

# The Effects of Hydrogen Gas Inhalation on Adverse Left Ventricular Remodeling After Percutaneous Coronary Intervention for ST-Elevated Myocardial Infarction

— First Pilot Study in Humans —

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**Background:** Hydrogen gas inhalation (HI) reduced infarct size and mitigated adverse left ventricular (LV) remodeling in a rat model of acute myocardial infarction (AMI). We designed a prospective, open-label, rater-blinded clinical pilot study in patients experiencing ST-elevated MI (STEMI).

**Methods and Results:** The 20 patients with an initial diagnosis of STEMI were assigned to either an HI group (1.3% H<sub>2</sub> with 26% oxygen) or a control group (26% oxygen). There were no HI-related severe adverse events. In the full analysis set, the cardiac salvage index as evaluated using cardiac magnetic resonance imaging at 7 days after primary percutaneous coronary intervention (PCI), showed no significant between-group difference (HI: 50.0±24.3%; control: 60.1±20.1%; P=0.43). However, the improvement from day 7 in the HI group was numerically greater than that in the control group in some of the surrogate outcomes at 6-month follow-up, including the LV stroke volume index (HI: 9.2±7.1 mL/m<sup>2</sup>; control: −1.4±7.2 mL/m<sup>2</sup>; P=0.03) and the LV ejection fraction (HI: 11.0%±9.3%; control: 1.7%±8.3%; P=0.11).

**Conclusions:** The first clinical study has shown that HI during PCI is feasible and safe and may also promote LV reverse remodeling at 6 months after STEMI. The study was not powered to test efficacy and a further large-scale trial is warranted. (Clinical trials registration: UMIN00006825)

**Key Words:** Cardiac magnetic resonance imaging; Hydrogen gas; Left ventricular remodeling; Myocardial infarction

The prognosis for acute myocardial infarction (AMI) has improved dramatically with the advent of the intensive care system and revascularization therapy such as primary percutaneous coronary intervention (PCI) for ischemic tissue.<sup>1</sup> As the number of survivors has increased, a concomitant increase in heart failure patients after MI has become a problem. LV dysfunction is the single strongest predictor of death following ST-elevated MI (STEMI).<sup>2</sup> Therefore, reducing the infarct size by mitigating myocardial ischemia-reperfusion (IR) injury

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during primary PCI is an important therapeutic target to prevent adverse left ventricular (LV) remodeling after MI. Studies in animal models of acute MI show that reperfusion injury accounts for up to 50% of the final size of the infarct.<sup>3</sup> Increasing understanding of the mechanism underlying acute protection from cardiac IR injury, particularly with regard to the inhibition of mitochondrial

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permeability transition pore opening, has led to the development of new pharmacologic interventions. However, until now, the efficacy that has been shown for most cardioprotective agents in animal models has been difficult to confirm in clinical trials. Intravenous administration of atrial natriuretic peptide for 3 days in patients who had acute MI and were undergoing reperfusion treatment reduced infarct size and prevented adverse LV remodeling.<sup>4</sup> The administration of cyclosporine, a pharmacologic inhibitor of cyclophilin D, immediately before primary PCI reduced the MI size in patients with STEMI in a proof-of-concept phase 2 trial.<sup>5</sup> However, in a phase 3 trial with a total of 970 patients with anterior STEMI who had been referred for primary PCI, intravenous cyclosporine did not prevent adverse LV remodeling.<sup>6</sup> To date, the development of novel therapeutic approaches to minimize infarct size or subsequent adverse LV remodeling is an unmet medical need in the age of PCI.

