# Expression and secretion of human apolipoprotein A-I in the heart

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**Abstract** **Various studies have correlated apolipoprotein (apo) A-I, the major component high-density lipoprotein, with protection against development of cardiovascular disease. Although apoA-I expression has been previously detected in the liver and intestine, we have discovered that the human apoA-I gene is also expressed in the heart. Using transgenic (Tg) mice generated with the human apoA-I/C-III/A-IV gene cluster and Tg mice produced with just the 2.2 kb human apoA-I gene, we have detected significant levels of apoA-I expression in the heart. Furthermore, the detection of apoA-I expression in the hearts of human apoA-I Tg mice indicates that the minimal regulatory elements necessary for cardiac expression of the gene are located near its coding sequence. To determine if the apoA-I gene is also expressed in the human heart, similar analyses were performed, where apoA-I expression was found in both adult and fetal hearts. Furthermore in-depth investigation of the various regions of human and Tg mouse hearts revealed that the apoA-I mRNA was present in the ventricles and atria, but not in the aorta. In situ hybridization of Tg mouse hearts revealed that apoA-I expression was restricted to the cardiac myocyte cells. Finally, heart explants and cardiac primary culture experiments with Tg mice showed secretion of particles containing the human apoA-I protein, and metabolic labeling experiments have also detected a 28 kDa human apoA-I protein secreted from the heart. From these novel findings, new insights into the role and function of apoA-I can be extrapolated.**


*Key words:* Heart; Transgenic mice; Human apoA-I expression; Lipids regulation

## 1. Introduction

Numerous clinical and epidemiological studies have demonstrated that the level of high-density lipoprotein (HDL) is an important cardiovascular risk factor [1] and it has also been shown that HDL has a protective role against coronary artery disease [2].

Apolipoprotein (apo) A-I is the major structural and functional protein component of HDL and constituting 75% of the HDL protein fraction. To a large extent, apoA-I determines the levels of HDL and protects against the development of atherosclerosis [3,4]. However, the precise mechanisms of its antiatherogenic action are not yet fully elucidated. The main hypothesis proposes that the protein plays a key role in the reverse cholesterol transport (RCT) pathway by promoting cholesterol efflux and activating lecithin cholesterol acyltransferase [5]. Other evidence suggests that apoA-I protects the arterial wall against atherosclerosis through antioxidant [6,7], antithrombotic [8] and antiinflammatory [9,10] actions.

Studies have shown that overexpression of human apoA-I in mice increases HDL levels and reduces the atherosclerosis that is induced by a high-fat, high-cholesterol diet or by the absence of the apoE gene [11,12]. Moreover, apoA-I deficient mice in an apoB transgenic (Tg) background show more severe atherosclerosis [13]. Transfer of the human apoA-I gene by recombinant adenovirus increases the HDL concentration and prevents the development of atherosclerotic lesions in different mouse models [14–17].

The human apoA-I gene is grouped together with the apoC-III, apoA-IV, and apoA-V genes in a cluster located on chromosome 11 [18,19]. The major sites of apoA-I expression are the liver and intestine. The main control regions involved in its expression are a proximal promoter/enhancer 250 bp upstream of the human apoA-I gene and a distal enhancer located 7 kb downstream of the gene, in the 5′ region of the apoC-III gene [3]. In Tg mouse models, the proximal promoter/enhancer is enough to achieve hepatic, but not intestinal, expression [20]. The distal enhancer is required to achieve intestinal expression [20] and to increase hepatic expression [21].

We report herein that, in addition to the liver and intestine, the apoA-I is expressed and secreted by the heart. In our studies, we observed cardiac expression of human apoA-I in humans and in two different previously generated Tg mouse models [22,23].

## 2. Materials and methods

### 2.1. Animal models

Five lines of mice transgenic for the human gene cluster apoA-I/C-III/A-IV were used: lines 11, 12 and 21 already described [23] and two recently generated lines, 7 and 25. Transgenic Alliance (IFFA CREDO, Charles River Company, France) furnished line 427 of human apoA-I Tg mice [22]. Tg lines were established and maintained in a C57BL/6 genetic background. When not otherwise specified, experiments with the cluster Tg mice were performed with animals of line 12. Seven-month-old animals were used. Non-Tg littermates were used as controls. Mice were housed in a temperature-controlled room with alternating 12 h light (7 a.m.–7 p.m.) and dark periods (7 p.m.–7 a.m.). The animals had access to regular mouse chow (UAR, France)

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and water ad libitum. In the high-fat, high-cholesterol diet experiments, a group of cluster Tg mice were subjected to a diet supplemented with 10% cocoa butter and 1.25% cholesterol (TD*88051; Teklad Premier Laboratory Diets, Madison, WI, USA) for 20 weeks. All procedures involving animal handling and care were conducted in accordance with Pasteur Institute Guidelines for Husbandry of Laboratory Mice.

