Fasting-induced changes in the expression of genes controlling substrate metabolism in the rat heart

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Abstract During fasting, when overall metabolism changes, the contribution of glucose and fatty acids (FA) to cardiac energy production alters as well. Here, we examined if the heart is able to adapt to such fasting-induced changes by modulation of its gene expression. Rats were fed *ad libitum* or fasted for 46 h, resulting in reduced circulating glucose levels and a 3-fold rise in FA. Besides changes in the cardiac activity or content of proteins involved in glucose or FA metabolism, mRNA levels also altered. The cardiac expression of genes coding for glucose-handling proteins (glucose transporter GLUT4, hexokinase I and II) was up to 70% lower in fasted than in fed rats. In contrast, the mRNA levels of various genes involved in FA transport and metabolism (FA translocase/CD36, muscle-type carnitine palmitoyl transferase 1, long-chain acyl-CoA dehydrogenase) and of the uncoupling protein UCP-3 increased over 50% in hearts of fasted rats. Surprisingly, mRNA levels of the fatty acid-activated transcription factors PPARα and PPARβ/δ were reduced in hearts of fasted rats, whereas in livers, fasting led to a marked rise in PPARα mRNA. Reducing FA levels by nicotinic acid administration during the final 8 h of fasting did not affect the expression of the majority of metabolic genes, but totally abolished the induction of UCP-3. In conclusion, the adult rat heart responds to changes in nutritional status, as provoked by 46 h fasting, through adjustment of glucose as well as FA metabolism at the level of gene expression.—Van der Lee, K. A. J. M., P. H. M. Willemsen, S. Samec, J. Seydoux, A. G. Dulloo, M. M. A. L. Pelsers, J. F. C. Glatz, G. J. Van der Vusse, and M. Van Bilsen. **Fasting-induced changes in the expression of genes controlling substrate metabolism in the rat heart. *J. Lipid Res.* 2001.** 42: **1752–1758.**

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Under physiological conditions, glucose and fatty acids are the main substrates for energy conversion in the heart (1, 2). During conditions in which whole-body metabolism changes, the relative contribution of individual substrates to cardiac energy production will alter. For example, during fasting or diabetes the contribution of fatty acids to cardiac energy production increases, whereas the utilization of glucose is reduced (3, 4). Under both conditions, circulating fatty acid concentrations are increased (5, 6), implicating an enhanced supply of these substrates to the myocardium.

Recently we have demonstrated that long-term exposure of primary cultures of rat neonatal cardiac myocytes to fatty acids resulted in an increased fatty acid oxidation capacity (7). This was accompanied by a specific and coordinated up-regulation of the expression of genes involved in cellular fatty acid transport and metabolism, whereas the expression of the insulin-sensitive glucose transporter GLUT4 declined (7, 8). These data point to metabolic plasticity of cardiac muscle cells, that is, the ability to respond to changes in substrate supply by adjusting the expression of genes encoding proteins involved in substrate transport and metabolism.

In the present study we have examined whether metabolic plasticity is an inherent feature of the cardiac muscle in vivo. Because circulating substrate levels and, hence, substrate supply, to the heart are altered during prolonged fasting, the effect of food deprivation was studied in rats that were not fed for 46 h. The ability of the heart to adapt to metabolic changes was monitored by analyzing the contents or enzymatic activities of a number of proteins concerned in cardiac transport and metabolism of either fatty acids or glucose. Moreover, the mRNA levels of a variety of metabolic genes were determined to explore the possibility that the plasticity, if present, was related to alterations in gene expression. The expression of the uncoupling proteins UCP-3 and UCP-2, which have been proposed to uncouple mitochondrial oxidative phosphorylation (9, 10), was determined, as we recently observed that the expression of these proteins seems to be respon-

Abbreviations: FA, fatty acids; FAT, fatty acid translocase; H-FABP, heart-type fatty acid binding protein; PPAR, peroxisome proliferator-activated receptor.

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sive to fatty acids too, at least in cardiac and slow oxidative muscle (11, 12). In addition, the effect of fasting on the expression of the fatty acid-activated transcription factors peroxisome proliferator-activated receptor α (PPARα) and PPARβ/δ (also referred to as FAAR or NUCI) was examined, since these factors are thought to play a regulatory role in the expression of genes involved in lipid handling (13). Finally, it was investigated whether reduction of plasma fatty acids by nicotinic acid treatment during the last 8 h of the fasting period could reverse the potential changes in mRNA and protein levels in the fasted heart.