Molecular hydrogen (H<sub>2</sub>) gas has versatile therapeutic effects through its reducing capacity, including effects on oxidative stress, inflammation, cell death and metabolism.<sup>7</sup> These favorable effects have brought significant benefits to various pathophysiological conditions in the field of emergency medicine such as brain infarction,<sup>8</sup> post-cardiac arrest syndrome,<sup>9–11</sup> and contrast-induced acute kidney injury (AKI).<sup>12</sup>

We previously reported that H<sub>2</sub> gas inhalation (HI) during reperfusion reduced infarct size in a rat model of cardiac IR injury.<sup>13</sup> A similar protective effect has been observed in dogs.<sup>14</sup> The study in dogs adequately mimics the clinical situation, namely, 1.3% H<sub>2</sub> with 26% oxygen was inhaled from 10 min prior to the start of reperfusion until 1 h of reperfusion. After adjustment for systemic hemodynamic parameters and coronary collateral circulation, HI reduced the infarct size normalized by the risk area (20.6±2.8% vs. control gas 44.0±2.0%; *P*<0.001).

To translate animal experimental data to the clinical setting, we designed a single-center prospective, open-label, blinded study to investigate the feasibility and effects of HI on infarct size and adverse LV remodeling after primary PCI for STEMI.

## Methods

### Study Design

A single-center, prospective, open-label, rater-blinded pilot study was performed at Keio University School of Medicine in Japan. The study was performed according to the principles of the Helsinki Declaration of Good Clinical Practice. Approval was obtained from the Ethical Committee of Keio University. The study was supported by Taiyo Nippon Sanso Corporation, but had no influence on the design or execution of the study; the collection, monitoring, analysis or interpretation of the data; or the writing of the report. The study was registered at UMIN: 00006825.

### Study Population

Patient recruitment occurred between November 2011 and March 2016. The eligible patients were men and women, aged 20–80 years, who presented within 24 h of the onset of symptoms and signs of STEMI, defined as significant ST-segment elevation in at least 2 contiguous leads. The following ST-segment elevation criteria were used: 1-mV ST-segment elevation in the limb leads (II, III and aVF, I,

aVL) and V4–6, and 2-mm ST-segment elevation in V1–3. The patients were not considered for enrollment if they presented with cardiogenic shock, severe hypoxia, sustained ventricular tachycardia or ventricular fibrillation, or had return of spontaneous circulation or had an absolute contraindication to contrast-enhanced cardiac magnetic resonance imaging (CMR). All patients received standard medical therapy per the discretion of the attending cardiologist. All patients were informed about the study orally and in writing, and all gave their written consent before inclusion.

### Study Intervention and Monitoring

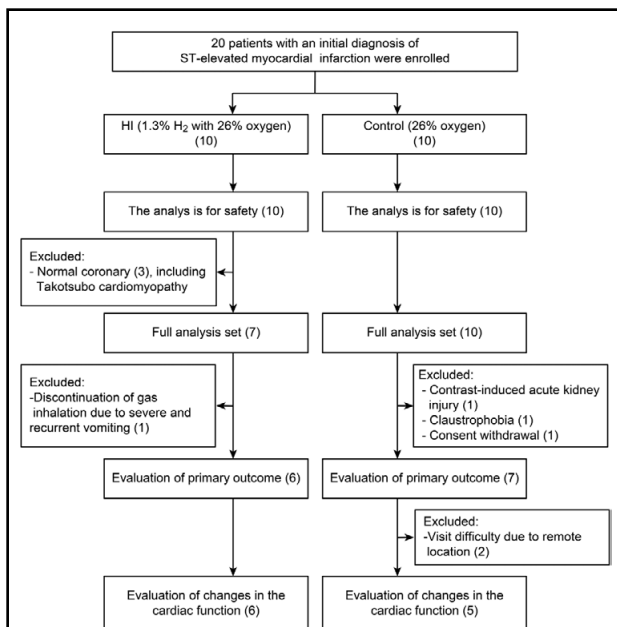
It was assumed that 1.3% H<sub>2</sub> had to be achieved before reperfusion, which required the treatment to be started before coronary angiography (CAG). Consequently, the first 10 patients were assigned to the HI group (1.3% H<sub>2</sub> with 26% oxygen) and the latter 10 patients were assigned to the control group (26% oxygen). The HI and control treatments were initiated through a mask upon arrival at the emergency room and were continued during primary PCI. Patients were blinded to the allocated treatment before, during, and after the inhalation. Taiyo Nippon Sanso Corporation provided the H<sub>2</sub> gas for this study. All patients, including those meeting the angiographic exclusion criteria, were treated with HI during CAG with or without primary PCI in order to evaluate any adverse effects (AEs). In all patients, blood samples were collected, and ECG and X-rays performed immediately upon arrival at the emergency room and at 7 days and 6 months after the primary PCI procedure. Creatine kinase (CK) was measured before and every 4 h after intervention for 48 h. Contrast-enhanced CMR was performed 7 days and 6 months after intervention. AEs were evaluated daily during admission and at every clinic visit.<sup>11</sup> Severe AEs were indicated as those when the patient's outcome was one of the following: death, life-threatening, hospitalization, disability, congenital anomaly and permanent impairment or damage.