### 2.2. RNA isolation and Northern blot experiments

After an overnight fast, mice were killed by cervical dislocation and perfused with phosphate-buffered saline (PBS). The heart, liver, and intestine were quickly dissected out and immediately frozen in liquid nitrogen. Total RNA from each organ was isolated using the TRI-Reagent kit (Euromedex, Souffefweyersheim, Belgium). J.J. Mercadier (INSERM U460, Paris, France) kindly supplied RNA samples from human. Twenty-four μg of total RNA was used for Northern blot analysis by standard procedures [24]. The blots were hybridized with the $^{32}$P-labeled cDNA probes previously described for human apoA-IV [25] and apoC-III [26]. Human and mouse apoA-I-specific probes were generated by polymerase chain reaction (PCR) amplification using the following primer sets: 5′-CAAGAACGGCGGCGCCAGA-3′ and 5′-GCTCTCCAGCACGGGCAGCAGG-3′ to give a 130 bp probe for human apoA-I; and 5′-GCATGCGCACACACGTAGACTCTCT-3′ and 5′-CGTCTCCAGCATGGGCATCAGACTA-3′ to give a 215 bp probe for mouse apoA-I. The human ubiquitin probe (Clontech Laboratories, Inc., Palo Alto, CA, USA) was used as control. Hybridization was carried out according to the QuickHyb Hybridization Solution instruction manual (Stratagene, La Jolla, CA, USA). Autoradiographs were scanned with the NIH Image program. Signals were normalized according to the ubiquitin signal. The results were analyzed with the Statview computer program. Differences were tested by the Student's $t$-test, and $P < 0.05$ was considered significant. To analyze apoA-I expression in different parts of the human heart, a positively charged membrane with immobilized poly $(A)^+$ RNAs from fetal and adult human cardiovascular tissues (Clontech Laboratories, Inc., Palo Alto, CA, USA) was hybridized with the human apoA-I probe described above and with a glyceraldehyde-3-phosphate dehydrogenase control probe.

### 2.3. Reverse transcription (RT)-PCR amplification

Five μg of total mRNA was reverse transcribed according to the Super Script II RT protocol (Gibco BRL, France). One-twentieth μl of the cDNA product was amplified by PCR using the primers used to generate the 130 bp human apoA-I probe and the 215 bp mouse apoA-I probe described above (30 cycles of: 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C). Tg mouse liver samples were used as a positive control for the presence of human apoA-I mRNA. A β-actin control transcript of 279 bp was also amplified using the primers: 5′-GTGGGGCGCCCCAGGCACCA-3′ and 5′-TGGCCTTGGGGTTCAGGGGG-3′. PCR products were separated by 1% agarose gel electrophoresis and visualized after ethidium bromide staining.

### 2.4. In situ hybridization

Mouse hearts were dissected in cold PBS, fixed in 4% paraformaldehyde overnight at 4°C and embedded in paraffin. Sections were hybridized with the riboprobes specific for human or mouse apoA-I described below. Psoralen–biotin-labeled antisense and sense RNA probes were transcribed using the BrightStar psoralen–biotin labeling kit (Ambion, CliniSciences, France), according to the instruction manual. The antisense and sense probes were obtained by separately cloning the 130 bp fragment of human apoA-I and the 215 bp fragment of mouse apoA-I (described in Section 2.2) into the vector pCRII-TOPO (TOPO TA cloning kit, Invitrogen, France). A restriction fragment containing either the Sp6 or T7 promoter was recovered and the probes were generated using the corresponding polymerases. The in situ hybridization procedure was adapted from Hemmati-Brivanlou et al. [27]. Streptavidin–alkaline phosphatase conjugate was used to detect biotinylated probe hybridized to the target mRNA, and NBT (4-nitrobluetetrazolium chloride) and BCIP (5-bromo-4-chloro-3-indolyl-phosphate) were used as substrates, generating a blue/purple color. Samples were examined by light microscopy.

