Cardiac induction of the thyroid-hormone degrading enzyme type III deiodinase in human ischemic heart failure

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**Abstract**

*Background*  Type III deiodinase (DIO3) is a thyroid-hormone (TH) degrading enzyme, which activity is stably induced in the left ventricle (LV) following myocardial infarction in mice. The subsequent LV remodeling and development of cardiac dysfunction is associated with decreased LV tissue TH content and action. This local hypothyroid condition may contribute to the development of heart failure, given the known regulation by TH of several key genes implicated in contractile dysfunction and heart failure. Here, we investigated whether DIO3 is also induced in the myocardium of patients with heart failure as a result of ischemic heart disease (ISHD).

*Methods and Results*  Tissue samples from patients (n=36) were obtained during heart transplantation surgery and non-failing heart tissue was obtained from donor hearts (n=75). Paraffin sections of tissue microarrays comprising up to 120, 1 mm LV cores of patients and donors were analyzed for DIO3 expression using quantitative immunohistochemistry. Levels of cardiac mRNAs were determined by RT-PCR and tissue levels of T3 and T4 were determined by tandem mass spectrometry. Post-mortem cardiac tissue from infants and aborted fetuses was analyzed for DIO3 enzyme activity. Immunohistochemistry showed a 1.9 fold increase in DIO3 expression in the ISHD group relative to the donor group. This was associated with a 2.2 fold increase in DIO3 mRNA levels and a 25% reduction of LV T3 content, with no change in RV T3 content. The suggested local hypothyroid condition in the ISHD was supported by a 7 fold increase in the ratio of MYH6 and MYH7 mRNA expression levels. DIO3 enzyme activity could not be detected in the tissue samples, but analysis of fetal cardiac tissue showed that DIO3 activity is expressed in the human myocardium.

*Conclusions*  This study demonstrates for the first time in human cardiac tissue that transient expression of DIO3 is part of the fetal gene program and that expression is upregulated in ISHD. The associated reduction of tissue T3 content suggests that the DIO3-dependent local hypothyroid condition of the heart reported in a mouse model of ISHD may also apply to the human situation and contribute to the development of heart failure.
In several animal models of heart failure the thyroid-hormone (TH) degrading enzyme type III deiodinase (DIO3) is induced in the heart (Wassen et al. 2002; Trivieri et al. 2006; Simonides et al. 2008; Pol et al. 2011). In models of pathological remodeling due to chronic pressure overload (Simonides et al. 2008) and myocardial infarction (MI) (Pol et al. 2011), the induction of DIO3 was associated with decreased ventricular tissue TH content and decreased in vivo TH-dependent transcription activity in cardiomyocytes. This aspect of remodeling is expected to influence the progression of heart failure.

Pathological ventricular remodeling resulting in congestive heart failure typically results from hypertension, valvular disease, or ischemic heart disease (ISHD). The chronic mechanical and metabolic stress imposed on the heart results in structural and functional changes and although initially adaptive, can progress to dysfunction and failure. A complex interplay of signal-transduction systems drives gene expression that results in the changes in myocyte morphology, contractile properties, metabolism and extracellular matrix composition that ultimately underlie dysfunction (Bernardo et al. 2010; Kehat and Molkentin 2010; Cohn, Ferrari, and Sharpe 2000; Opie, Commerford, Gersh, Pfeffer, et al. 2006).

A partial recapitulation of the fetal gene program is a hallmark of pathological ventricular remodeling. Re-expression of this gene program is also an aspect of the hypothyroid heart, and consequently a role for impaired cardiac TH signaling in ventricular remodeling has been suggested (Rajabi et al. 2007; Klein and Ojamaa 2001; Oka, Xu, and Molkentin 2007; Vergaro and Emdin 2008). Examples of TH-regulated cardiac genes that show a hypothyroid profile during remodeling are sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase, voltage-gated potassium channel, β-1 adrenergic receptor, phospholamban, the myosin heavy chain isoforms α (MYH6) and β (MYH7), the Na\(^+\)/K\(^+\)-ATPase, and the Na\(^+\)/Ca\(^{2+}\) exchanger (Klein and Ojamaa 2001).