### Study Outcomes

The primary outcome was the cardiac salvage index, measured by CMR at 7 days after primary PCI. Secondary outcomes included (1) changes in LV end-systolic volume index (LVESVI), LV end-diastolic volume index (LVEDVI), LV stroke volume index (LVSVI), and LV ejection fraction (LVEF) as assessed by CMR at 7 days and 6 months after primary PCI; (2) angiographic myocardial blush scores; (3) resolution of ST-segment elevation on 12-lead ECG; and (4) peak plasma level of CK.

### CAG and Primary PCI

Patients underwent primary PCI according to standard guidelines.<sup>2</sup> They were pretreated with heparin (intravenously) and with aspirin (200 mg orally), clopidogrel (300 mg orally) or prasugrel (20 mg orally). After initiation of the treatment of HI or control, CAG was performed to identify the culprit lesion. Direct stenting, thrombectomy and choice of bare-metal or drug-eluting stents were left to the discretion of the operator. Predilatation with a small-sized balloon was allowed before stenting. Ischemic post-conditioning was not allowed, and balloon angioplasty alone was limited to cases in which a stent could not be deployed or was considered harmful. All patients were treated with 75 mg clopidogrel or 3.75 mg prasugrel daily over 6 months and 100 mg aspirin daily for life. Two



**Figure 1.** Study flowchart. The analysis of safety included all patients who underwent hydrogen gas inhalation (HI) (n=10) or control treatment (n=10). The population for the full analysis set included patients with a final diagnosis of ST-elevated myocardial infarction after coronary angiography, except for 3 patients in the HI group with normal coronary arteries, including 1 case of takotsubo cardiomyopathy (HI: n=7; control: n=10). The population for the evaluation of cardiac function as assessed by cardiac magnetic resonance (CMR) imaging at 7 days after primary percutaneous coronary intervention (PCI) included 6 patients in the HI group and 7 patients in the control group. The changes in cardiac function as assessed by CMR at 7 days and 6 months after primary PCI were compared between 6 patients in the HI group and 5 patients in the control group.

blinded observers analyzed the angiograms for stenoses other than the culprit lesion, myocardial blush grade and TIMI flow grade.

### CMR Imaging

CMR was performed on a 3-T scanner (Discovery MR750, GE Healthcare, Waukesha, WI, USA) equipped with a 32-channel cardiac coil. All images were obtained during breath-holding at end-inspiration using ECG triggering. The first scan was performed on day 7 after primary PCI to assess the myocardial area at risk (AAR), and the second examination was performed 6 months later to assess the changes in cardiac function and the area of late-gadolinium enhancement (LGE). The 7th day was selected for the first scan because previous studies revealed that this time point was suitable for evaluating the AAR in humans admitted with STEMI.<sup>15–18</sup>