### 2.5. Metabolic labeling experiments

Hearts of cluster Tg and control mice were dissected from the thoracic cavity, opened longitudinally, and flushed with ice-cold incubation medium (methionine and cysteine-free DME, D-0422; Sigma Chemical Co., St. Louis, MO, USA) supplemented with 7% fetal calf serum, 1.6 mM glutamate, and 1.6 mM sodium pyruvate). Metabolic labeling experiments were performed as described [28]: Briefly, freshly minced heart tissues of Tg and control mice were first washed three times with 1.0 ml of the incubation medium and then incubated in 1.0 ml of labeling medium containing 0.6 mCi [$^{35}$S]Promix (Amersham Corp., Arlington Heights, IL, USA). After 4 h incubation at 37°C, the minced heart was pelleted by centrifugation and the media was collected. Two hundred μl of each medium was partially purified by immunoprecipitation with antibodies directed against the human apoA-I (Vector Laboratories, Burlingame, CA, USA) and in the presence of protein A/G-agarose beads (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Once the immunoprecipitates were collected by centrifugation and the supernatants were discarded, the pellets were washed five times with TNEM buffer (125 mM NaCl, 50 mM Tris–HCl pH 7.5, 5 mM EDTA, 0.1% NP-40, 1 mM PMSF) and resuspended in electrophoresis sample buffer (0.5 ml glycerol, 0.25 ml β-mercaptoethanol, 1.5 ml 10% sodium dodecyl sulfate (SDS), 0.6 ml 1.0 M Tris–HCl pH 6.7 and 1 mg bromophenol blue). After boiling 5 min, samples were centrifuged and supernatants were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) and autoradiography. The remaining 800 μl of each radioactive incubation medium was analyzed by SDS–PAGE. After transfer and autoradiography, the membranes were incubated overnight at 4°C with a 1:10 000 dilution of a polyclonal goat anti-human apoA-I antibody. This antibody was detected with a 1:6500 dilution of peroxidase-conjugated anti-goat IgG (H+L) (Vector Laboratories, Burlingame, CA, USA) and the ECL detection system (Amersham, Little Chalfont, UK). Antibodies directed against human apoA-I were shown by Western blot to interact only with the human protein. No cross-reaction was observed with samples containing only the mouse, horse or fetal bovine apoA-I (data not shown).

### 2.6. Agarose gel electrophoresis

Freshly minced heart tissues of Tg mice were incubated in complete medium. After 4 h, the incubation medium was analyzed by agarose gel electrophoresis at 4°C in 0.75% agarose on Gelbond (FMC, Rockland, ME, USA) in 50 mM barbital buffer (pH 8.6). Twenty μl of media and human plasma (used as a migration control) was loaded into 1.5 × 0.2 cm sample channels [29]. After transfer, species containing apoA-I were immunologically identified, as described above. The electrophoretic migration was compared to that of the human plasma lipoproteins stained with Sudan Black.

### 2.7. Mouse cardiac myocyte cultures

Cardiac myocytes were obtained from the hearts of 1–3-day-old mice (cluster Tg or non-Tg littermate), by serial trypsinization of minced tissue as described [30]. Myocytes were plated at a density of 400 cells/mm$^2$ in low-glucose DMEM (BioWest) supplemented with 17% M199 (Sigma), 10% horse serum (Sigma), 5% fetal calf serum (Gibco), 2 mM glutamine (Gibco) and 50 units/ml penicillin–streptomicin (Gibco). The human apoA-I cardiac expression for cluster Tg mice was assessed by immunoprecipitation, followed by immunodetection.

## 3. Results

### 3.1. Expression of human apoA-I gene in the heart of Tg mice

The human apo expression in the heart was analyzed in apoA-I/C-III/A-IV gene cluster Tg mice. Cluster Tg mice were generated using a 33 kb human genomic fragment containing the 17 kb of the apoA-I/C-III/A-IV cluster, 8 kb of its 5′ region and about 7 kb of its 3′ flanking sequences [23]. The three previously reported Tg lines (lines 11, 12 and 21) and the two newly generated lines (7 and 25) expressed the three human genes in liver and intestine. Northern blot analysis also revealed the presence of a significant amount of human apoA-I mRNA in the heart of all five Tg lines (Fig. 1A). The amount of human apoA-I mRNA in the heart was 36% relative to that measured in the liver. Neither human apoC-III

