Cholesterol diet-induced hyperlipidemia influences gene expression pattern of rat hearts: a DNA microarray study

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Abstract To profile gene expression patterns involved in the direct myocardial effect of cholesterol-enriched diet-induced hyperlipidemia, we monitored global gene expression changes by DNA microarray analysis of 3200 genes in rat hearts. Twenty-six genes exhibited significant up-regulation and 25 showed down-regulation in hearts of rats fed a 2% cholesterol-enriched diet for 8 weeks as compared to age-matched controls. The expression changes of 12 selected genes were also assessed by real-time quantitative polymerase chain reaction. Genes with altered expression in the heart due to hyperlipidemia included procollagen type III, cofilin/destrin, tensin, transcription repressor p66, synaptic vesicle protein 2B, Hsp86, chaperonin subunit 5ε, metallothionein, glutathione *S*-transferase, protein kinase C inhibitor, ATP synthase subunit c, creatine kinase, chloride intracellular channel 4, NADH oxidoreductase and dehydrogenase, fibronectin receptor β chain, CD81 antigen, farnesyltransferase, calreticulin, disintegrin, p120 catenin, Smad7, etc. Although some of these genes have been suspected to be related to cardiovascular diseases, none of the genes has been previously shown to be involved in the mechanism of the cardiac effect of hyperlipidemia.
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Key words: Cholesterol diet; Hyperlipidemia; Heart; Gene expression; Procollagen type III; Chloride intracellular channel 4; Tensin; Hsp86; Hsp105; Farnesyltransferase; Metallothionein; NADH dehydrogenase; CD81 antigen; Catenin

1. Introduction

A high-cholesterol diet is regarded as an important factor in the development of cardiac diseases, since it leads to development of hyperlipidemia, atherosclerosis, and ischemic heart disease. The heart of the hyperlipidemic/atherosclerotic patient adapts poorly to oxidative or other kinds of stress, suggesting that the endogenous adaptive mechanisms against myocardial stress are impaired (see [1] for review). The focus of research so far has been mainly the coronary effects of cholesterol, i.e. coronary sclerosis, and the possible direct effect of hypercholesterolemia on the heart was neglected. Very few studies looked at the cellular effects of cholesterol-enriched diet on the myocardium. However, intracellular lipid accumulation in cardiomyocytes and several alterations in the structural and functional properties of the myocardium have been observed [2,3]. We and others have previously shown that hyperlipidemia induced by a cholesterol-enriched diet attenuates the cardioprotective effect of ischemic preconditioning via a mechanism independent of atherosclerosis and other vascular effects of hyperlipidemia [4,5] (see [6,7] for reviews). Furthermore, we have recently shown that hyperlipidemia leads to a moderate contractile dysfunction of the heart characterized by an elevation of left ventricular end-diastolic pressure [8]. These results show that hyperlipidemia acts directly on the myocardium.

The underlying molecular mechanisms of the direct effects of cholesterol diet-induced hyperlipidemia on the myocardium have been addressed by a few studies, but the exact biochemical mechanisms are still a question of debate. A variety of mechanisms, i.e. inhibition of the mevalonate pathway [9], decrease in NO bioavailability and cGMP metabolism [5,10], increase in free radical and peroxynitrite formation [8], inhibition of heat shock response [11], and expression of oxidized low-density lipoprotein receptors which induces apoptosis [12,13], have been shown to play a role in the cardiac effects of hyperlipidemia. However, the traditional biochemical and pharmacological approaches have been insufficient so far to explore the key cellular events in the heart in hyperlipidemia. Recent studies have attempted to identify gene activity changes in atherosclerotic plaques in human and animal blood vessel samples [14,15]. However, the gene expression pattern of the heart in response to hyperlipidemia induced by chronic cholesterol-enriched diet is not known.

Therefore, to profile the gene expression pattern of the heart associated with hyperlipidemia, we have used cDNA microarrays with 3200 genes to monitor transcript levels in rat hearts, in the hope of identifying new cellular pathways involved in the direct cardiac effects of hyperlipidemia induced by dietary cholesterol.