MATERIALS AND METHODS

Animals

In this study, 6-week-old male Sprague-Dawley rats (Tierzucht, Zürich, Switzerland) were used, as described in detail by Samec et al. (11). Animals were maintained on a commercially available diet (Provimi-Lacta, Cossonay, Switzerland) consisting (by energy) of 24% protein, 66% carbohydrates, and 10% fat, and had free access to water. Rats were either fed ad libitum or fasted for 46 h. During fasting, all animals had free access to water containing 0.45% NaCl. In a subgroup of rats, the antilipolytic agent nicotinic acid (Fluka Biochimica, Buchs, Switzerland) was administered at 100 mg/kg i.p. every 2 h during the last 8 h of the fasting period, according to Lowell (14). Sterile saline was administered to the control animals. This resulted in four experimental groups (fed/-, fasted/-, fed/NA, and fasted/NA, n = 5 per group). Rats were killed by decapitation 2 h after the last i.p. injection. Blood was collected to assess serum fatty acids and glucose. Hearts and livers were excised, immediately frozen in liquid nitrogen, and stored at −80°C. Frozen tissue was homogenized by grinding with a precooled mortar and pestle. The powder was divided into two portions, one for RNA analysis and the other for preparation of 5% (w/v) homogenates in SET buffer (0.25 M sucrose, 2 mM EDTA, 10 mM Tris, pH 7.4). The latter was used for analysis of enzyme activities and protein contents.

Biochemical analyses

Serum fatty acids were measured using a NEFA C kit (Wako Chemicals, Neuss, Germany), and serum glucose concentrations were determined on a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA, USA).

Hexokinase (HK; EC 2.7.1.1), 3-hydroxyacyl-CoA dehydrogenase (HAD; EC 1.1.1.35), and citrate synthase (CS; EC 4.1.3.7) activities in cardiac muscle homogenates were analyzed on a Cobas Bio centrifugal analyzer (Roche Diagnostics, Basel, Switzerland) using an UV-assay (15–17). Enzyme activities are expressed per mg total protein as determined with the micro BCA method (Pierce, Rockford IL, USA), using BSA as standard. Absorbance was read at 450 nm using a Titertek Multiscan MKII microplate reader.

ELISAs

For the quantification of fatty acid translocase (FAT/CD36), an ELISA was used according to Pelsers et al. (18). In brief, a microtiter plate was coated overnight at 4°C with a monoclonal antibody against human platelet FAT, cross-reacting with rat FAT. After washing with 200 μl PBS containing 0.1% BSA (w/v) and 0.05% (v/v) Tween-20 (PBT), wells were blocked with PBS/2% Marvell dried milk powder (Chivers and Sons Ltd., Dublin, Ireland). After washing with PBT, 100 μl of homogenate (pretreated with 2% Triton X-100 for 1 h at 37°C, centrifuged for 2 min at 13,000 rpm, and diluted in PBT) or standard (0 to 100 μg/l) was added and incubated for 90 min at room temperature. Wells were washed and incubated with phage M13 (2×10^{11} c.f.u./ml in PBS/2% Marvell) for 1 h at room temperature. After washing, 1:5000-diluted monoclonal anti-M13 (peroxidase labeled) in PBS/2% Marvell was added. Incubation for 1 h was followed by 5 washes and addition of 100 μl substrate. After 5 min, the reaction was stopped by adding 50 μl stop solution. The absorbance was read at 450 nm using a microplate reader.

To determine the tissue content of heart-type fatty acid-binding protein (H-FABP), a sandwich-type ELISA for rodent H-FABP [according to Wodzig et al. (19)] was provided by Hycult Biotechnology, Uden, The Netherlands. In short, first 50 μl of the second monoclonal antibody against H-FABP (conjugated with horseradish peroxidase) and 50 μl of either diluted homogenates or standards of 0 to 25 μg/l H-FABP were added to the wells, which were precoated with the first monoclonal antibody. After incubation at room temperature for 2 h, the wells were washed 4 times with 200 μl buffer followed by the addition of 100 μl substrate solution. After 15 min, the reaction was stopped by adding 100 μl stop solution. Absorbance was read at 450 nm using a microplate reader.

