Microsomal Triglyceride Transfer Protein Gene Expression and Triglyceride Accumulation in Hypoxic Human Hearts

Lars B. Nielsen, Mario Perko, Henrik Arendrup, Claus B. Andersen

Objectives—Cardiac myocytes secrete apolipoprotein (apo)B-containing lipoproteins. Their function may be the removal of triglycerides when β-oxidation of fatty acids is decreased, eg, during hypoxia. To test this hypothesis, we examined heart biopsies from patients undergoing coronary artery bypass graft (CABG, n=13) or valve replacement (n=6) surgery.

Methods and Results—Ventricular microsomal triglyceride transfer protein (P=0.02) and apoB (P=0.04) mRNA levels were both ≈2-fold higher in CABG compared with valve replacement patients. In CABG patients, ventricular microsomal triglyceride transfer protein mRNA levels were negatively associated with the triglyceride content in ventricular myocytes (r=−0.70; P=0.02) and with mRNA levels of sterol regulatory element binding protein-1 (r=−0.74; P=0.004).

Conclusions—The results are compatible with the notion that cardiac lipoprotein production is increased in hypoxic human ventricle, possibly as a result of decreased sterol regulatory element binding protein-1 expression. This might attenuate accumulation of triglycerides in cardiac myocytes. (Arterioscler Thromb Vasc Biol. 2002;22:1489-1494.)

Key Words: hypoxia ▪ lipids ▪ lipoproteins ▪ myocytes

Formation and secretion of triglyceride-rich lipoproteins from cells depend on expression of apolipoprotein (apo)B and microsomal triglyceride transfer protein (MTP). ApoB forms the structural backbone of the triglyceride-rich lipoproteins acquiring neutral lipids during its translation and translocation into the lumen of the endoplasmic reticulum.1 MTP transfers the lipids onto the apoB polypeptide.2 The apoB and MTP genes are expressed in the liver, intestine, and yolk sac.1–4 In addition, the apoB and MTP genes also are expressed by cardiac myocytes, which secrete apoB-containing lipoproteins.5–7 In the intestine the apoB mRNA is edited, resulting in formation of a truncated apoB, ie, apoB48. The apoB mRNA is not edited in the heart,5 and the heart secretes lipoproteins containing the full-length apoB, ie, apoB100.7 The physiological importance of lipoprotein secretion from the heart is unknown. It has been proposed that lipoprotein formation in the heart serves as a pathway for its export of triglycerides during states with reduced oxidation of fatty acids, eg, cardiac hypoxia.

In the present study, we collected fresh biopsies of atrial and ventricular myocardium from patients undergoing coronary artery bypass graft (CABG) or valve replacement (VR) surgery to study a putative role of lipoprotein formation in cardiac triglyceride metabolism. The specific aims were to examine whether expression of apoB and MTP might be upregulated during cardiac hypoxia and whether variations in gene expression might be associated with lipid accumulation in the hypoxic heart.

Methods

Patients

Myocardial biopsies were collected from 2 men and 11 women who underwent CABG surgery; the preoperative cardiac ejection fraction...
was 51±4%. Biopsies were also collected from 6 women with normal coronary arteriography examinations who underwent cardiac VR surgery. The cardiac ejection fraction was 58±2% in the VR patients. All patients gave informed consent, and the study protocol was approved by the Danish ethics committee system (protocol [KF] 01-101/99).

Assessment of Oxygen Tension in the Myocardium

The oxygen tension in the left ventricular muscle was measured with a REVOXODE PO2 probe (Licox CMP Instruments). The probe was fixed with a suture in a noninfarcted area of the anterior wall of the left ventricle shortly after sternectomy and connected to an external computer. Oxygen tension was measured before and 24 hours after cardiac surgery.

Biopsies

Biopsies from the left ventricle (10 to 30 mg) were collected immediately on cardioplegia with a scalpel or a 5-mm muscle biopsy cannula (Stille) from an area immediately adjacent to the PO2 probe. Great care was taken to avoid inclusion of periadventitial fat. Atrial biopsies (40 to 200 mg) were taken from the auricula of the right atrium and unavoidably contained visible fat. No adverse effects of the procedures were observed. Within 1 minute after removal, the biopsies were separated into 2 to 3 portions. One-two portions were frozen in liquid nitrogen and stored at −141°C until RNA and lipid analyses. One portion was processed for histology.