2. Materials and methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and was approved by local ethics committees.

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2.1. Induction of hyperlipidemia

Male Wistar rats (18 weeks old), housed in a room maintained at a 12-h light–dark cycle and a constant temperature of 22 ± 2°C, were fed laboratory chow enriched with 2% cholesterol or standard chow for 8 weeks as described [5,8,11]. Hearts of rats were chosen for the study, since this species shows a moderate increase in serum cholesterol level due to a high-cholesterol diet, and no substantial atherosclerosis develops. However, the increased concentration of tissue cholesterol leads to strong metabolic effects [16,17]. At the end of the 8-week diet period, body weights of the animals were 350–400 g, and there was no significant difference between groups. Plasma cholesterol and triglyceride levels increased by approximately 20% and 300%, respectively, which was consistent with our previous findings [5,8,11]. At the end of the diet period, hearts were isolated for measurement of cardiac function and biochemical parameters.

2.2. Perfusion protocol of isolated rat hearts

Animals were anesthetized with diethylether and given 500 U/kg heparin. Hearts from normal and hyperlipidemic rats ($n = 8$ in each group) were then isolated and perfused in Langendorff mode with oxygenated, normothermic Krebs–Henseleit buffer for 5 min. Subsequently, the perfusion was switched to a working mode [18,19] to measure cardiac mechanical functional and hemodynamic parameters including heart rate, coronary flow, aortic flow, left ventricular developed pressure and its first derivatives ($+dP/dt_{max}$, $-dP/dt_{max}$), and left ventricular end-diastolic pressure as described [20]. Lactate dehydrogenase release was measured from coronary effluent collected for 5 min at the beginning of the perfusion [18]. At the end of the perfusion protocols, the ventricles of hearts from both groups were cut off, immediately frozen, and powdered with a pestle and mortar in liquid nitrogen for RNA preparation.

2.3. RNA preparation

Total RNA was purified from each group (25–25 mg tissue from each heart) with NucleoSpin RNA purification kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions as described [21]. The quantity and quality of RNA from each sample was assessed by gel electrophoresis as well as spectrophotometry (NanoDrop spectrophotometer, NanoDrop, USA). Two RNA pools were prepared from each group ($n = 4$, randomly selected from each group) and used in replica experiments. Total RNA was used for microarray analysis as well as for reverse transcription quantitative polymerase chain reaction (QRT-PCR).

2.4. Microarrays and probes

Construction and use of microarrays were performed as described [22,23]. Briefly, 3200 amplified cDNA inserts from different mouse cDNA libraries were amplified with vector-specific primers, analyzed with agarose gel electrophoresis, and purified with Millipore PCR purification plates. Purified PCR products were reconstituted in 50% dimethylsulfoxide/water and arrayed in duplicate on FMB cDNA slides (Full Moon Biosystems, Sunnyvale, CA, USA) using a MicroGrid Total Array System spotter (BioRobotics, Cambridge, UK) with 16 pins in a 4×4 grid format. After printing, DNA was UV cross-linked to the slides with 700 mJ energy (Stratalinker, Stratagene). Microarray probes were generated by a modified version of a linear amplification technique described before [22,24]. Briefly, 2 μg total RNA from each pooled sample was amplified. Three μg of amplified RNA was labeled with both Cy5 and Cy3 fluorescent dyes (dye-swap experiments) during RT with RNase H (−) point mutant M-MLV reverse transcriptase (Fermentas, Vilnius, Lithuania) and random nonamers. After RT, RNA was alkali hydrolyzed and labeled cDNA was purified with NucleoSpin™ PCR purification kit (Macherey-Nagel) according to the manufacturer's instructions. Probes generated from the control and treated samples were mixed, reconstituted in 16 μl hybridization buffer (50% formamide, 5×SSC, 0.1% SDS, 100 μg/ml salmon sperm DNA) and applied onto the array after denaturation by heating for 1 min at 90°C. Prior to hybridization, the slides were blocked in 1×SSC, 0.2% SDS, 1% bovine serum albumin for 30 min at 45°C, rinsed in water and dried. The slide was covered by a 22 mm×22 mm coverslip, and sealed with DPX Mountant (Fluka, Buchs, Switzerland) in order to prevent evaporation. Slides were incubated at 42°C for 20 h in a humid hybridization chamber. After hybridization the mountant was removed and the arrays were washed by submersion and agitation for 10 min in 1×SSC with 0.1% SDS, for 10 min in 0.1×SSC with 0.1% SDS and for 10 min in 0.1×SSC at room temperature, then rinsed briefly in water and dried.

