A previous report examining total CPT activity showed low activity levels in patients with end-stage heart failure.³² Gene expression analysis of mCPT-I and several enzymes involved in β -oxidation showed a coordinated decrease in these genes in

the right ventricle from patients with pulmonary hypertension.²¹ Previous studies using positron emission tomography found an accumulation of [¹¹C]palmitate in patients with congestive cardiomyopathy³³ and a reversion of this metabolic imprint when patients were treated with a β -blocker.³⁴ This is consistent with our findings, which showed that mCPT-I and MCAD are decreased in the failing human heart.

Mitochondrial Function

Mitochondrial function changes during development and is altered in the failing human heart. Ultrastructural studies in the rabbit heart have shown that at midgestation, mitochondrial content and mitochondrial oxygen consumption are low.^{35,36} During the transition from the fetal to the adult period, mitochondrial number and activity increase.³⁶ Activity levels of citrate synthase, a key regulator of the tricarboxylic acid cycle, increased during development. This is consistent with our finding of higher levels of citrate synthase mRNA in the nonfailing heart compared with the fetal heart. Previous studies have shown that despite the decrease in the activity of oxidative enzymes, no age-related differences are found in the efficiency of ATP production in the fetal lamb heart.³⁷ The efficiency of ATP synthesis is determined by the ratio of high-energy phosphate production to oxygen consumption. This efficiency can be modulated by the uncoupling proteins, which dissociate the electrochemical gradient from ATP synthesis. Indeed, we found no differences in UCP-2 or UCP-3 expression between the fetal and the nonfailing hearts. Mitochondrial function is impaired in the

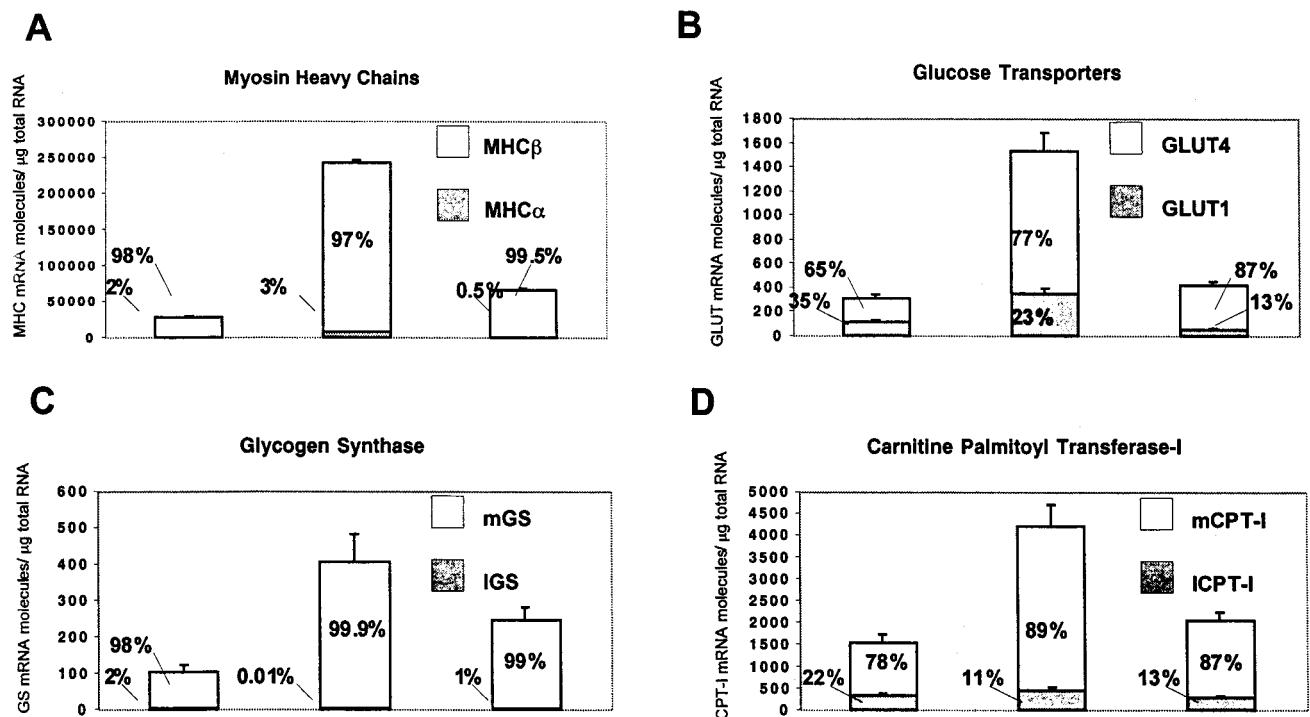


Figure 5. Isoform ratios of MHCs, GLUTs, CPT-I, and GS in fetal, nonfailing, and failing hearts (in each panel, from left to right). Adult isoforms predominated in all groups (A through D). Except for MHC- α , all adult isoforms showed a greater induction than their corresponding fetal isoforms (B through D). Because of higher abundance of adult isoforms, metabolic genes in failing heart were repressed mainly by a decrease of adult isoforms (A through D).

failing heart, and the activity of the respiratory chain is decreased in mitochondria isolated from patients with dilated cardiomyopathy.³⁸ Protein levels of several mitochondrial enzymes, including citrate synthase, are decreased in the failing human heart.³⁹ We now add transcript levels of citrate synthase and UCP-2 to the catalogue of proteins that are downregulated in the failing heart. Although the role of the uncoupling proteins in the heart is still not well understood, the decrease of UCP-2 in the failing heart may be associated with increased efficiency of ATP synthesis.

Study Limitations

Because of the small amount of tissue available to us, we were able to measure only steady-state levels of mRNA. We realize that mRNA turnover rates, posttranscriptional modifications, and changes in enzyme activity are additional modulators of energy metabolism. We cannot exclude the possibility that the human failing heart reactivates fetal isoforms, which were not investigated in this study. The main aim of this study, however, was to test whether the failing human heart reverts to a metabolic fetal gene expression profile, and the data in hand provide a new interpretation of an old phenomenon.

Conclusions

This study shows that the human fetal heart and the human adult failing heart exhibit striking similarities in the expression profile of ANF, MHC- α , and MHC- β and of key regulators of energy metabolism. In contrast to the rat heart, the human heart does not exhibit a true switch to fetal

metabolic isoforms. Rather, in the human heart, metabolic genes are constitutive or induced during the transition from the fetal to the adult stage. Thus, the failing heart reverts to a fetal metabolic gene profile by repressing metabolic adult genes and not by inducing fetal genes.

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