Northern blotting analysis

Total RNA was isolated with TRIzol reagent (Gibco BRL Life Technologies, Gaithersburg, MD, USA). Ten μg of total RNA was size-fractionated on a denaturing gel, transferred to a nylon membrane (Hybond-NX, Amersham, Slough, UK), and fixed with standard techniques. Following prehybridization, the filters were probed with fragments of cDNA of GLUT4, hexokinase II (HKII), FAT/CD36, H-FABP, acyl-CoA synthetase (ACS), long-chain acyl-CoA dehydrogenase (LCAD), or uncoupling protein-2 (UCP-2), as described previously (7, 12). A 2.1 kb EcoRI fragment of rat glucose transporter GLUT1 cDNA (a gift of Dr. A. Zorzano, University of Barcelona, Spain), a 0.6 kb BglII fragment of rat hexokinase I (HKI; a gift of Dr. J. E. Wilson, Michigan State University, USA), a 0.5 kb KpnI-BamHI fragment of muscle-type carnitine palmitoyl transferase 1 (mCPT1; kindly provided by Dr. F. van de Leij, University of Groningen, The Netherlands), a PstI fragment of mouse peroxisome proliferator-activated receptor α (PPARα; kindly provided by Dr. J. Auwerx, Institute Pasteur, Lille, France), and a 1.5 kb BamHI fragment of rat PPARβ/δ (a gift of Dr. P. A. Grimaldi, University of Nice, France) were also used for hybridization, as well as a 1.0 kb fragment of rat CS generated via reverse transcriptase PCR using forward (5′-ATCCGTTTCCGAGGCTWY AGT-3′) and reversed (5′-CGTGAY ACCCCRAACAGGACY-3′) primers, and a 0.7 kb fragment of rat UCP-3 also generated via RT-PCR using (5′-GGCCATCCTCCG GAA CCATGG-3′) and (5′-GCGGCCTGCTTGCCTTGTTCA-3′) as forward and reversed primers, respectively. The cDNA probes were labeled with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham) by random-priming (Radprime, Gibco BRL) to a specific activity of $>0.5 \times 10^9$ cpm/μg DNA. To correct for possible differences in loading and transfer, filters were also hybridized with ^{32}P-labeled ribosomal 18S probe. Following hybridization, filters were washed at the appropriate stringency to remove nonspecific binding. The filters were exposed for 18–36 hours to imaging screens and subsequently scanned on the Personal FX Imager (Bio-Rad Laboratories, Hercules, CA, USA). Signals were quantified using Quantity One software (Bio-Rad Laboratories).

Statistical analysis

Results are expressed as means ± SD. Data were analyzed using a two-way analysis of variance (ANOVA) for the main effects of group (fed versus fasted), treatment condition (saline versus nicotinic acid), and group X treatment condition interaction. Differences were considered significant at $P < 0.05$.

TABLE 1. Serum glucose and fatty acid concentrations in fed and fasted rats treated with saline or nicotinic acid

	Fed		Fasted		ANOVA	
	Saline	Nicotinic Acid	Saline	Nicotinic Acid	Fasting effect	NA Effect
Glucose (mM)	8.2 ± 0.2	7.2 ± 1.4	5.9 ± 0.7	5.9 ± 0.8	$P = 0.0003$	NS
Fatty acids (mM)	0.45 ± 0.13	0.29 ± 0.08	1.33 ± 0.25	0.77 ± 0.49	$P < 0.0001$	$P = 0.015$

Rats were fasted for 46 h or fed ad libitum and treated with either nicotinic acid (NA) or saline for the last 8 h of the fasting period. Data are presented as means ± SD (n = 5). NS, statistically not significant.

RESULTS

Effects of fasting

To explore the plasticity of cardiac metabolism to adapt to changes in whole body metabolism, fasting was used as a mean to modulate extracellular substrate availability. As shown in **Table 1**, fasting had pronounced effects on serum substrate concentrations. After 46 h of fasting, glucose concentrations were approximately 30% lower and fatty acid concentrations 3-fold higher in fasted rats than in fed rats.

The fasting-induced decline in serum glucose and rise in fatty acid concentrations were associated with a small, though statistically significant, reduction in the cardiac activity of the glycolytic enzyme hexokinase (HK) and a rise in the activity of 3-hydroxyacyl-CoA dehydrogenase (HAD), an enzyme involved in mitochondrial fatty acid β-oxidation (**Fig. 1**). The activity of the citric acid cycle enzyme citrate synthase (CS) did not significantly differ between hearts of fed and fasted rats, indicating that the mitochondrial population in the heart was unaltered by fasting.