2.5. Scanning and data analysis

Each array was scanned under a green laser (543 nm for Cy3 labeling) or a red laser (633 nm for Cy5 labeling) using a ScanArray Lite (GSI Lumonics, Billerica, MA, USA) scanning confocal fluorescent scanner with 10 μm resolution (laser power: 85% for Cy5 and 90% for Cy3, gain: 75% for Cy5 and 70% for Cy3) [21]. Scanned output files were analyzed using the GenePix Pro 3.0 software (Axon Instruments, Foster City, CA, USA). Each spot was defined by automatic positioning of a grid of circles over the image. The average and median pixel intensity ratios calculated from both channels and the local background of each spot were determined. An average expression ratio (MeaR, denotes the average of local background corrected pixel intensity ratios) was determined for each spot. Normalization was performed by the global Lowess method [25]. Those data were flagged and excluded where the replicate spots from a different site of the same array or results from the replicate experiments were significantly different. Data analysis was done by the significance analysis of microarrays method [26] and visualization of scatter images was performed with the Microsoft EXCEL software. The cholesterol-regulated genes were determined by calculating the average fold change between heart samples from untreated and cholesterol-fed animals. From two biological replicates and two hybridizations altogether four data points were gathered from each gene. Genes for which the average change (increase or decrease) of the four data points was at least 1.9-fold were considered genes regulated by cholesterol diet.

2.6. Real-time QRT-PCR

Confirmatory real-time QRT-PCR was performed on a RotorGene 2000 instrument (Corbett Research, Sydney, Australia) with gene-specific primers and SYBR Green protocol to confirm the gene expression changes observed by DNA microarrays as described [21]. In brief, 10 μg of total RNA from each pool was reverse transcribed in the presence of oligo(dT) primer in a total volume of 20 μl. After dilution of the mix with 80 μl of water, 2 μl of this mix was used as template in the QRT-PCR. Reactions were performed in a total volume of 20 μl (8 pmol/each forward and reverse primer, 1× Bio-Rad

Table 1
Primers used in real-time PCR analysis

Gene product	Forward primer	Reverse primer
β-Actin	GGAAATCGTGCGTGACATTAAA	TGCGGCAGTGGCCATC
Chaperonin subunit 5ε	TACAGCTCTGCAGATGAAGGATGCTT	TGACATCCGTAAGCCTGGAGAATCTG
Procollagen, type III, α1	TGGAAGTCAAGGAGAAAGTGGTC	AAACCCATGACACCAGGCTG
Synaptic vesicle protein 2B	CCACCAACCAGAGGGCC	GATGGCACCAAGTTTGCACA
Tensin	CGGGTCAAGCTCCGCA	TTTCGTGGCCAAGAAGCC
Heat shock protein, Hsp86	GTTGTGTCAAACCGATTGGTGACATCC	TGTAGATCCTGTTAGCATGGGTCTGG
Protein kinase C inhibitor ι	TCATTCACCACCATCCGGT	GGCAAGAAATGTGCTGCAGA
Glycogen synthase kinase 3	GCTCCCCAACGACCGC	TGGATGGACAGTTCACCAGGA
Metallothionein II	TCGCCATGGACCCCAACTGCTCCTGTG	GAAGCCTCTTTGCAGATGCAGCCCTG
ATP synthase subunit c	TCAAGCAGCAGCTCTTCTCCT	GCCCCATGGCCTCAGAC
Chloride intracellular channel 4	GGCCATCTTAAACTCCCGTG	CCCAGTCTGATGCACATGGA
ND5 respiratory NADH dehydrogenase	ATCGAAGCCATCAACACGTG	GCAGTTATGGATGTGGCGATT
CD81 antigen	CACCGCCGTGCTGAGG	TCCTTCAGAAGCTGAGTGAATGAG