Quantitation of mRNA

Total RNA was extracted from biopsies with TRIzol (Life Technologies) after homogenization with a Polytron PT1200CL (Buch & Holm). First-strand cDNA was synthesized from 1 μg of RNA with M-MULV reverse transcriptase (40 U, Roche A/S) and random hexamer primers.

Forward and reverse primers for amplification of apoB, MTP, sterol regulatory element binding protein (SREBP)-1 and -2, glyceroldehyde-3-phosphate dehydrogenase (GAPDH), β-actin, and adipose most-abundant gene transcript 1 (apM1) cDNAs were as follows: h-apoB-31 (5'-CTGTGGAAGAGAGACACTCA-3') and h-apoB-51 (5'-TTGGATCTATCGAGTGATGCGTCTTT-3'), h-MTP-32 (5'-GAAGCTGTAGTACATC-3') and h-MTP-51 (5'-TCACACAACGCTGTCCTTC-3'), h-SREBP-1-31 (5'-GGCCGCAAGACAGACGATTTAT-3'), h-SREBP-1-51 (5'-TAGTGGCAGAAGATCTGCTG-3'), h-GAPDH-51 (5'-GTACTGGGACCTGCTGCTTTA-3'), h-GAPDH-31 (5'-TCGTCAGCTGCTGTCCTTGA-3'), h-β-actin-51 (5'-AGAAAATCTGGGACCCACC-3') and h-β-actin-31 (5'-GGGGTGTTAGGTCTTAAA-3'), and h-apM1-51 (5'-GCTGGAAAGCCTGTCCTGAGC-3') and h-apM1-31 (5'-GCTGGAGAGCCTGTCCTGAGC-3').

The Lightcycler and DNMastEr SYBR GREEN kit (Roche A/S). This alternative method and the SYBR green method hybridization probe after the protocol provided by the manufacturer correlated (ie, \( r^2=0.97 \) in ventricular biopsies). Accordingly, normalization with either mRNA produced essentially identical results. On repeated analyses, interassay coefficients of variation were 6% (n=6) and 7% (n=8) for apoB and MTP mRNA levels, respectively. Comparisons between samples from CABG and VR patients are all based on results obtained within the same run. The data are expressed as percentage of mean expression in the VR patients in the same tissue. For each mRNA, the presented values are the average of 2 to 4 measurements of each tissue sample.

To investigate regional variation in gene expression among different regions of the heart, we analyzed an explanted heart from a patient with dilated cardiomyopathy (provided by Dr. J. P. Götzke, Rigshospitalet). The coefficients of variation between mRNA copies in 7 samples randomly harvested from left and right ventricle were 13% for apoB and 17% for MTP.

Lipid Analysis

Lipids were quantitated by thin-layer chromatography. Plasma lipid concentrations were determined with enzymatic kits (free fatty acids: WAKO NEFA C kit, Trichem Aps; triglycerides: GPO-TRINDER, SIGMA; total cholesterol: CHOD-PAP, Roche, Mannheim, Germany; and HDL cholesterol: HDL-C, Roche).

Histology

Neutral lipids in heart biopsies were stained with an imidazole-based technique. After Epon embedding, sections (50 to 70 nm) for electron microscopy (EM) were cut with a Reichard Jung, Ultra WTE 701704 microtome and inspected in a Philips 201 electron microscope. Sections (1 μm) for light microscopy (LM) were counter-stained with toluidine blue. To assess the relative amounts of lipid-staining material in ventricular biopsies, pictures were taken with a GC-X1E JVC digital camera and a Leica Diaplan microscope by using an original magnification ×400. Three random pictures from each biopsy were inspected. The amount of lipid-staining material in cardiac myocytes was graded from 0 to 5, and the median value of the three grades was used in the comparison between CABG and VR patients. Grading was performed blinded with respect to patient identity and biochemical lipid data.