ELISAs were performed to investigate whether the fasting-induced rise in fatty acid availability was also reflected by changes in the levels of fatty acid transport proteins. **Figure 2** illustrates that the protein content of the cytosolic H-FABP did not change significantly as a result of fasting. However, the protein content of the transsarcolemmal fatty acid translocase FAT/CD36 had nearly doubled after 46 h of food deprivation.

To investigate whether the changes in enzyme activity and protein content were related to changes in mRNA levels of metabolic proteins, Northern blotting was applied. mRNA levels of both cardiac glucose transporters GLUT1 and GLUT4 and of the hexokinase isozymes hexokinase I and II (HKI and HKII) were determined. Quantification of GLUT1 appeared not to be possible, since the signal was below the level of detection (data not shown). mRNA levels of the insulin-sensitive glucose transporter GLUT4 and the glycolytic enzyme HKII were 56% and 67% lower in the fasted group than in the fed group, respectively (**Fig. 3A**). The expression of HKI was slightly but significantly decreased in hearts of fasted animals. In contrast, the mRNA levels of proteins that are involved in fatty acid transport and metabolism increased up to 1.7-fold in the cardiac muscle of fasted animals, the only exception being acyl-CoA synthetase (ACS), the level of which did not change significantly (Fig. 3B and C). The expression of CS was also not affected by fasting (Fig. 3C).

Whereas all above mentioned proteins are involved in the transport and conversion of substrates in order to produce energy, uncoupling proteins (UCPs) are thought to dissipate energy by uncoupling oxidative phosphorylation (9, 10, 20). As opposed to UCP-2, the expression of UCP-3 was markedly induced in the heart muscle after 46 h of fasting (**Fig. 4**).

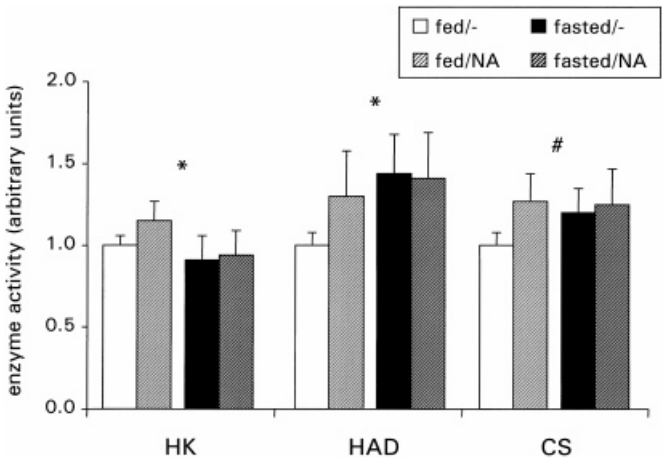

Fig. 1. Effects of 46 h fasting and 8 h nicotinic acid treatment on enzyme activities in the rat heart. Activities of 3-hydroxyacyl-CoA dehydrogenase (HAD), hexokinase (HK), and citrate synthase (CS) amounted to 487 ± 40, 33 ± 2, and 743 ± 56 U/g protein in fed/- hearts, respectively, and were normalized by setting the control group (fed/-) at 1.0. Data are presented as means ± SD (n = 5); * indicates significant ($P < 0.05$) effect of fasting, and # indicates significant ($P < 0.05$) effect of nicotinic acid by two-way ANOVA.

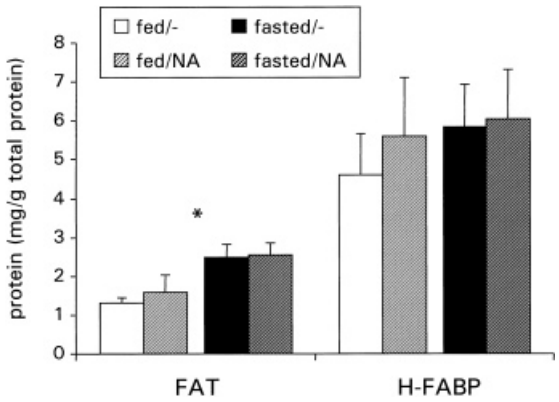

Fig. 2. Effects of 46 h fasting and 8 h nicotinic acid treatment on contents of fatty acid translocase/CD36 (FAT) and heart-type fatty acid-binding protein (H-FABP) in the rat heart. Data are presented as means ± SD (n = 5); * indicates significant ($P < 0.0001$) effect of fasting by two-way ANOVA.