SYBR Green buffer, Bio-Rad, Hungary) with the following protocol: 10 min denaturation at 95°C, and 45 cycles of 25 s denaturation at 95°C, 25 s annealing at 59°C, and 25 s extension at 72°C. Fluorescent signals were gathered after each extension step at 72°C. Curves were analyzed by the RotorGene software using dynamic tube and slope correction methods ignoring data from cycles close to baseline. Relative expression ratios were normalized to β-actin and calculated with the Pfaffl method [27]. The PCR primers used in this study are listed in Table 1. Primers were designed using the ArrayExpress software (Applied Biosystems). All the PCRs were performed four times in separate runs.

3. Results and discussion

Relative gene expression changes in rat hearts in response to cholesterol diet-induced hyperlipidemia were compared to the expression profiles of rats on a normal diet. Changes of 3200 genes were followed by mouse-specific cDNA microarrays. Among the 3200 genes examined in the present study an average of 1324 showed significant intensity (see Section 2 for statistical calculations) and 4.0% (51 genes) showed altered expression: 26 genes exhibited significant up-regulation (Table 2A) and 25 were down-regulated (Table 2B) after 8 weeks of high-cholesterol diet. Out of the 51 genes, 43 genes of known function and eight expressed sequence tags (ESTs) or hypothetical protein genes with unknown function were detected. The gene expression changes ranged from −4.4-fold to +5.5-fold (Table 2).

Table 2
Genes with altered expression due to high-cholesterol diet-induced hyperlipidemia in rat hearts

Function	Gene product	Accession number	Microarray (average fold)	S.D.
A: Up-regulated genes				
Structural proteins	Cofilin/destrin (actin depolymerizing factor)	W17549	3.15	0.21
	Calsarcin-1, myozenin-like 2	XM_215692	2.35	0.52
	Procollagen, type III, α1	W89883	2.14	0.50
Regulatory proteins	Protein phosphatase 1, regulatory subunit 9A	AA087542	4.80	1.52
	Transcription repressor p66	XM_227388	3.38	0.80
	Similar to developmentally regulated protein	AA259357	3.52	0.39
	Pleiotropic regulator 1	AA286018	2.77	0.20
	Glycogen synthase kinase 3	NM_032080	1.95	0.04
	Protein kinase C inhibitor ι	NM_022192	1.90	0.03
Adhesion molecules, membrane proteins	p120 catenin isoform 4B	AW545658	5.48	1.84
	SH3-containing protein SH3P4	W34672	5.11	0.74
	Clara cell phospholipid binding protein	W36838	4.51	0.71
	NIPSNAP2 protein	W15931	3.00	0.34
	Tensin	U26310	1.97	0.19
Stress proteins	Glutathione *S*-transferase	AA231621	3.26	0.20
	Metallothionein II	H32024	1.97	0.06
	Heat shock protein, Hsp86	AW536140	1.95	0.14
Others	Engulfment and cell motility 2	AA087542	4.80	0.34
	Disintegrin, metalloprotease domain 10	AA267983	3.21	0.32
	Mad-related protein Smad7	AA068440	2.70	0.28
	Homologous to ribosomal RNA processing 4	NM_144886	2.58	0.20
	Synaptic vesicle protein 2B	AF372834	1.96	0.09
Hypothetical	Hypothetical protein	AA245492	4.57	0.56
	KIAA0719 protein	AW545304	3.70	0.93
	Hypothetical	XM_220393	2.27	0.41
	Homo sapiens HSPC137	AW545388	1.92	0.18
B: Down-regulated genes				
Energy metabolism	Sarcomeric mitochondrial creatine kinase	XM_226693	−3.58	0.81
	Enolase 3β	W11965	−3.15	0.27
	NADH-ubiquinone oxidoreductase	W83085	−2.59	0.26
	Muscle form glycogen phosphorylase	W16286	−2.34	0.26
	ND5 respiratory NADH dehydrogenase	S46798	−2.08	0.08
	ATP synthase subunit c	D13123	−2.07	0.09
Ion channels, receptors	Fibronectin receptor β chain	AW544628	−3.29	0.53
	Sodium/potassium ATPase β	AW544502	−2.02	0.34
	Chloride intracellular channel 4	NM_031818	−2.00	0.12
	Intracellular chloride channel protein	AW539790	−1.95	0.20
Protein degradation, folding (chaperones)	Proteasome component C9	AA277958	−3.60	0.92
	Ubiquitin-like protein FUBI	AA239437	−3.47	0.57
	105-kDa heat shock protein	AW544862	−2.71	0.32
	Calreticulin	AW545345	−2.26	0.35
	Chaperonin subunit 5ε	AA955792	−2.00	0.08
Cholesterol synthesis, transport	START domain containing 7	W36450	−3.61	0.43
	Farnesyltransferase β subunit	AA259357	−2.05	0.09
	CEA-related cell adhesion molecule 9	AW545543	−1.98	0.12
Others	Fibroblast inducible secreted protein	W36541	−2.68	0.02
	Basigin	AW544934	−2.26	0.43
	CD81 antigen	NM_013087	−2.09	0.35
Hypothetical	Hypothetical protein	AA403436	−4.45	0.51
	mKIAA0475 protein	AA274981	−2.33	0.99
	EST	W44032	−2.33	0.21
	EST	AA068436	−2.17	0.37