The suggested reduction of TH signaling is supported by several studies showing changes in cardiac TH metabolism as well as alterations in TH receptor expression. With respect to the latter, both up- and downregulation of receptor mRNA and protein has been reported, both in animal models (Kinugawa, Yonekura, et al. 2001; Constantinou Pantos, Mourouzis, et al. 2007; Belke et al. 2007; C Pantos et al. 2005; C. Pantos et al. 2010) and humans (Kinugawa, Minobe, et al. 2001; Modesti et al. 2008; d’Amati et al. 2001; Sylvén et al. 1996). These data indicate that possible involvement of TH receptors in altered TH action, apart from species differences, depends on the type and stage of pathological remodeling studied. In contrast, in all animal models studied thus far, upregulation of DIO3 activity has been found (Wassen et al. 2002; Trivieri et al. 2006; Simonides et al. 2008; Pol et al. 2011). DIO3 converts thyroxine (T4) and 3,5,3′-triiodothyronine (T3) to the inactive metabolites 3,3′,5′-triiodothyronine (rT3) and 3,3′-diiodothyronine (T2), respectively, and it is primarily expressed in fetal tissues (Gereben et al. 2008). With the exception of brain and skin, DIO3 is virtually absent in adult tissues. However, DIO3 has been shown to be upregulated in conditions of cellular stress as well as in certain pathologies, both in rodents and humans, leading to a reduction of local TH levels (Dentice and Salvatore 2011; Bianco and Kim 2006; S. A. Huang and Bianco 2008). The relevance of DIO3 induction for cardiac TH metabolism was indicated by studies in rat and mouse showing substantial reduction of tissue T3 levels in failing RV (Simonides et al. 2008) and LV (Pol et al. 2011) respectively, which were not related to changes in plasma TH levels. Moreover, the local hypothyroid condition was confirmed in these models by decreased in vivo TH-dependent transcription in cardiomyocytes. Although to date, DIO3 activity as not been reported for human cardiac tissue, the data from multiple animal models suggest that expression of DIO3 might also occur in human heart failure, if indeed DIO3 is also part of the fetal
cardiac gene program in man. We therefore examined DIO3 expression in fetal and neonatal myocardium as well myocardium of donors and end-stage heart-failure patients who underwent cardiac transplantation.
Methods and materials

Human myocardium

Human cardiac tissue was obtained during heart transplantation surgery from patients with end-stage heart failure. LV free wall was dissected and tissue samples were frozen immediately and stored in liquid nitrogen, or fixed in 5% formaldehyde for examination by immunohistochemistry.

Non-failing cardiac tissue was obtained from potential but unused transplant donor hearts. Hearts were perfused with ice-cold cardioplegic solution, transported on ice to the University of Sydney usually within 2-3 hours of the declaration of brain death. Transmural sections of the free wall of the LV were frozen immediately and stored in liquid nitrogen. Part of the tissue was fixed in 5% buffered formaldehyde for examination by immunohistochemistry.

Fetal and neonatal cardiac tissue was obtained as described before (Kester et al. 2004) from 7 fetuses (postmenstrual age 18-24 weeks) and 3 infants who died between 3 and 13 weeks of age.

All studies were approved by the local ethical committees and in accordance with Australian, Dutch or British law. The investigations conform to the principles outlined in the Declaration of Helsinki (World Medical Association General Assembly 1997).

Tissue microarrays

Five tissue microarrays (TMAs) were constructed as described by others (Kononen et al. 1998). The Beecher-MTA1 instrument was used to make an assembly of up to 120 tissue samples from donor and patient hearts. Each heart was represented by two 1 mm diameter cores cut from each fixed-embedded tissue block. Tissue samples were paraformaldehyde (5%)-fixed and paraffin-embedded. Thus, our TMAs contained 60 duplicated samples from each patient or donor. Sections of the resulting TMA block were cut at 4 μm thickness and used for immunohistochemistry.