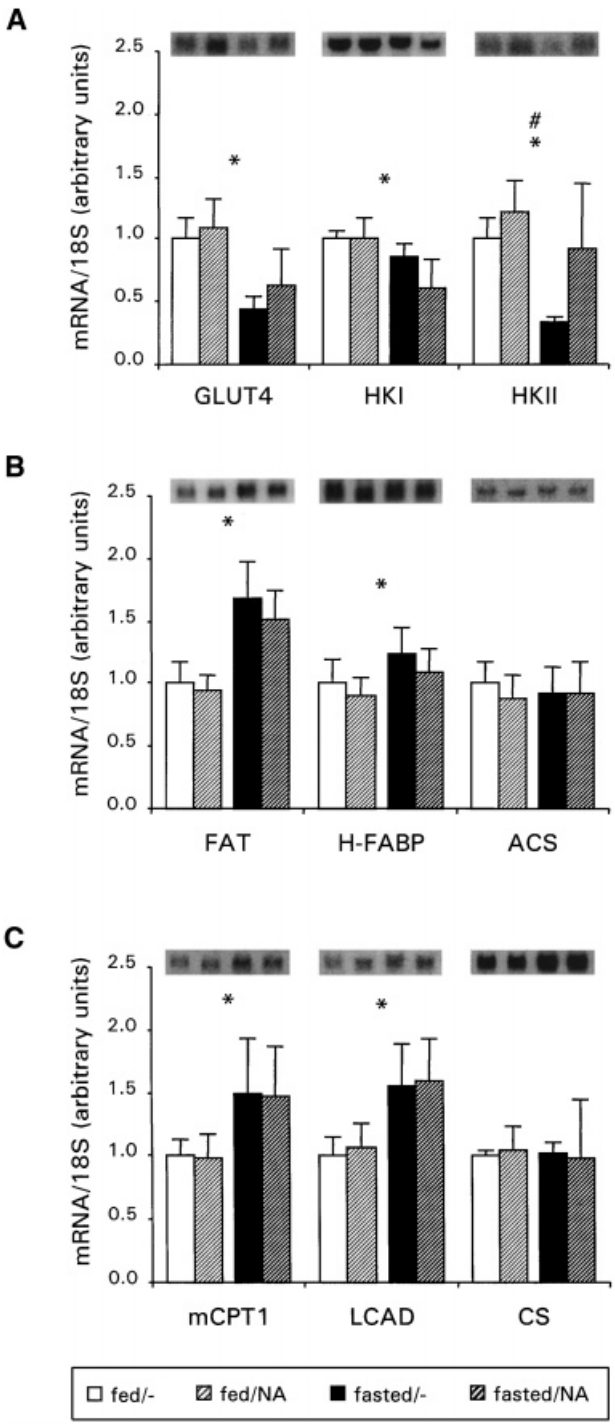

Fig. 3. Effects of 46 h fasting and 8 h nicotinic acid treatment on mRNA levels of proteins involved in glucose utilization (A), fatty acid transport and activation (B), mitochondrial fatty acid handling, and the citric acid cycle (C). mRNA levels of the glucose transporter GLUT4, hexokinase (HK) I and II, fatty acid translocase/CD36 (FAT), heart-type fatty acid-binding protein (H-FABP), acyl-CoA synthetase (ACS), muscle-type carnitine palmitoyl-transferase 1 (mCPT1), long-chain acyl-CoA dehydrogenase (LCAD), and citrate synthase (CS) were determined by Northern blotting and normalized to 18S ribosomal RNA levels to correct for possible loading differences. The level of expression of the control group (fed/-) was arbitrarily set at 1.0. Data are presented as means ± SD (n = 5); * indicates significant ($P < 0.05$) effect of fasting, and # indicates significant ($P < 0.001$) effect of nicotinic acid by two-way ANOVA.

As the fatty acid-activated transcription factors PPARs play a regulatory role in lipid metabolism (13, 21), the mRNA levels of the PPAR isoforms PPARα and PPARβ/δ were also measured. Surprisingly, the expression of both PPAR subtypes was found to decrease significantly in hearts of fasted rats (normalized values for PPARα are 1.00 ± 0.34 in fed and 0.73 ± 0.25 in fasted animals ($P < 0.01$), and for PPARβ/δ, 1.00 ± 0.33 and 0.63 ± 0.09 in fed and fasted animals, respectively ($P < 0.05$) (**Fig. 5**). Since it has been reported that in liver PPARα, mRNA levels are increased in fasted mice (22), PPARα expression in livers of fed and fasted rats were determined as biological controls. As expected, the expression of liver PPARα was markedly induced by fasting (Fig. 5).