Cine images were acquired with a balanced steady-state free precession cine sequence in long vertical and horizontal planes and a short axis covering the entire LV with the following parameters: TR/TE, 3.5/1.6 ms; flip angle 45°; matrices, 224×224; bandwidth, 125 kHz; phases per cycle, 20. After the cine images, the black blood T2W image with fat suppression (BBT2WI) was acquired using fast spin-echo following the inversion-recovery technique for suppressing

the signal in the LV lumen with the following parameters: TR/TE, 1,800/85 ms; flip angle, 90°; matrix, 320×224; echo train length, 20; bandwidth, 83 kHz. Approximately 10 min later, after the administration of 0.1 mmol/kg of gadodiamide hydrate (Omniscan; Daiichi Pharmaceutical, Tokyo, Japan), LGE was achieved using an inversion-recovery technique with the following parameters: TR/TE, 4/1.2 ms; flip angle 15°; matrix, 256×192; bandwidth 31.25 kHz in LGE. The inversion time was optimized to the null point of normal myocardium in each patient. The field of view was adjusted to each patient's body size and utilized throughout the examination, which ranged from 32 cm to 38 cm. A slice thickness of 7 mm without gap was used for image acquisition, and the slice locations were adjusted to the same in the cine, BBT2WI and LGE images for each patient.

The LV functional parameters were analyzed on a commercially available workstation (AdvantageWorkstation, GE Healthcare). The endocardial and epicardial borders were manually traced to obtain LVESV and LVEDV, LVEF and the LV mass. Papillary muscles were included in the LV lumen.

BBT2WI and LGE images were transferred to another workstation (Ziostation 2, Zio, Tokyo, Japan), and the results are the consensus of 2 experienced observers blinded to the treatment and angiographic data. The AAR was defined as a hyperintense area on the T2W images (i.e., myocardium with a signal intensity 2 standard deviations (SD) above the mean signal obtained in remote non-infarcted myocardium).<sup>16,17</sup> Any area with a lower signal within the AAR was considered part of the AAR. Hyperintense areas in the LV cavity adjacent to the endocardium caused by slow flow were excluded as artifact. The size of the AAR was expressed in grams and as a percentage of the total LV mass.

The enhanced area of myocardium was measured on LGE images.<sup>19,20</sup> The size of the enhanced area was determined using an automatic approach when the signal intensity was higher than +5 SD of the signal intensity in remote non-infarcted myocardium.<sup>16,21</sup> A dark zone in the subendocardial side of the hyperenhanced region was considered a microvascular obstruction and included in the infarct volume. The infarct size was expressed in grams and as a percentage of the total LV mass. The cardiac salvage index was calculated as follows: [AAR (g)–infarct size (g)]/AAR (g).<sup>18,22</sup> BBT2WI and LGE were evaluated separately with an interval of 1 month to avoid any bias.

### Sample Size and Statistical Analysis

Based on previous results of ischemic post-conditioning in patients with STEMI, we assumed the average salvage index measured by CMR to be 0.50 with a SD of 0.16. Therefore, 40 patients per group were needed to achieve 80% power ( $\alpha$  is 2-sided 5%) in detecting a 20% difference in the salvage index. However, when a total of 20 patients were enrolled with 10 patients assigned to the HI group and 10 to the control group, this clinical trial was terminated because patient recruitment was very time-consuming and excessive extension of the study period was thought to decrease the reliability of the data. In a post-hoc evaluation, this study size could detect a 30% relative difference with 80% power for the primary outcome.

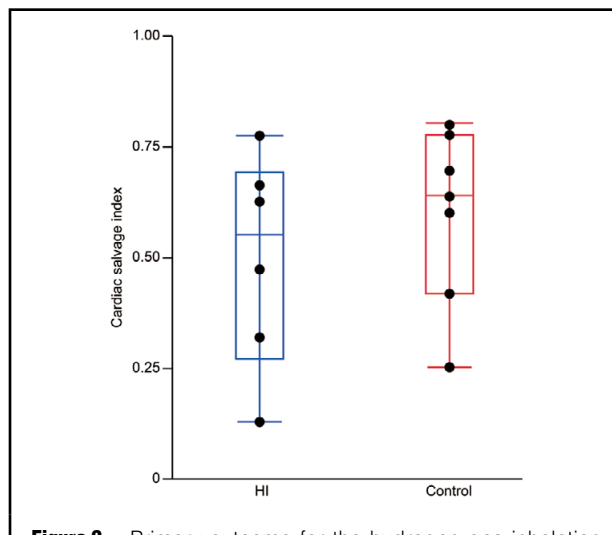
All patients who participated in the study treatment were included in the safety analysis. In the efficacy evaluation, the primary population was the full analysis set (FAS)