Histochemistry

For DIO3 immunohistochemistry, 4 μm TMA sections were deparaffinized and rehydrated, exposed to 0.02 M HCl (20 min) to block endogenous peroxidase activity and microwaved at 93 °C for 10 min in 10 M citrate buffer (pH 6.0) for epitope retrieval. Sections were incubated with the validated, affinity-purified polyclonal rabbit anti-DIO3 antibody 718 (S. A. Huang et al. 2002) at 1:50 dilution for 1 hour at 37 °C and then processed with Envision®+ reagents (Dako, Glostrup, Denmark) using 3,3’-diaminobenzidine (DAB) as chromagen yielding brown staining. Images were converted to black (no staining) and blue, and the mean pixel intensity of each core was quantified using Image J. Of all hearts, 63 were present on 1 TMA, 57 on two TMAs, and 15 on 3 TMAs. Damaged cores were not included in the analysis, and because of this, of 3 hearts only one core was analyzed. On average 3 cores per heart (range 1 to 8) were analyzed for DIO3 staining and these measurements were averaged.

Quantitative real-time PCR

Total RNA was extracted from LV using TriPure (Roche Applied Science, Basel, Switzerland) and treated with DNase I (Invitrogen, Carlsbad, CA, USA). Two μg of total RNA was used to generate cDNA strands in a 20 μl reaction volume using the Cloned AMV First Strand Synthesis Kit (Invitrogen, Carlsbad, CA, USA). An equivalent of 31.25 ng of total RNA was subsequently used for the preamplification reaction with 15 nM primers for DIO3 (DIO3 sense primer, 5’-CCTGGGACTCTGCTTCTGTAAC-3’; DIO3 anti-sense primer, 5’-GGGGTGAAGAAATGCTGTAGAG-3’) and 1x MESA GREEN qPCR MasterMix Plus for SYBR assay (Eurogentec, Seraing, Belgium) in a total volume of 5 μl. Preamplification was performed under the following conditions: 2’ at 50 °C, 10’ at 95 °C, 14 cycles 15’ at 95°C and 4’ at 60°C. The preamplification product was diluted 3 x and 2.5 μl was used for the amplification reaction with 300 nM primers for DIO3 and 1x MESA GREEN qPCR MasterMix Plus for SYBR assay (Eurogentec,
Seraing, Belgium) or 1x TaqMan Gene Expression assay for PPIA with VIC probe (Hs99999904_m1; Applied Biosystems, Foster City, CA, USA) and 1x Eurogentec qPCR MasterMix (Eurogentec, Seraing, Belgium) in a total volume of 10 µl, using standard cycle parameters. Both amplification reactions were performed on an Applied Biosystems model 7700 (Applied Biosystems, Foster City, CA, USA). DIO3 expression levels were normalized to the level of Peptidylprolyl isomerase A (PPIA) mRNA expression in each sample and are shown as relative expression units. Expression levels of MYH6 and MYH7 were analyzed on an equivalent of 25 ng of total RNA with 100nM final concentration of primers for MYH6 (MYH6 sense primer, 5’-CAACAATCCCTAC-GACTACG-3’; MYH6 anti-sense primer, 5’-ACGTCAAAGGCCTACGTG-3’) and MYH7 (MYH7 sense primer, 5’-CCTTGAGTAGCCAGCACA-3’; MYH7 anti-sense primer, 5’-CTTCTAGCCCTCCTCCTCGT-3’) and 1x MESA GREEN qPCR MasterMix Plus for SYBR assay (Eurogentec, Seraing, Belgium) in a total volume of 10 µl. MYH expression levels were normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression in each sample (GAPDH sense primer, 5’-.GGTCTCCTCTGACTTCA-3’; GAPDH anti-sense primer, 5’-AGCCAAATTCGTTGTCATAC-3’). The Ct values were normalized using the formula ΔCt = Ct target – Ct reference gene. The ΔCt values were then converted to relative expression (2ΔCt) for each sample.