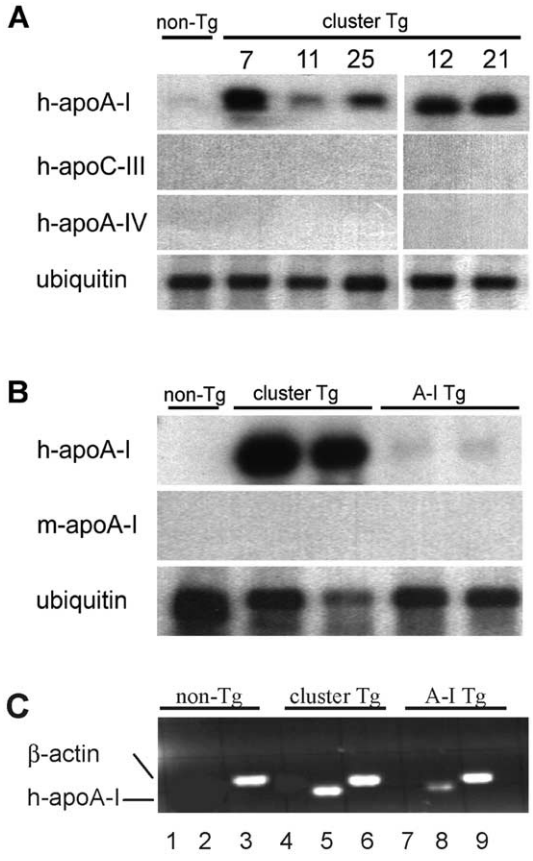


Fig. 1. Apo expression in mouse heart. A: Northern blot analysis of non-transgenic (non-Tg) mice and five different lines of cluster transgenic (cluster Tg) mice: lines 7, 11, 25, 12 and 21. The membranes were hybridized independently with four radioactive probes: $_{h}$apoA-I, human apoA-I (exposure time: 2 h); $_{h}$apoC-III, human apoC-III (exposure time: overnight); $_{h}$apoA-IV, human apoA-IV (exposure time: overnight) and ubiquitin (exposure time: 2 h). B: Northern blot analysis of non-Tg mice, of cluster Tg mice line 12 and of $_{h}$apoA-I Tg mice line 427. The membrane was hybridized independently with three radioactive probes: $_{h}$apoA-I (exposure time: 2 h), $_{m}$apoA-I, mouse apoA-I (exposure time: overnight) and ubiquitin (exposure time: 4 h). C: RT-PCR analysis of non-Tg mice, of cluster Tg mice line 12 and of $_{h}$apoA-I Tg mice line 427. Experiments were performed with $_{m}$apoA-I (lanes 1, 4, 7), $_{h}$apoA-I (lanes 2, 5, 8) and β-actin (lanes 3, 6, 9) primers.

nor apoA-IV expression was detected in the heart, even after long periods of autoradiographic exposure.

To further characterize the DNA sequence necessary for cardiac apoA-I expression, we analyzed hearts of human apoA-I Tg mice previously generated by Walsh et al. [22]. The fragment used to generate this apoA-I line is 2.2 kb long and contains the apoA-I gene, 248 bp of the 5′ flanking region, and 80 bp of the 3′ flanking region. We also found expression of the human apoA-I gene in the heart of these mice, although to a lesser extent than that observed in cluster Tg mice (Fig. 1B). The presence of the human apoA-I messenger in the heart of both Tg models was confirmed by RT-PCR (Fig. 1C). The corresponding mouse mRNA was not detected either in Tg or in control littermate mice (Fig. 1B,C).