S.D., standard deviation.

Table 3
Cardiac functional parameters in control and cholesterol-fed groups

	HR	CF	AF	LVDP	$+dP/dt_{max}$	$-dP/dt_{max}$	LVEDP
Control	273 ± 6	23.0 ± 0.5	44.4 ± 2.0	18.5 ± 0.4	844 ± 46	456 ± 31	0.51 ± 0.05
Chol	271 ± 8	21.9 ± 0.5	45.1 ± 1.3	18.9 ± 0.5	935 ± 50	478 ± 43	0.87 ± 0.05*

HR, heart rate (beats/min); CF, coronary flow (ml/min); AF, aortic flow (ml/min); LVDP, left ventricular developed pressure (kPa); LVEDP, left ventricular end-diastolic pressure (kPa); $+dP/dt_{max}$ (kPa/s); $-dP/dt_{max}$ (kPa/s). Values are means ± S.E.M. ($n = 8$ in each group). *$P < 0.05$, significant difference compared to control by Student's t-test.

Similarly to our previous results [5,8], left ventricular end-diastolic pressure was significantly increased in the hyperlipidemic group showing a mechanical dysfunction of the heart (Table 3). Other hemodynamic parameters including coronary flow were not changed by hyperlipidemia (Table 3) and no lactate dehydrogenase release was detected (data not shown) indicating that hyperlipidemia did not result in restriction of coronary circulation and development of myocardial ischemia. Therefore, gene expression changes observed in this study can be attributed to a direct effect of chronic hyperlipidemia on the myocardium.

We have found that hyperlipidemia significantly affected cardiac energy metabolism and the ATP generating machinery at the level of gene expression, as sarcomeric mitochondrial creatine kinase, enolase 3β, ND5 respiratory NADH dehydrogenase, NADH-ubiquinone oxidoreductase, muscle form glycogen phosphorylase, and ATP synthase subunit c were markedly down-regulated (Table 2B) as evidenced by microarray data and in the case of ATP synthase subunit c by RT-PCR results as well (Table 4). These data are consistent with recent observations showing that hypercholesterolemia leads to an increase in mitochondrial damage in cardiovascular tissues [28]. Reduced energy metabolism and ATP synthesis may lead to functional deterioration of hyperlipidemic hearts as observed in our present and previous studies [5,8].

We have observed down-regulation of genes involved in cholesterol synthesis and transport in the heart after a high-cholesterol diet. Steroidogenic acute regulatory protein-related lipid transfer (START) domains can bind sterol and perform critical functions in moving the sterol substrate to the mitochondrial inner membrane, and stimulate steroidogenesis [29]. The marked down-regulation of a START domain in the present study suggests that excess exogenous cholesterol inhibits intracellular cholesterol transport and steroidogenesis in the heart. The down-regulation of farnesyltransferase β subunit can be attributed to inhibition of the mevalonate pathway (the pathway for cholesterol synthesis) due to excess exogenous cholesterol [30]. We have also observed a moderate repression of carcinoembryonic antigen-related cell adhesion molecule 9 (CEACAM9). Members of the CEACAM family of proteins play a role in the biliary cholesterol crystallization, promoting low-density protein–lipid complex [31], and serve as a potent angiogenic factor and a major effector of vascular endothelial growth factor [32]. These data suggest that dietary cholesterol reduces cardiac cholesterol synthesis and transport, and confirm our previous assumptions that high-cholesterol diet inhibits the mevalonate pathway thereby reducing protein prenylation and ubiquinone synthesis [9].