**Tissue T3 and T4 content**  Intra-cardiac T3 and T4 were analyzed using HPLC tandem mass spectrometry as described before (Saba et al. 2010) with the following modification. After evaporation to dryness, samples were reconstituted with 200 µL of 3.0 N HCl in n-butanol, incubated for 60 minutes at 60°C, dried again under a gentle stream of N2, reconstituted with 100 µL of MeOH/HCl 0.1 M 50/50, and centrifuged at 21,500 g for 10 minutes. The supernatant was used for injection into the LC-MS system.

**Control for effect of cardioplegic solution on DIO3 expression**  Housing of animals and all experiments complied with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication no. 86-23, Revised 1996) and were approved by the Institutional Animal Care and Use Committee of VU University Medical Center Amsterdam.

A total of 7 male C57Bl/6J mice, between 11 and 13 weeks of age, were weighed, anesthetized with isoflurane [2.5-3.0% (vol/vol)] and MI was induced by permanent ligation of the left coronary artery (LCA) as described previously (Pol et al. 2011; van den Bos et al. 2005; de Waard et al. 2007). Mice were sacrificed 8 days after the procedure when MI-induced DIO3 expression has been shown to be high. Animals were anesthetized and the heart was excised. In 3 of the 5 animals that survived the MI surgery permanent ligation of the LCA resulted in an infarcted area of the LV of 30-50%. The heart was briefly perfused in retrograde fashion via the aorta with ice-cold cardioplegic solution (119 mM NaCl, 1.2 mM NaH2PO4, 4 mM KCl, 25 mM NaHCO3, 1.2 mM MgSO4, 1 mM CaCl2, 11 mM glucose). The LV noninfarcted tissue of each heart was cut in four pieces: one was frozen in liquid nitrogen and stored at −80°C until DIO3 activity analysis; one was fixed in 4% paraformaldehyde and processed for histochemistry; and two were incubated for 4 hours in cold cardioplegic solution after which one of the two was frozen in liquid nitrogen and stored at −80°C until DIO3 activity analysis and the other one was fixed in 4% paraformaldehyde and processed for histochemistry. The 4 hr incubation is the maximum time that donor hearts are kept in this solution.

For immunohistochemistry, 4 µm LV sections were stained for DIO3 as described above.
**DIO3 enzyme activity**  DIO3 enzyme activity in human and mouse cardiac tissue was determined by analysis of inner-ring deiodination of radiolabeled T3 essentially as described previously (Kuiper, Klootwijk, and Visser 2003).

**Statistics**  Data analysis was performed with GraphPad Prism version 5.01 for Windows (GraphPad, San Diego, CA, USA) and SPSS 15.0 for windows (SPSS Inc, Chicago, IL, USA). For analyses of DIO3 mRNA expression and DIO3 immunostaining of human heart samples, unpaired *t* testing was used, with Grubbs’ test for outlier detection. Age and sex were used as covariates in ANCOVA analysis of DIO3 immunostaining. Analysis of DIO3 activity measurements of mouse heart samples used paired *t* testing. Significance was accepted at *p*<0.05. Data are presented as means ± SEM of *n* observations.
As part of an earlier analysis of deiodinase expression in fetal and postnatal tissues, cardiac tissue had been procured and was analyzed for DIO3 activity by one of us (T.J. Visser, unpublished data). The results of this analysis are shown in Figure 1, indicating substantial DIO3 activity in the fetal heart, which decreases within the first weeks of life by more than 80%.

Characteristics of patients and donors included in the present analysis are given in Table 1. All ISHD patients undergoing transplant surgery suffered from end-stage heart failure.