Effects of nicotinic acid treatment

In order to examine in which way the heart responds to changes in circulating fatty acids, in a subset of experiments, fed and fasted rats were treated with the anti-lipolytic agent nicotinic acid for 8 h. As anticipated (14), nicotinic acid reduced fatty acid levels in both fed and fasted rats, but did not affect serum glucose concentrations (Table 1).

Nicotinic acid treatment per se did not affect the fasting-induced changes in the expression of the majority of genes coding for proteins involved in cardiac substrate transport and metabolism (Fig. 3). However, nicotinic acid treatment partially restored HKII mRNA levels in hearts of fasted animals (Fig. 3A). In line with the absence of changes in mRNA levels, neither protein contents nor enzyme activities were affected by nicotinic acid treatment, with exception of CS, the activity of which slightly though significantly increased (Figs. 1 and 2).

In contrast to UCP-2, nicotinic acid treatment affected the expression of UCP-3 profoundly (Fig. 4). When the animals were treated with nicotinic acid for 8 h, the fasting-induced increase of UCP-3 mRNA in the heart was completely abolished. As far as the fatty acid-activated transcription factors are concerned, both PPARα and PPARβ/δ mRNA levels were not significantly altered (data not shown).

DISCUSSION

Fasting-induced changes in the heart

During fasting, whole body metabolism dramatically changes. Along with alterations in plasma hormone levels, the concentration of circulating fatty acids substantially increases and glucose levels decline. In this study, the heart is shown to adapt to these changes in substrate supply by modulating the expression of a variety of genes involved in cardiac glucose and fatty acid transport and metabolism. Overall, mRNA levels of proteins involved in cellular glucose transport and metabolism declined in myocardial tissue of fasted rats, whereas the expression of the majority of genes encoding proteins involved in cellular fatty acid transport and metabolism increased. These changes were accompanied by altered protein contents or enzyme activities of a variety of proteins involved in substrate transport and metabolism.

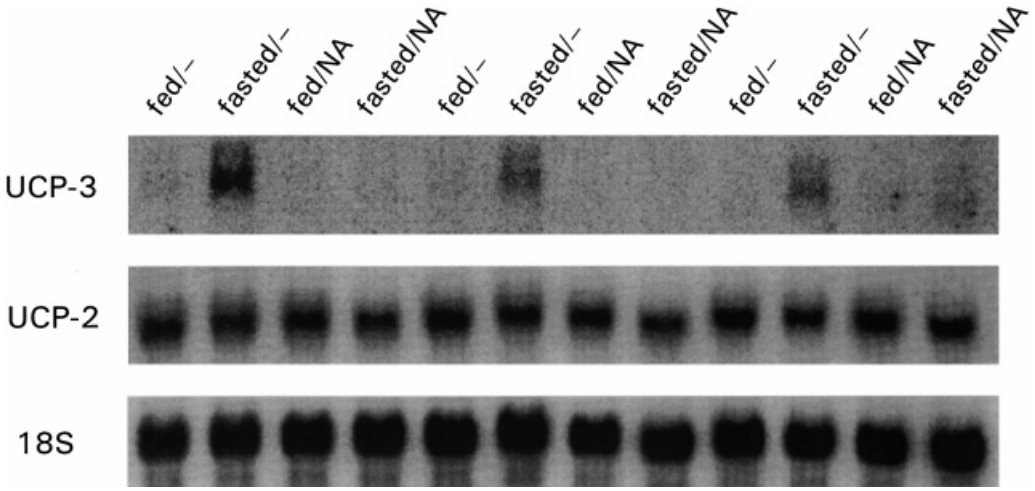

Fig. 4. Representative Northern blot, showing the effects of 46 h fasting and 8 h nicotinic acid treatment on mRNA expression of the uncoupling proteins UCP-3 and UCP-2. The 18S ribosomal RNA signal demonstrates possible loading differences. Normalized values for UCP-2 (mean ± SD) are 1.00 ± 0.17, 0.92 ± 0.35, 0.92 ± 0.23, and 0.95 ± 0.45 for the control fed group, fasted group, fed nicotinic acid-treated group, and fasted nicotinic acid-treated group, respectively. UCP-3 data were not normalized since the contents in control fed hearts were close to background.