Statistics

Differences between CABG and VR patients were analyzed with Student t test for correction for different variances in the two groups whenever appropriate. These and the linear regression analyses were performed with the Prism software (version 2.0, GraphPad).

Results

The oxygen tension in the anterior wall of the left ventricle was ~50% lower in CABG patients than in VR patients before surgery. After surgery this difference was eliminated (Figure 1A). Plasma lipid concentrations were similar in the two patient groups (Table 1).

The relatively low mean plasma cholesterol concentration may be ascribed, at least in part, to surgical stress and the fact that 43% of the patients received cholesterol-lowering medication. The body mass index was higher in CABG than in VR patients (Table 1).
Increased ApoB and MTP mRNA in the Left Ventricle of Patients With Coronary Artery Disease

The ventricular levels of both MTP and apoB mRNA were ∼2-fold higher in CABG compared with VR patients (Figure 1B and 1C). In contrast, there was no difference in either MTP mRNA or apoB mRNA levels in the atrium of the 2 patient groups. The GAPDH mRNA levels were almost identical in CABG and VR patients, both in the ventricle and in the atrium (Figure 1D).

Association Between MTP and SREBP-1 Expression

In cultured liver cells, the SREBP-1 and -2 transcription factors both reduce expression of a luciferase gene driven by the MTP gene promoter. On real-time PCR quantification, the levels of SREBP-1 and -2 mRNA in the heart were ∼1% and ∼4%, respectively, of that in HepG2 cells. SREBP-1 gene expression appeared to be regulated oppositely to the expression of MTP: Ventricular SREBP-1 mRNA levels were lower in CABG patients than in VR patients (Figure 2A), and there was a highly significant

Table 1. Age, Body Mass Index, and Plasma Lipid Concentrations

<table>
<thead>
<tr>
<th></th>
<th>CABG Patients (n=13)</th>
<th>VR Patients (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65 (48–77)</td>
<td>65 (45–85)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.2±1.4</td>
<td>23.8±1.7*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.4±0.25</td>
<td>3.21±0.33</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.73±0.07</td>
<td>0.96±0.15</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.05±0.18</td>
<td>0.88±0.32</td>
</tr>
<tr>
<td>Free fatty acids, mmol/L</td>
<td>1.14±0.23</td>
<td>0.86±0.18</td>
</tr>
</tbody>
</table>

Values are mean and range (age) or mean±SEM (body mass index and plasma lipids).

*P<0.04 compared with CABG patients.
TABLE 2. Ventricular Lipid Concentrations in CABG and VR Patients

<table>
<thead>
<tr>
<th>Lipid</th>
<th>CABG Patients (n=10)</th>
<th>VR Patients (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides, nmol/mg w.w.</td>
<td>38±7.0</td>
<td>9.2, 6.8</td>
</tr>
<tr>
<td>Diglycerides, nmol/mg w.w.</td>
<td>0.8±0.5</td>
<td>0.8, 1.0</td>
</tr>
<tr>
<td>Sphingomyelin, µg/mg w.w.</td>
<td>0.22±0.04</td>
<td>0.22, 0.24</td>
</tr>
<tr>
<td>Phosphatidylcholine, µg/mg w.w.</td>
<td>10.6±1.3</td>
<td>11.6, 9.6</td>
</tr>
<tr>
<td>Phosphatidylserine, µg/mg w.w.</td>
<td>1.2±0.3</td>
<td>1.3, 1.3</td>
</tr>
<tr>
<td>Phosphatidylinositol, µg/mg w.w.</td>
<td>1.8±0.3</td>
<td>1.0, 1.8</td>
</tr>
<tr>
<td>Phosphatidylethanolamine, µg/mg w.w.</td>
<td>5.6±1.6</td>
<td>7.1, 4.0</td>
</tr>
<tr>
<td>Cardiolipin, µg/mg w.w.</td>
<td>4.6±1.6</td>
<td>4.2, 6.8</td>
</tr>
<tr>
<td>Lyso phosphatidylcholine, µg/mg w.w.</td>
<td>0.20±0.10</td>
<td>0.22, 0.22</td>
</tr>
<tr>
<td>Free cholesterol, nmol/mg w.w.</td>
<td>3.7±0.8</td>
<td>3.8, 3.8</td>
</tr>
<tr>
<td>Cholesterol esters, nmol/mg w.w.</td>
<td>0.8±0.7</td>
<td>0.4, 1.0</td>
</tr>
</tbody>
</table>