Immunohistochemical analysis of LV tissue cores from 75 donor and 36 patients present on the 5 TMAs showed low DIO3 staining in most donor samples, whereas substantial DIO3 staining was found in most of the ISHD samples. Some striking examples are depicted in Figure 2. In the majority of the positive ISHD samples, all cardiomyocytes showed DIO3 expression [Fig. 2b-1], but in some samples both DIO3-positive and negative cardiomyocytes were present [Fig. 2b-2, 3], similar to what is found in remodeled mouse myocardium following MI (Pol et al. 2011). Interstitial cells did not show DIO3 expression in any of the samples. Quantification of the mean intensity of DIO3 staining of the cores indicated a 1.9 fold increase in the ISHD group relative to the donor group [Fig. 3]. The covariates, age and sex, were not significantly related to the DIO3 staining.

Table 1  Patient and donor characteristics

<table>
<thead>
<tr>
<th>n</th>
<th>Donor</th>
<th>ISHD</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>35±2</td>
<td>52±1</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>42/33</td>
<td>31/5</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>23±2 (26)</td>
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<tr>
<td>CO (L/min)</td>
<td>3.7±0.5 (7)</td>
<td></td>
</tr>
<tr>
<td>CI (L/min/m2)</td>
<td>2.1±0.3 (5)</td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>68±3 (9)</td>
<td></td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>58±4 (9)</td>
<td></td>
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<tr>
<td>FS (%)</td>
<td>18±3 (8)</td>
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Parameters of heart function of (n) patients were available: LVEF: left ventricular ejection fraction, CO: cardiac output, C: cardiac index, LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular systolic diameter. Data are mean ± SEM.

Because of limited tissue availability, mRNA levels of DIO3, MYH6 and MYH7 were determined in LV tissue of 20 ISHD patients and 17 donors. Figure 4 shows the reciprocal change in mRNA levels of the T3-responsive genes MYH6 and MYH7 in the ISHD relative to the donor group, characteristic of pathological remodeling. Expression levels of DIO3 were found to be low and a pre-amplification of 14 cycles was needed to obtain Ct values of approximately 24. Figure 5 shows the 2.2 fold increase in DIO3 mRNA level in the ISHD group relative to the donor group.

Cardiac tissue levels of thyroid hormone were determined in order to assess the physiological relevance of the apparent increase in DIO3 expression in ISHD. Tissue availability limited the analysis to 6-7 samples in each group. The results are presented in Figure 6a, showing a 25% decrease in LV T3 content in the ISHD group, whereas RV T3 content did not change significantly. There was an
Figure 1  DIO3 activity in fetal and neonatal myocardium. Cardiac tissue of 7 aborted fetuses (postmenstrual age 18-24 wks) and 3 infants (aged 3-13 wks) were analyzed for DIO3 enzyme activity. Data are the means ± SEM, *: p < 0.05.

Figure 2  DIO3 immunohistochemistry. [a] Examples of tissue cores of LV samples from donors and ISHD patients stained for DIO3. Details of cellular staining in ISHD sections are shown in b, with high DIO3 staining in all cardiomyocytes in b1 and cells with high and low DIO3 staining in b2 and b3. Scale bar indicates 1 mm in a and 10 μm in b.

Figure 3  Quantification of DIO3 immunostaining. Tissue cores of LV samples from donors (n=75) and ISHD patients (n=36) stained for DIO3 were analysed using ImageJ as described in the Materials and Methods section. Mean pixel intensities of cores were determined and plotted for both groups. Whiskers in the boxplot present the minimum and maximum values of the data, *: p<0.05.

Figure 4  LV myosin heavy chain mRNA expression. MYH6 [a] and MYH7 [b] mRNA expression levels, normalized to GAPDH, were determined by RT-PCR in a random sub-set of donor samples (n=17) and ISHD samples (n=20). The ratio of MYH6 and MYH7 mRNA expression levels are depicted in c. Whiskers in the boxplot present the minimum and maximum values of the data, *: p<0.05.