**Table 1. Baseline Characteristics of the Full Analysis Set**

	HI (n=7)	Control (n=10)
<b>Demographic and anthropometric data</b>		
Age (years)	50.7±8.6	60.0±11.4
Male sex	4 (57.1)	6 (60.0)
Body mass index (kg/m <sup>2</sup> )	26.7±3.5	24.7±4.0
Body surface area (m <sup>2</sup> )	1.8±0.2	1.7±0.1
Heart rate (beats/min)	84.0±35.2	73.1±16.4
Systolic BP (mmHg)	137.1±34.7	145.3±20.8
Diastolic BP (mmHg)	93.3±25.4	89.1±14.8
Hypertension	5 (71.4)	4 (40.0)
Diabetes mellitus	3 (42.7)	3 (30.0)
Hyperlipidemia	3 (42.7)	4 (40.0)
Previous myocardial infarction	0 (0)	2 (20.0)
Previous heart failure	0 (0)	1 (10.0)
<b>Metabolic data</b>		
Creatinine (mg/dL)	0.9±0.2	0.9±0.3
Hb	15.0±0.5	15.1±1.7
<b>Catheter data</b>		
Culprit vessel		
LAD artery	2 (28.6)	6 (60.0)
Left circumflex artery	1 (14.3)	1 (10.0)
Right coronary artery	4 (57.1)	3 (30.0)
Stenting	7 (100.0)	10 (100.0)
Multivessel disease	3 (42.7)	4 (40.0)
<b>Medication data</b>		
Dual antiplatelet drug	5 (71.4)	9 (90.0)
ACEI or ARB	2 (28.6)	5 (50.0)
β-blocker	4 (57.1)	7 (70.0)
Aldosterone antagonists	0 (0)	1 (10.0)
Calcium antagonists	0 (0)	3 (30.0)
Statin	4 (57.1)	7 (70.0)
Diabetes drugs	1 (14.3)	1 (10.0)
Diuretics	0 (0)	1 (10.0)

Data are the mean±standard deviation, median (interquartile range), or number (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BP, blood pressure; Hb, hemoglobin; LAD, left anterior descending.

in which all patients who met the major eligibilities with a final diagnosis of STEMI after CAG were included. In the FAS, 3 patients in the HI group with normal coronary arteries, including 1 case of takotsubo cardiomyopathy, were excluded because they seemed to be independent of the efficacy evaluation (HI: n=7; control: n=10 in the FAS). In the analysis of the primary outcome (the cardiac salvage index), 1 and 3 patients were excluded from the HI group and the control group, respectively. Of them, 1 patient withdrew from the study because of severe and recurrent vomiting in the catheter laboratory during HI. This patient could not undergo CMR at 7 days after primary PCI in the HI group. A total of 3 patients in the control group could not participate: 1 patient had a contrast-induced AKI after primary PCI, 1 was diagnosed with claustrophobia just before CMR scanning, and 1 withdrew before the scanning for personal reasons. In the analysis of some of the secondary outcomes (e.g., CMR), all patients who had serial CMR data obtained on day 7



**Figure 2.** Primary outcome for the hydrogen gas inhalation group (HI: n=6) and control treatment group (n=7): the cardiac salvage index at 7 days after primary percutaneous coronary intervention between the HI and control groups. The cardiac salvage index was defined as [area at risk (AAR) (g)–infarct size (g)]/AAR (g). The myocardial AAR was assessed as edema using a T2-weighted short tau inversion-recovery sequence. The infarct size was defined as the area of hyper-enhanced myocardium on the delayed-enhancement images.

and in month 6 were included. In addition to the previously mentioned patients, 2 were excluded from the control group because of difficulty attending outpatient visits because of remote residence. The changes in cardiac function as assessed by serial CMR at 7 days and 6 months after primary PCI were compared in the remaining patients (HI: n=6; control: n=5). There was no effect on the evaluation of the primary and secondary outcomes because the excluded patients had unexpected complications that were not related to HI and withdrew their consent (**Figure 1**).