### 3.2. Lipid regulation of the human apoA-I gene in the heart of cluster Tg mice

Previous reports have shown apoA-I gene expression to be regulated by lipids in cluster Tg mice [31]. We tested the effect

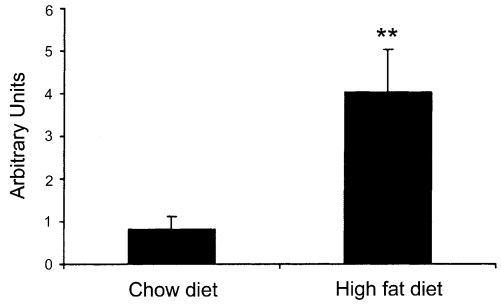


Fig. 2. Effect of a high-fat, high-cholesterol diet on cardiac apoA-I expression. Human apoA-I mRNA levels in hearts of cluster Tg mice under a standard chow diet or a high-fat, high-cholesterol diet for 20 weeks. Values, normalized for ubiquitin mRNA levels, are expressed in arbitrary units and correspond to the mean ± S.D. of five measurements in cluster Tg mice on each diet. Error bars represent S.E.M. $^{**}P < 0.005$.

of a high-fat, high-cholesterol diet on cardiac apoA-I gene expression in these animals. After 20 weeks, cluster Tg mice fed with an atherogenic diet showed a significant increase of human apoA-I mRNA levels in the heart (five-fold), when compared with those fed with a standard chow diet (Fig. 2).

### 3.3. Expression of apoA-I gene in human heart

To test whether the presence of human apoA-I mRNA observed in the heart of Tg mice reflects the human gene expression pattern, we analyzed several samples from human adult hearts. By Northern blot, we demonstrated clearly that the apoA-I gene was expressed in all the analyzed human heart samples (Fig. 3A), while neither apoC-III nor apoA-IV expression was detected, even after long periods of expo-

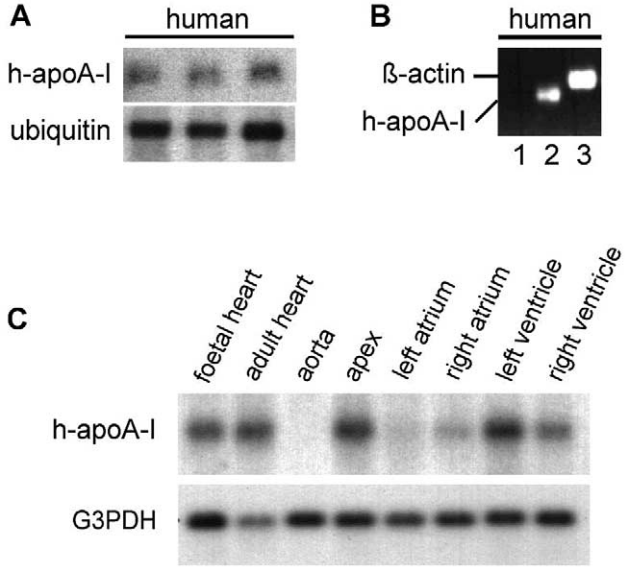


Fig. 3. ApoA-I expression in human heart. A: Northern blot of human heart mRNA of three different individuals. The membrane was hybridized independently with two radioactive probes, $_{h}$apoA-I and ubiquitin (exposure time: overnight). B: Representative RT-PCR of mRNA sample from human heart. Experiments were performed with $_{m}$apoA-I (lane 1), $_{h}$apoA-I (lane 2) and β-actin (lane 3) primers. C: Northern blot analysis of a commercial membrane containing poly(A)$^{+}$ RNAs isolated from human tissues. The membrane was hybridized with two distinct radioactive probes: $_{h}$apoA-I (exposure time: 29 days) and glyceraldehyde-3-phosphate dehydrogenase (exposure time: 3 h).

sure (data not shown). RT-PCR experiments also confirmed apoA-I expression in human hearts (Fig. 3B). Furthermore, the analysis of a commercial membrane carrying human cardiac mRNA from different subjects and from different regions of the heart revealed that the gene was expressed in multiple regions of the organ. The apoA-I mRNA was clearly found in the apex, the ventricles, and at a lower level in the atria. ApoA-I mRNA was also detected in total fetal heart. Interestingly, no expression was observed in the aorta (Fig. 3C).

### 3.4. Human apoA-I is expressed by cardiac myocytes

In order to identify the cell types responsible for the human apoA-I expression, we performed in situ hybridization on the hearts of Tg mice. The results show that the human apoA-I messenger was found only in cardiac myocytes, in both atria and ventricles. No expression was detected in endothelial, fibroblast or smooth muscle cells or in any of the aortic cells (Fig. 4). The same pattern of expression was observed in both cluster Tg and apoA-I Tg mice. The mouse apoA-I gene expression was not detected in any heart tissue, whereas it was clearly evident in the liver (data not shown).