Genes encoding ion transport proteins and ion channels such as sodium/potassium ATPase β, chloride intracellular channel 4 (CLIC4), and intracellular chloride channel genes were also repressed. Decreased activity of sodium/potassium ATPase increases cardiac contractility, and may therefore be an adaptive response to attenuate hyperlipidemia-induced loss of cardiac function [33]. The role of the CLIC family of proteins is poorly understood, and no data are available on the role of CLIC in the heart; however, cellular volume control, cellular motility, and apoptosis have been suspected [34–36]. The possible role of CLIC in hyperlipidemia in the heart is an entirely new observation that needs further study. Another gene encoding a membrane-bound protein, fibronectin receptor β chain gene, was also markedly down-regulated. Integrins are heterodimeric receptors that couple the extracellular matrix to intracellular signaling pathways and the cytoskeleton. Integrins have been suggested to play a role in cardiac development and several cardiovascular disorders [37,38]. Our present study is the first to suggest that down-regulation of fibronectin receptor plays a role in the cardiac effects of hyperlipidemia.

The most significantly up-regulated genes were membrane

Table 4
Confirmation of gene expression changes due to high-cholesterol diet-induced hyperlipidemia by real-time PCR in rat hearts

Gene product	Accession number	Microarray (average fold)	Real-time PCR (average fold)	Average C_t value	Confirmed by real-time PCR
Procollagen, type III, α1	W89883	2.14	4.15	15.24	Yes
Synaptic vesicle protein 2B	AF372834	1.96	3.86	14.32	Yes
Tensin	U26310	1.97	2.76	16.40	Yes
Heat shock protein, Hsp86	NM_175761	1.95	2.45	16.43	Yes
Protein kinase C inhibitor ι	NM_022192	1.90	1.74	14.95	Yes
Glycogen synthase kinase 3α	NM_032080	1.95	−1.60	12.65	No
Metallothionein II	H32024	1.97	2.46	16.74	Yes
ATP synthase subunit c	D13123	−2.07	−1.92	10.52	Yes
Chloride intracellular channel 4	NM_031818	−2.00	−1.74	16.14	Yes
ND5 respiratory NADH dehydrogenase	S46798	−2.08	−2.16	8.56	Yes
Chaperonin subunit 5ε	AA955792	−2.00	−2.20	16.98	Yes
CD81 antigen	NM_013087	−2.09	−3.44	14.35	Yes

C_t: threshold cycle.

proteins, p120 catenin isoform 4B, SH3-containing protein SH3P4 (endophilin), Clara cell phospholipid binding protein, and NIPSNAP2 protein coding genes. The exact role of these proteins in the heart is not known. P120 catenin affects cell–cell adhesion, as it controls internalization and degradation of classical cadherins in endothelial cells [39], and it is likely to have additional roles in the nucleus [40]. It is plausible to speculate that the expression changes of these membrane protein genes may be partly the consequence of the possible alterations in membrane composition of the heart due to cholesterol diet-induced hyperlipidemia.

Several regulatory protein genes were induced after cholesterol treatment: a protein kinase, a pleiotropic regulator, the regulatory subunit 9A of the protein phosphatase 1, and a transcriptional repressor. Among the regulatory protein coding genes, the protein kinase C inhibitor ι gene was slightly induced, which was confirmed by QRT-PCR as well. Induction of this gene could have a dramatic effect on apoptosis of cardiomyocytes, as protein kinase C inhibitor ι is the critical downstream target of Bcr-Abl, which mediates the anti-apoptotic effects of Bcr-Abl [41]. Moreover, Wang et al. have shown that diet-induced hypercholesterolemia in rabbits was associated with a markedly increased activation of caspase-3 and an increased myocardial Bcl-2/Bax ratio (markers of apoptosis) within the ischemic myocardium, showing the increased extent of cardiomyocyte apoptosis [42].