Continuous data are presented as the mean±SD for normally distributed data. Categorical data are presented as numbers (percentages). Categorical variables were compared with Fisher's exact test. For continuous variables, means were compared between 2 groups using a 2-sample t-test. The paired t-test was used to compare the means within a group. A 2-sided P value <0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS, Cary, NC, USA).

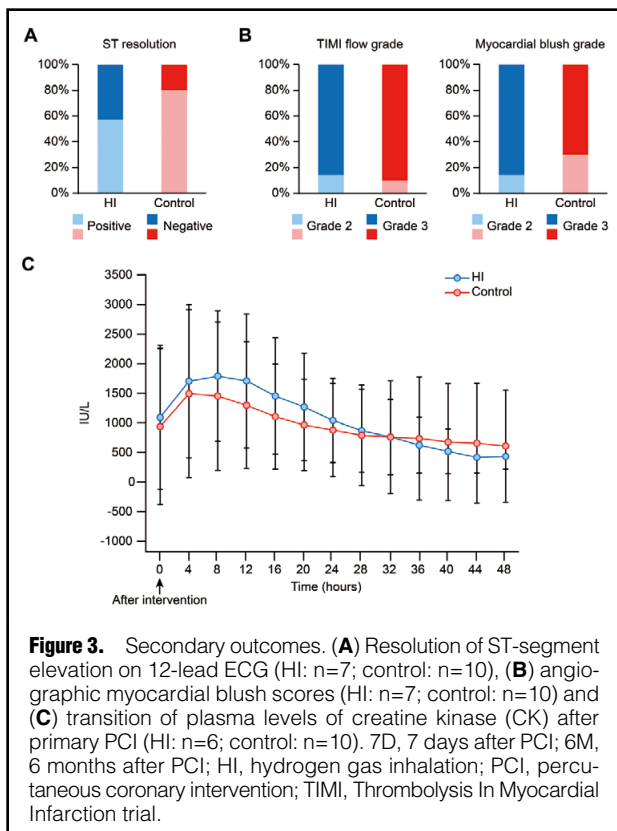
## Results

### Study Population

Patients' demographic factors and baseline characteristics of the FAS population (HI: n=7; control: n=10) are shown in **Table 1**. In the patients who underwent primary PCI, the site of the infarct was in the left anterior descending coronary artery in 60% (n=6) of the control group and in 28.6% (n=2) of the HI group. Multivessel disease incidence was 40% (n=4) in the control group and 42.7% (n=3) in the HI group. Stenting was performed for all patients in both groups.

### IR Injury

Regarding the efficacy of the primary outcome, the cardiac



salvage index, there was no statistically significant difference in the eligible patients (HI:  $n=6$ ; control:  $n=7$ ) between the HI group and the control group ( $50.0 \pm 24.3\%$ ,  $60.1 \pm 20.1\%$ , respectively;  $P=0.43$ ) (Figure 2). For the efficacy of the secondary outcomes, there were no significant between-group differences in the resolution of ST-segment elevation on the 12-lead ECG (Figure 3A; HI:  $57.1\%$ ; control:  $80.0\%$ ;  $P=0.59$ ) or angiographic myocardial blush scores (Figure 3B; TIMI flow grade (grade 3): HI:  $85.7\%$ ; control:  $90.0\%$ ;  $P=1.00$ ; myocardial blush grade (grade 3): HI:  $85.7\%$ ; control:  $70.0\%$ ,  $P=0.60$ ). With respect to the temporal change in serum CK levels, there were no significant between-group differences (Figure 3C; peak CK: HI:  $2,033.7 \pm 1,249.9$  IU/L; control:  $1,649.5 \pm 1,496.1$  IU/L;  $P=0.61$ ; Area under the CK curve: HI:  $1,079.9 \pm 734.2$  IU/L; control:  $969.9 \pm 875.7$  mg/dL;  $P=0.80$ ), although the serum CK levels in the HI group tended to return to the normal range faster than in the control group despite a higher peak serum CK level. The integrated value of CK in blood during the first 48 h after PCI was comparable between the 2 groups.