### 3.5. Human apoA-I synthesis by the heart of Tg mice

To study the synthesis of human apoA-I in the heart, minced pieces of cluster Tg mouse hearts were incubated in complete media and the resulting supernatant was subjected to agarose gel electrophoresis. The results show the appearance of pre-beta particles, as judged by their electrophoretic mobility and confirmed by immunodetection of human apoA-I (Fig. 5). However, the apoA-I in the secreted particles could have originated from other tissues and subsequently been trapped in the heart. To rule out this possibility, primary cardiac myocyte cultures were developed from hearts of newborn cluster Tg mice or controls. At 5 days of age, primary cardiac myocyte cultures from Tg (in which apoA-I mRNA was previously detected) secreted the human protein in the media (data not shown). In addition [$^{35}$S]methionine/cysteine metabolic labeling experiments were performed on fresh heart explants from cluster Tg mice and control littermates (Fig. 6). The incubation media were analyzed by SDS–PAGE and a faint supplementary radioactive band was found only in the Tg media (Fig. 6A). The same gel was transferred to a membrane and submitted to a Western blot analysis. Using a specific apoA-I antibody, we found that the supplementary radio-

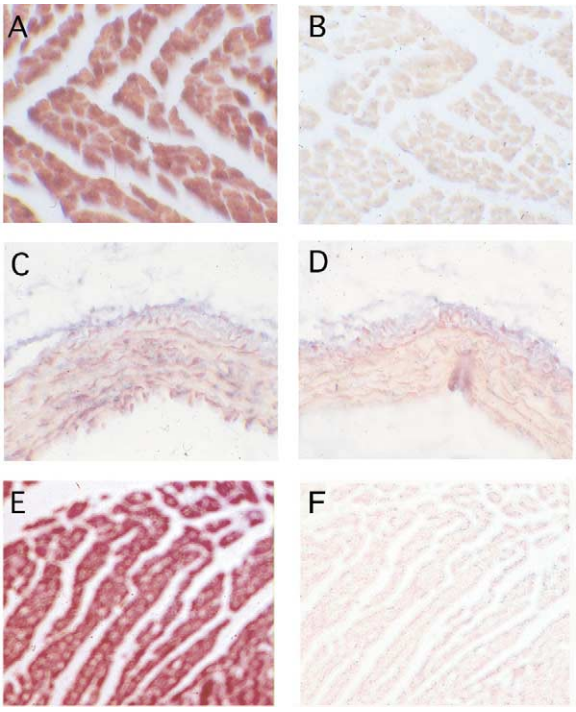


Fig. 4. In situ hybridization experiments. Human apoA-I in situ hybridization experiments in sections of heart (A,B) and of aorta (C,D) of cluster Tg mice, line 12, and in sections of heart of $_{h}$apoA-I Tg mice, line 427 (E,F). A,C,E: $_{h}$apoA-I antisense probe. B,D,F: $_{h}$apoA-I sense probe. Positive signals in A and E indicating the presence of $_{h}$apoA-I mRNAs in cardiac myocytes appear as purple staining. Amplification ×400. (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.)

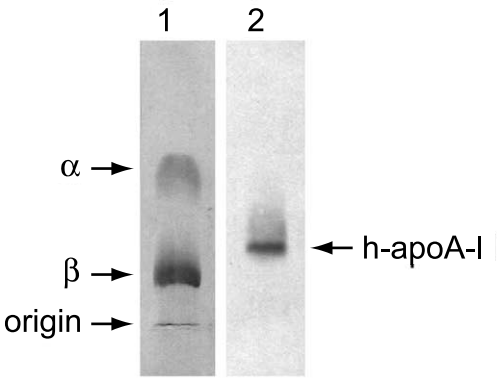


Fig. 5. Analysis of $_{h}$apoA-I in cluster Tg mice. Agarose gel electrophoresis of incubation media of minced heart tissue from Tg mice. α and β electrophoretic mobilities were positioned after electrophoresis of human plasma lipids stained with Sudan Black (lane 1). Western blot of proteins secreted by minced heart tissue of cluster Tg mice (lane 2). The incubation medium was submitted to agarose gel electrophoresis and immunoblotted with anti-human apoA-I antibodies.