Figure 5  LV DIO3 mRNA expression. DIO3 mRNA expression levels, normalized to PPIA, were determined by RT-PCR in a random sub-set of donor samples (n=17) and ISHD samples (n=20). Whiskers in the boxplot present the minimum and maximum values of the data, *: p<0.05.
unexpected 60% increase in T4 content in both LV and RV of ISHD samples as compared to donor samples [Fig. 6b]. Since plasma thyroid hormone levels of the patients and donors are unknown and present a possible confounding factor, T3 and T4 content of LV and RV were compared for individual hearts. This was possible for 5 donor and 6 ISHD hearts. The ratio of LVT4/RVT4 was identical in donor and ISHD samples, but the ratio of LVT3/RVT3 indicated a 34% reduction in the ISHD group relative to the donor group [Fig. 6c].

Effect of cardioplegia on DIO3 expression

LV tissue from explanted hearts is immediately frozen, whereas donor hearts are perfused with cold cardioplegic solution and kept on ice for up to 4 hours needed to transport the heart to the transplant theaters where the tissue was immediately dissected into small pieces and frozen in liquid nitrogen. To exclude the possibility that healthy hearts also express DIO3 but that the cardioplegic solution affects DIO3 staining, the effect of cardioplegia on DIO3 detection was tested on infarcted mouse hearts. DIO3 activity and DIO3 immunostaining were analyzed after brief perfusion with cold cardioplegic solution (t = 0) and after 4 hours of incubation on ice in this solution (t = 4). DIO3 activities at t = 0 and t = 4 were 0.8 ± 0.2 fmol/min/mg (n = 3) and 1.8 ± 0.5 fmol/min/mg (n = 3), respectively. This demonstrated that cold cardioplegic solution does not lower DIO3 activity. Accordingly, no difference was observed in the DIO3 immunostaining pattern or intensity between tissue sections of these hearts at t = 0 and t = 4 (data not shown).
This study demonstrates for the first time that cardiac DIO3 expression is increased in human ischemic heart disease and that this is associated with a ventricle-specific reduction of T3 content. This suggests that the DIO3-dependent local hypothyroid condition of the heart found in a mouse model of chronic heart failure following MI, also applies to human hearts and may contribute to the development of heart failure.

The immunostaining showed that DIO3 is expressed in cardiomyocytes and although we cannot exclude that DIO3 is also expressed by other cell types, such as inflammatory cells which are known to be able to express DIO3 (Boelen, Kwakkel, and Fliers 2011), we did not observe DIO3 staining in cells other than cardiomyocytes in the immunohistochemistry images.

Although we found substantial cardiac expression of DIO3 in most ISHD patients with end-stage heart failure, some patients showed levels of DIO3 staining comparable to donor hearts. This may be explained by the limited number of randomly sampled tissue cores in combination with the heterogeneity of ventricular remodeling, i.e., the hearts of ISHD patients will also have areas with normally perfused, non-remodeling tissue as well as hibernating tissue distal to severe stenoses.

Despite the evident increase in DIO3 immunostaining in ISHD samples, which was furthermore associated with a 1.9-fold increase in DIO3 mRNA expression levels, DIO3 activity was not detectable in whole homogenates of cardiac tissue (data not shown). It should be noted that the significant DIO3 activity in freshly prepared mouse cardiac samples, showing similar DIO3 staining intensity, is in the lower fmol/min/mg range (Pol et al. 2011). This is close to the detection limit of the current DIO3 activity assay and a likely explanation for the lack of DIO3 activity of the tissue used in the present study is that these samples have been stored in liquid nitrogen for one to four years. The previously performed analysis of DIO3 activity on fetal and neonatal hearts, which is presented here for the first time, clearly shows DIO3 activity in human myocardium. These results furthermore confirm data from animal studies that DIO3 expression is part of fetal gene program, with little DIO3 activity left in adult cardiac tissue. The observed increase in DIO3 protein and mRNA expression in ISHD is therefore in line with the proposed re-expression of the fetal gene program in pathological remodeling (Pol et al. 2011; Pol, Muller, and Simonides 2010).