Values for CABG patients are mean±SD (range). Values for VR patients are individual values from two patients. w.w. indicates wet weight.

negative association between ventricular SREBP-1 and MTP mRNA levels within the group of CABG patients (Figure 2B). Ventricular SREBP-1 mRNA levels were not associated with apoB mRNA levels or any of the cardiac lipid included in Table 2. The SREBP-2 mRNA levels were similar in the CABG and VR patients in both atrium and ventricle (data not shown).

Triglyceride Accumulation in Viable Ventricular Myocytes

Microscopy was used to examine neutral lipid-staining material in myocardial biopsies. In contrast with atrial sections where lipid-filled adipocytes were abundant, we did not observe adipocytes in ventricular sections. This morphological observation was supported by real-time PCR amplification of an adipocytes specific gene transcript (apM1) that encodes adiponectin.17 ApM1 could readily be amplified from a pool of atrial cDNA, even when the cDNA pool was diluted 1:1000. In contrast, the apM1 transcript was undetectable in ventricular biopsies from all but 1 patient. Exclusion of data from this patient did not affect the overall results and conclusions.

On LM, ventricular myocytes often contained clustered droplets of neutral lipid-staining material adjacent to the cellular nuclei (Figure 3A). The relative amount of LM-visible neutral lipid-staining material in each ventricular biopsy was scored on a scale from 0 to 5 (see Methods). There was no difference in the mean lipid content scores of biopsies from CABG (3.0±0.5) and VR (2.0±0.4) patients (Mann-Whitney test; P=0.2).

EM showed neutral lipid-staining material as heterogeneous droplets adjacent to myocyte nuclei (Figure 3B). In addition, lipid-staining material included scattered uniformly staining spherical inclusions resembling triglyceride droplets (Figure 3C) and grape-like clusters of lipid-staining material adjacent to mitochondria (Figure 3D).

We had sufficient biopsy material for biochemical lipid analyses from 10 CABG patients and 2 VR patients (Table 2). In accordance with the morphological examinations, the ventricular triglyceride concentration varied considerably between the patients, whereas the ventricular concentrations of phospholipids and free cholesterol varied to a lesser extent (Table 2).

MTP Expression Is Inversely Related to Triglyceride Content in the Ventricles of CABG Patients

Among the CABG patients, the MTP mRNA level in the left ventricle was negatively associated with the triglyceride concentration (Figure 4). Inclusion of the two VR patients in which ventricular triglycerides had been determined did not change this result. There was no association between the MTP mRNA level and the concentration of any of the other lipids listed in Table 2 or the tissue-oxygen tension. Also, there was no association between triglycerides and apoB mRNA levels or tissue-oxygen tension. Ventricular MTP and apoB mRNA levels as well as triglyceride content were not associated with patient body mass index.

Discussion

It has previously been documented that the apoB and MTP genes are expressed in the heart.5,6 In this study, we observed...
that the mRNA levels of both MTP and apoB were ~2-fold higher in the left ventricle of CABG patients compared with VR patients. This provides the first indication that lipoprotein secretion from the human heart might be regulated. This conclusion rests on the assumption that the gene-expression levels affect lipoprotein-formation rates. Studies of MTP knockout mice suggest that MTP is rate limiting for hepatic secretion of apoB100-containing lipoproteins and that changes in MTP gene expression are closely associated with changes in MTP activity. Thus, it is quite conceivable that changes in MTP gene expression result in changes in lipoprotein formation in the heart as well. In support of this, we found a negative association between the left ventricular MTP mRNA level and its presumed physiological correlate, i.e., the triglyceride content. However, as a result of the limited amount of biopsy material that could be safely collected, we were unable to ascertain that the observed differences in MTP gene expression also affected MTP activity and lipoprotein secretion in the heart. Whether a 2-fold change in apoB gene expression would also affect lipoprotein secretion from the heart is less clear. Studies of liver cells suggest that the apoB protein is produced in abundance, with posttranslational degradation being a major mechanism that regulates how many apoB-containing lipoproteins are actually secreted from the cells. Still, heterozygous apoB knockout mice display decreased plasma lipoprotein levels, indicating that relatively small changes in apoB gene expression under certain circumstances might affect secretion of apoB-containing lipoproteins.