Previous studies already showed that differences in nutritional state lead to changes in cardiac substrate utilization (3, 4, 23–25). The question whether this was merely a mass effect (less glucose, more fatty acids supplied via the blood) or due to intrinsic changes in cardiac metabolism has previously been tackled by using isolated hearts of fed and fasted animals. These studies consistently reported a marked reduction in glucose utilization (3, 4) along with an increase in fatty acid utilization (4). Although the latter observation did not corroborate the earlier findings of Opie et al. (3), the general picture that emerges is that of a shift in substrate preference from glucose to fatty acids in isolated hearts of fasted animals, suggesting that intrinsic changes in cardiac metabolism are involved. Indeed, scattered data are available demonstrating that mRNA levels of mCPT1 (26) and acyl-CoA dehydrogenases (27) increased and GLUT4 gene expression (28, 29) decreased in the heart during fasting. The present study extends these findings and shows that, in addition to factors such as intracellular redistribution of transport proteins, phosphorylation of enzymes, and allosteric modulation, the shift in cardiac substrate preference can be attributed to a reciprocal adjustment at the level of gene expression of a variety of transport proteins and enzymes. Thus, mRNA levels of GLUT4, HKI, and HKII, proteins involved in glucose transport and metabolism, are down-regulated, whereas the expression of genes coding for FAT/CD36, H-FABP, mCPT1, and LCAD, proteins involved in fatty acid transport and metabolism, significantly increases in myocardial tissue of the fasted rat.

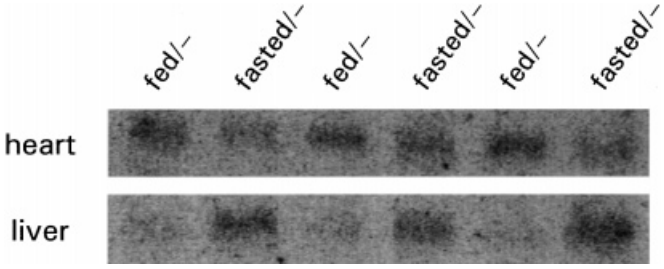

Fig. 5. Representative Northern blot, showing the effects of 46 h fasting on mRNA expression of the transcription factor PPARα in heart and liver tissue.

Fatty acids

The factors that are responsible for this metabolic remodeling remain to be elucidated. Since circulating fatty acid concentrations are markedly increased in fasted animals, and fatty acids have been shown to act as signaling molecules (30), it is tempting to speculate that these substrates are implicated in the remodeling process. This speculation is supported by previous in vitro observations. We found that the mere addition of fatty acids to the culture medium of neonatal cardiomyocytes leads to a selective and coordinate induction of the expression of proteins involved in fatty acid uptake and metabolism, thereby showing that fatty acids themselves are able to modulate gene expression in cardiac myocytes (7).

To obtain more insight into the role of fatty acids per se in the fasting-induced changes in cardiac gene expression, nicotinic acid was administered to reduce the serum fatty acid concentrations in rats. Decreasing circulating fatty acid concentrations in fasted rats by an 8 h nicotinic acid treatment only affected the expression of some of the genes investigated (HKII, UCP-3). The mRNA levels of the majority of the metabolic genes did not alter when circulating fatty acid levels were reduced. At first sight, this would argue against a predominant role of circulating fatty acids in the modulation of the expression of these cardiac genes. It should, however, be taken into account that the half-lives of these mRNAs may be substantially longer than

the duration of nicotinic acid treatment. For instance, the half-life for FAT/CD36 mRNA was estimated to be approximately 13 h in cultured adipocytes (31). Accordingly, it is likely that the duration of the nicotinic acid-induced reduction of circulating fatty acid levels was simply too short to show significant effects on cardiac content of these mRNAs.

Uncoupling proteins

Cardiac UCP-3 mRNA levels, which are very low in rats fed ad libitum, markedly increase during fasting, an induction that is completely reversed by nicotinic acid treatment. This strongly suggests that UCP-3 expression in the heart, a highly oxidative muscle, is regulated by fatty acids. This is consistent with our previous findings in soleus muscle, an oxidative muscle as well, in which we found UCP-3 expression to be dependent on fatty acid supply (11, 32). Interestingly, this analogy does not hold for UCP-2. Similar to UCP-3, this isoform is clearly responsive to fatty acids in soleus muscle, but in cardiac muscle it is marginally responsive to changes in circulating fatty acid levels. The reason for this difference in behavior is incompletely understood.