In animal models of heart failure, cardiac DIO3 induction was associated with decreased tissue T3 levels and reduced TH-dependent transcription in vivo. The significant decrease in LV T3 content in the ISHD patient samples relative to donor samples in this study suggests that this is also be the case in human heart failure. This is furthermore underscored by the reciprocal changes in expression of the T3-regulated myosin heavy chain genes MYH6 and MYH7, typical of reduced cardiac T3 action. Although it should be noted that other factors contribute to these changes in pathological remodeling.

An unexpected finding was the substantial increase in T4 content of both the LV and RV in the ISHD patient samples as compared to donor samples. This may be related to the fact that transplant patients are treated with T4 and/or T3 in some transplant centers, but these data were not available for our patient group. Because possible differences in plasma TH levels are an important confounder in our analyses, we also looked at LV/RV ratios in those hearts in which data for both ventricles were available. These results confirmed the LV-specific reduction of T3 content, albeit that the significance was borderline (p=0.05) as a result of the smaller sample size.

Taken together, the present data support our suggestion that an increase of LV DIO3 activity leads to a local hypothyroid condition in ischemic heart disease. The ISHD patient used in this study were severely ill and induction of DIO3 in human liver and skeletal muscle is known to occur in critically ill patients (Peeters et al. 2003).
was associated with decreased T3 and increased rT3 concentrations in plasma, which were prognostic for mortality (Rothwell et al. 1995; Peeters et al. 2005). The prognostic value of low plasma T3 levels also applies to cardiac patients (Friberg et al. 2002; Pingitore et al. 2005; Passino et al. 2009; Coceani et al. 2009; Lymvaios et al. 2011). Whereas the induction of DIO3 expression in liver and skeletal muscle appears to be a general response, the expression of DIO3 in some but not all cardiomyocytes in remodeled myocardium indicates regional or cell-specific mechanisms. Potential regulators of DIO3, which are also known to be involved in cardiac remodeling and hypertrophy, are transforming growth factor β (S. A. Huang et al. 2005), MAPK, sonic hedgehog (SHH) (Dentice et al. 2007; Kusano et al. 2005) and hypoxia-inducible factor 1α (HIF1α) (Kido et al. 2005; G. H. Li et al. 2009). The latter is a likely candidate regulating DIO3 induction during cardiac remodeling as it was shown to induce DIO3 in cardiomyocytes as well as in other cell types in response to hypoxia (Simonides et al. 2008). In addition, HIF1α interacts directly with the DIO3 gene (Simonides et al. 2008). Furthermore, HIF1α induction was found in several animal models post-MI (Kido et al. 2005; Bai et al. 2008; Jürgensen et al. 2004) and associated with DIO3 induction in a rat model of right ventricular hypertrophy and heart failure (Simonides et al. 2008). HIF1α expression is also upregulated in ISHD as a result of the inadequate myocardial perfusion and tissue hypoxia (S. H. Lee et al. 2000). In addition, increased ventricular wall tension may be an independent stimulus of HIF1α signaling in the diseased heart (C.-H. Kim et al. 2002).

A possible limitation of this study is that donor hearts underwent a different treatment than explanted failing hearts, which may have resulted in loss of any DIO3 activity present in healthy myocardium. Although DIO3 is a fetally expressed gene and activity in adult heart may be assumed to be as low as in adult rodent heart, DIO3-expressing post-MI mouse hearts were treated as donor hearts to assess the effect of cardioplegia on DIO3 expression. Perfusion with cardioplegic solution and short term storage on ice did not affect DIO3 activity or immunostaining, excluding the procurement protocol as a cause of the low or absent DIO3 staining in donor tissue.

Tissue-specific induction of DIO3 has now been shown to occur in several pathological situations in animal models and in humans, affecting local and systemic thyroid hormone metabolism. The present finding that cardiac DIO3 expression is also upregulated in ISHD patients, suggests that the impaired cardiac T3 signaling shown in animal models is present in human cardiac disease and may contribute to the development of heart failure.