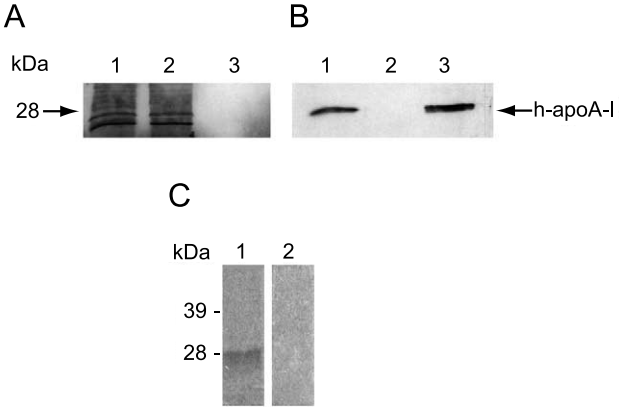


Fig. 6. Metabolic labeling experiments. A: SDS–PAGE autoradiography of [$^{35}$S]methionine/cysteine incubation media of minced heart from cluster Tg mice line 12 (lane 1), from non-Tg mice (lane 2) and as a control, human apoA-I purified protein (lane 3). B: Western blot analysis, using a specific anti-human apoA-I antibody, of the corresponding SDS–PAGE shown in A. C: Metabolic labeling of hearts from cluster Tg mice line 12 (lane 1) and from non-Tg mice (lane 2). Minced heart tissues were incubated in the presence of [$^{35}$S]methionine/cysteine. The incubation media was partially immunoprecipitated and analyzed by autoradiography after SDS–PAGE separation. Molecular weight markers are indicated on the left.

active band corresponded to the human apoA-I protein (Fig. 6B). Aliquots of the incubation media were also partially purified by immunoprecipitation using antibodies directed against human apoA-I and analyzed by SDS–PAGE. The results show that a 28 kDa radioactive protein with the molecular weight of apoA-I was present in media from cultures of Tg but not control heart explants (Fig. 6C).

## 4. Discussion

In this study, we demonstrate that the human apoA-I gene is expressed in the heart of humans and in two distinct Tg mouse models [22,23]. Whereas the human apoA-I, apoC-III, and apoA-IV genes are expressed in the liver and intestine of both humans and cluster Tg mice, expression in the heart is a specific feature only of apoA-I. The relative amount of human apoA-I mRNA in the heart represents about one-third of the liver expression. The apoA-I plasmatic level is regulated by a post-transcriptional mechanism and associated with changes in HDL distribution [45]. The production of human apoA-I protein by the heart might therefore be proportionately lower than the liver secretion.

In situ hybridization analyses showed that the expression of the human apoA-I gene in the hearts of cluster Tg and apoA-I Tg mice was restricted to cardiac myocytes. Neither other cardiac cells nor any aortic cells expressed the human gene. The human apoA-I transcription was not restricted to certain areas of the organ, since the presence of the mRNA was observed in ventricles and atria in adult tissues of both humans and Tg mice. This cell type-specific expression suggests that the presence of apoA-I in the heart could be linked to a cardiac myocyte function. These cells are not expected to synthesize proteins involved in lipoprotein metabolism; however, it has been shown that they synthesize the apoB and microsomal triglyceride transfer protein and consequently are involved in lipid transport regulation [32–34].

We also studied whether the apoA-I protein is synthesized by the cluster Tg heart. After incubation of Tg mouse hearts in complete media, particles that displayed a pre-beta HDL electrophoretic mobility and contained the human apoA-I protein were secreted. Moreover, 5-day-old primary cultures of Tg cardiac myocytes secreted human apoA-I into the media. Finally, when minced pieces of Tg hearts were incubated in the presence of radioactive precursors and media was partially purified via immunoprecipitation, a labeled protein with the apoA-I molecular weight of 28 kDa was observed. The radioactive band migrating at this position was shown to be the human apoA-I protein. These results strongly support the fact that the cardiac myocytes of cluster Tg mice secrete human apoA-I.