Since uncoupling proteins uncouple ATP synthesis from the respiratory chain, UCPs are thought to dissipate energy. However, the exact biological functions of UCP-3 and UCP-2 are still largely unknown. Many functional roles for these uncoupling proteins have been proposed, one of which is participation in the regulation of lipid metabolism (33, 34). The notions that UCP-3 and UCP-2 respond to fatty acids in oxidative but not in glycolytic muscles [(11, 32) and present study] and that genes coding for proteins involved in fatty acid metabolism are responsive to fatty acids (7, 35) further support the latter hypothesis. In this respect it is striking that both UCP-3 and UCP-2 are induced in cultured neonatal rat cardiomyocytes after exposure to fatty acids for 48 h (K. A. J. M. van der Lee and M. van Bilsen, unpublished observations) (12).

Peroxisome proliferator-activated receptors

In cultured neonatal cardiomyocytes, the fatty acid-induced up-regulation of the expression of genes coding for fatty acid-handling proteins could be mimicked through the addition of a selective PPARα ligand, suggesting that the effect of fatty acids is PPAR-mediated (7). Studies using PPARα knock-out mice indeed showed that the fasting-induced up-regulation of genes involved in lipid metabolism is blunted in livers (22, 36) as well as in hearts (37) of these mice.

In liver of fasted mice a substantial increase in the expression of PPARα was observed (22). In the present study, a similar response was demonstrated in the livers of fasted rats. In the cardiac muscle, however, mRNA levels of both PPARα and PPARβ/δ decline as a result of fasting. Thus, liver and heart appear to respond differently to fasting as far as PPARα expression is concerned. A corollary of this finding is that enhanced transcription of PPARs per se is not a prerequisite for the induction of genes involved in lipid metabolism. It is therefore noteworthy that recent findings indicate that, in addition to PPAR abundance, transcriptional activity is also dependent on the presence of auxiliary proteins that act as coactivator or corepressor, and on the phosphorylation state of PPARα (13, 38). Further studies are required to disclose whether the latter mechanisms are operational in differential responses of PPAR functioning in heart and liver of fasted rats. In this respect, the elucidation of the promoter sequence of the PPARα gene will be valuable.

Involvement of other factors

Cardiac mRNA levels of proteins involved in glucose transport and metabolism are markedly reduced in the fasted rat. So far, it seems quite unlikely that the decline in mRNA levels of the glucose transporter GLUT4 and of hexokinase II involves PPAR. The observation that Wy-14,643, a PPARα-specific ligand, was unable to affect the expression of GLUT4 in cultured cardiac myocytes is in support of this notion (7). Nonetheless, recent findings by Wu and colleagues (39, 40) demonstrate that the expression of the pyruvate dehydrogenase kinase isozyme PDK4, which phosphorylates and, hence, inactivates the PDH complex, is rapidly induced in skeletal and cardiac muscle of diabetic and fasted animals. Furthermore, these investigators showed that PDK4 expression in skeletal muscle is also induced by administration of the specific PPARα ligand Wy-14,643 (40). The latter findings suggest that PPARs are able to modulate glucose oxidation, albeit in an indirect manner.

Finally, it is unclear why, unlike other fatty acid metabolic genes, ACS is not up-regulated in the cardiac muscle during fasting. In vitro, ACS mRNA levels do increase after exposure of neonatal cardiomyocytes to fatty acids and ACS clearly behaves as a PPARα-responsive gene (7). In this context, it is worth mentioning that the in vivo administration of PPARα ligands to rats did not affect the expression of ACS in the heart, whereas in the liver a marked up-regulation was found (41, 42). These observations again point to a divergent response of liver and heart. Taken together, the present findings strongly suggest that, in addition to PPARs, other factors also play a role in the regulation of metabolic genes in the heart. During fasting, circulating levels of hormones, such as insulin, glucocorticoids, and thyroid hormone, change as well, and effects of these hormones cannot be excluded.

In summary, the present study demonstrates that changes in cardiac metabolism as found during severe fasting have their origin in altered metabolic gene expression in the heart.

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