Further studies are needed to resolve the molecular mechanism causing increased apoB and MTP gene expression in hypoxic myocytes. SREBPs might negatively regulate the MTP gene in HepG2 cells. The present studies revealed that SREBP-1 and MTP expression are closely coordinated in the heart. First, the higher MTP mRNA level in CABG patients compared with VR patients was paralleled by a lower SREBP-1 mRNA level. Second, within the group of CABG patients, the MTP and SREBP-1 mRNA levels were negatively associated. Whether these associations reflect a direct regulation of MTP gene expression by SREBP-1 in the heart remains to be investigated. In the liver, SREBP-1 mRNA and protein levels are regulated in parallel by metabolic factors. However, SREBP-1 can also be regulated by posttranslational modifications. Hypoxia induces posttranslational activation of hypoxia inducible factor-1, which in turn regulates the transcription of several genes. In addition, hypoxia deactivates PPAR-α by reducing the availability of its obligate partner RXR. A putative role of hypoxia inducible factor-1 activation and PPAR-α deactivation in regulation of cardiac lipoprotein secretion will have to be determined in the future. Finally, it is also possible that the larger body mass index in CABG compared with VR patients plays a role. Obesity is associated with increased MTP gene expression and secretion of VLDLs from the liver and could have similar effects in the heart. However, we did not find an association of the body mass index with either MTP gene expression or cardiac triglyceride content.

The border zone of myocardial infarcts accumulates neutral lipids. Thus, lipid accumulation might also occur in hypoxic left ventricular myocardium. On histological examinations, we could readily identify neutral lipid-staining material within cardiac myocytes in most biopsies. A semi-quantitative analysis of the amount of LM-visible neutral lipid-staining material could not demonstrate a difference in lipid content between CABG and VR patients. However, there was a large variation in the neutral lipid content between CABG patients. Thus, a large number of patients will have to be studied to establish whether the triglyceride content in ventricular myocardium is increased in CABG patients.

The results suggest that MTP gene expression is coordinated with triglyceride metabolism in the ventricle of patients with ischemic heart disease. First, MTP mRNA levels were inversely related to SREBP-1 mRNA levels in the CABG patient group. SREBP-1 is a potent stimulator of triglyceride synthesis in hepatocytes and adipocytes. Thus, in the heart lipoprotein secretion rates presumably tend to increase when triglyceride synthesis tends to decrease and vice versa. Second, MTP mRNA levels were inversely related to the triglyceride content in the ventricle. At this stage, it is unknown whether the latter association reflects a causal relationship. Nevertheless, genetically accelerated or attenuated cardiac lipoprotein production respectively decreases and increases heart triglyceride stores in mice.

Surplus free fatty acids can negatively affect cardiac contractility and conductivity. Obese rats and transgenic mice with cardiac overexpression of long-chain acyl CoA synthetase have emphasized that triglyceride accumulation in rodent myocardium leads to lipotoxic heart disease. This study supports the idea that transcriptional upregulation of MTP (and possibly apoB) gene expression might serve to attenuate triglyceride accumulation in the hypoxic heart. Recent studies in mice suggest that an increased cardiac lipoprotein formation may affect cardiac function. Diabetic wild-type mice displayed cardiac triglyceride accumulation and signs of cardiac dysfunction, whereas diabetic mice that overexpress a human apoB transgene in the heart had a normal cardiac triglyceride content and less pronounced cardiac dysfunction than the diabetic wild-type mice.

In summary, the results suggest that MTP gene expression is increased in the left ventricle of CABG patients. This might reflect that hypoxia leads to increased MTP gene expression to mitigate ischemia-induced triglyceride accumulation in
cardiac myocytes. This idea also correlates well with the finding that a high level of MTP gene expression was associated with a low level of triglyceride storage in the ischemic left ventricle.

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References
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