The human apoA-I expression observed in apoA-I Tg mice indicates that the regulatory elements sufficient for the cardiac expression are contained in a short 2.2 kb genomic fragment located between −248 bp upstream and +1934 bp downstream of the apoA-I cap site [22]. Therefore, this result demonstrates that the distal enhancer located near the apoC-III promoter, a locus control region necessary to achieve intestinal expression, is not required for apoA-I heart expression. Nevertheless, levels of human apoA-I mRNA observed in the heart of apoA-I Tg mice are lower than in cluster Tg mice. One explanation could be that the 33 kb DNA fragment used to generate the cluster Tg mice contains positive regulatory elements which are lacking in the small 2.2 kb DNA fragment used in generating the apoA-I Tg mice. One may also hypothesize hat enhanced apoA-I cardiac expression in cluster Tg mice is a consequence of the hyperlipidemic profile characteristic of these animals. In fact, it has been reported that the expression of the gene in cluster Tg mice is influenced by lipids in liver and intestine [31]. Furthermore, after 20 weeks in a high-fat, high-cholesterol diet, cluster Tg mice exhibited a five-fold increase in human apoA-I expression in the heart.

We did not observe expression of the endogenous mouse apoA-I gene in the heart of control or Tg animals. It is possible that the expression levels of the mouse mRNA in the heart are below the detection threshold of the techniques used. On the other hand, several-fold higher copy numbers of the human apoA-I transgene could by their numbers alone produce a higher 'basal' mRNA level. Another possible explanation is that, unlike human apoA-I, the mouse gene is not expressed in this organ. Along this line, it is well known that the rabbit apoA-I gene, unlike its corresponding gene in other species, is not expressed in the liver [35].

To try to understand the differential heart expression pattern observed between humans and mice, we aligned the human and murine promoter sequences [36,37]. We found that the first 230 bp upstream of the transcription initiation site of the human apoA-I gene are highly conserved and present about 83% homology. In these sequences, we also checked for the presence of potential motifs that could confer any cardiac tissue-specific expression. Between nucleotides −61 and −66 bp of the human apoA-I promoter, we localized a putative binding site (TGATAA) for GATA transcription factor members that is strictly conserved in mice. Among the GATA factors, the GATA-4 directs tissue-specific expression during mammalian cardiac development [38]. In addition, there is a PPAR-responsive element between nucleotides −210 and −190 bp of the human promoter (TGAACCCTTGACCCCTGCCCT) [39]. PPARα is a nuclear protein involved in the control of myocardial lipid metabolism [40] and is also known to modulate the hepatic expression of apoA-I [41]. It has been shown in humans that fibrates, which activate the PPAR-responsive element, increase plasma levels of HDL by the induction of apoA-I gene expression [42]. When compared to the PPAR-binding site present in the human gene, three and four nucleotide differences are observed in the rat and mouse apoA-I promoters, respectively [39]. Interestingly, these sequence differences abolish the binding of PPARα. Actually, the mechanisms responsible for the human apoA-I cardiac gene expression and its absence in mice seem to be quite complex, and much additional work is needed to shed light on this matter.

Several studies have indicated that the antiatherosclerotic mechanisms dependent on apoA-I are related to its promotion of RCT [43], its antioxidant effect on lipid peroxidation [6,7], or its inhibition of the expression of endothelial cell adhesion molecules [9,10]. Although these mechanisms may confer protection in heart tissue fluids via apoA-I arising from plasma, apoAI produced by cardiac myocyte cells may intervene as well. Even in a low proportion, this synthesis may increase the apoA-I concentration in heart fluids, thereby reinforcing the protective effect carried out by the plasma protein. Furthermore, it has been reported that even small amounts of retrovirus-mediated apoA-I synthesis in macrophages protects

against atherosclerosis in vivo without modifying plasma apoA-I and HDL cholesterol levels [44].

While it is believed that the heart is very active in lipid metabolism, the nature of the lipid-containing particles secreted by this organ is not very well known. Recent studies showing that the heart assembles and secretes apoB-containing lipoproteins introduced the concept that this organ has mechanisms for exporting lipids [32–34]. The apoA-I cardiac synthesis might also be associated with the secretion of lipoproteins particles (by myocytes) in an aim to prevent lipid accumulation in the heart. It is worth noting that apoA-I may be associated with apoB-secreted lipid particles, which were found in unusual composition and size in human placental extracts, as first described by Park et al. [46]. In this regard, much work needs to be done to determine the nature of these particles. Overall, we did not notice any obvious anatomical or functional differences between the hearts of Tg mice expressing the human apoA-I and the wild-type mice (not expressing any apoA-I).

Our results demonstrate that the human apoA-I gene is expressed in the heart in humans and in cluster Tg and apoA-I Tg mice. We also show that the protein is secreted by cardiac myocytes. Further studies are needed to address the specific role and physiological relevance of this cardiac protein.

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