We have previously shown that transcription of hsp70 was significantly higher in hearts of rats fed a 2% cholesterol-enriched diet compared to normal controls, although the HSP70 protein level was not different [11]. We have also shown that hyperlipidemia inhibits expression of cardiac Hsp70 in response to heat stress and ischemia [11]. Here, we have detected extensive changes in the expression of stress proteins due to hyperlipidemia. The induction of heat stress proteins is well known in response to myocardial, renal, and cerebral ischemia [43–45], but this is the first demonstration that hsp86 and the antioxidants metallothionein II and glutathione *S*-transferase can be induced by cholesterol diet. However, other stress proteins, such as chaperonin subunit 5ε, 105-kDa heat shock protein, calreticulin, and a ubiquitin-like protein were significantly down-regulated. The mechanisms underlying the opposite regulation of these stress proteins due to high-cholesterol diet would be interesting to clarify, as still little is known about the function of these proteins in the heart [46,47].

Opposite changes in transcription of structural protein genes have been observed in the present study. Genes encoding a fibrogenic gene (fibroblast-inducible secreted protein) was down-regulated, while genes for cofilin/destrin (actin depolymerizing factor), calsarcin-1, myozenin-like 2, and procollagen III α1 were induced by high-cholesterol diet. The function of these proteins in the heart might be some remodeling process in response to hyperlipidemia. However, very little is known about the exact role of these genes in the heart.

The activity of numerous genes with diverse function was also significantly altered in hearts of rats fed cholesterol-rich diet as shown in Table 2. For example, we have observed overexpression of Mad-related protein Smad7. Recent reports have implicated Smad7 as a crucial regulator of transforming growth factor β activity in human disease [48] and found that Smad6 and Smad7 constitute a novel class of MAD-related proteins, termed vascular MADs, which are induced by fluid mechanical forces and can modulate gene expression in response to both humoral and biomechanical stimulation in vascular endothelium [49].

Eight clones encoding hypothetical proteins or ESTs having no homology to known proteins exhibited significant up- or down-regulation. The function of these genes and their relationship to myocardial function and to the effects of cholesterol need to be elucidated.

In order to confirm the differential expression of genes revealed by microarray analysis of rat hearts after cholesterol diet, several genes were analyzed by RT-PCR. We have selected 12 genes differentially expressed in hearts from cholesterol-fed animals for real-time RT-PCR analysis (Table 4). The results for 11 genes were in agreement with the microarray data, while in the case of glycogen synthase kinase 3α gene, the moderate alteration in its expression (a statistically borderline case) could not be confirmed. Genes encoding procollagen type III α1, synaptic vesicle protein 2B, tensin, heat shock protein Hsp86 and metallothionein II had very significant rises in transcription rate. In all these cases, more pronounced induction could be detected by QRT-PCR than with the microarray technique. A higher degree of change was also observed in the repression of CD81 antigen. Gene expression changes obtained by QRT-PCR for genes encoding ATP synthase subunit c, chloride intracellular channel 4, protein kinase C inhibitor ι, ND5 respiratory NADH dehydrogenase, and chaperonin subunit 5ε were repressed and values were very similar to those obtained in the microarray measurements. Discrepancies in some cases between DNA microarray and RT-PCR studies have also been reported previously [22,50] and could be explained by cross-hybridization of homologous sequences or by other experimental variables introduced by hybridization, labeling, data processing, and normalization variations during microarray analysis.

We conclude that cholesterol-enriched diet-induced hyperlipidemia leads to significant changes in expression of several genes in rat hearts. As hyperlipidemia does not lead to coronary sclerosis and myocardial ischemia in our rat model, gene expression changes can be attributed to a direct effect of hyperlipidemia on the myocardium. The role of most of the genes we have found to be regulated by hyperlipidemia is not exactly known in the heart, therefore, our present findings may open new directions in the research of the cardiac effects of hyperlipidemia.

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