#### LV Volume and Function at 7 Days and 6 Months

Among the patients who underwent serial CMR scanning (HI:  $n=6$ ; control:  $n=5$ ), there were no significant differences in LVEDVI, LVESVI, LVSVI, or LVEF between the 2 groups on the 7th day (Table 2). In contrast, a significantly higher LVSVI (HI:  $45.5 \pm 6.9$  mL/m<sup>2</sup>; control:  $32.2 \pm 6.3$  mL/m<sup>2</sup>,  $P=0.009$ ) and LVEF (HI:  $65.7 \pm 5.2\%$ ; control:  $48.3 \pm 18.2\%$ ;  $P=0.05$ ) were found in the HI group compared with the control group at 6 months (Table 2). Next, we focused on each individual patient's change in LV volume and function between 7 days and 6 months (Figure 4A). This analysis

**Table 2. Cardiac Magnetic Resonance Imaging Data Obtained at 7 Days and 6 Months**

	HI (n=6)	Control (n=5)	P value
<b>7 days</b>			
LVEDVI (mL/m <sup>2</sup> )	66.4±8.6	76.4±24.0	0.366
LVESVI (mL/m <sup>2</sup> )	30.1±5.3	42.8±23.8	0.234
LVSVI (mL/m <sup>2</sup> )	36.3±5.1	33.6±5.0	0.404
LVEF (%)	54.7±4.4	46.7±12.4	0.169
<b>6 months</b>			
LVEDVI (mL/m <sup>2</sup> )	68.9±6.5	75.6±30.8	0.611
LVESVI (mL/m <sup>2</sup> )	23.4±2.8	43.4±36.6	0.211
LVSVI (mL/m <sup>2</sup> )	45.5±6.9	32.2±6.3	0.009
LVEF (%)	65.7±5.2	48.3±18.2	0.05

Data are mean±standard deviation. Statistical significance between the patients is shown. \*If  $P<0.05$  (Student's t-test). HI, hydrogen gas inhalation; LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end-systolic volume index; LVSVI, left ventricular stroke volume index.

revealed that the LVSVI significantly increased from  $36.3 \pm 5.1$  mL/m<sup>2</sup> to  $45.5 \pm 6.9$  mL/m<sup>2</sup> in the HI group ( $P=0.03$ ), but no change was found in the control group ( $33.6 \pm 5.0$  mL/m<sup>2</sup> to  $32.2 \pm 6.3$  mL/m<sup>2</sup>;  $P=0.69$ ). The LVEF also significantly increased from  $54.7 \pm 4.4\%$  to  $65.7 \pm 5.2\%$  in the HI group ( $P=0.03$ ), but there was no change in the control group ( $46.7 \pm 12.4\%$  to  $48.3 \pm 18.2\%$ ;  $P=0.67$ ). Furthermore, we observed a significant increase in the LVSVI in individual patients of the HI group compared with the patients in the control group ( $+9.2 \pm 7.1$  mL/m<sup>2</sup>,  $-1.4 \pm 7.2$  mL/m<sup>2</sup>, respectively;  $P=0.04$ ) and a significant increase in LVEF in the HI group compared with the control group ( $11.0 \pm 9.3\%$ ,  $1.7 \pm 8.3\%$ , respectively;  $P=0.11$ ) (Figure 4B). Regarding LVESVI, there was a nonsignificant trend towards lower values in the HI group compared with the control group ( $-6.7 \pm 7.1$  mL,  $0.7 \pm 14.6$  mL, respectively;  $P=0.30$ ) (Figure 4B). There were no significant between-group differences in the medication therapy at discharge (Table S1) and at the 6-month follow-up (Table S2).

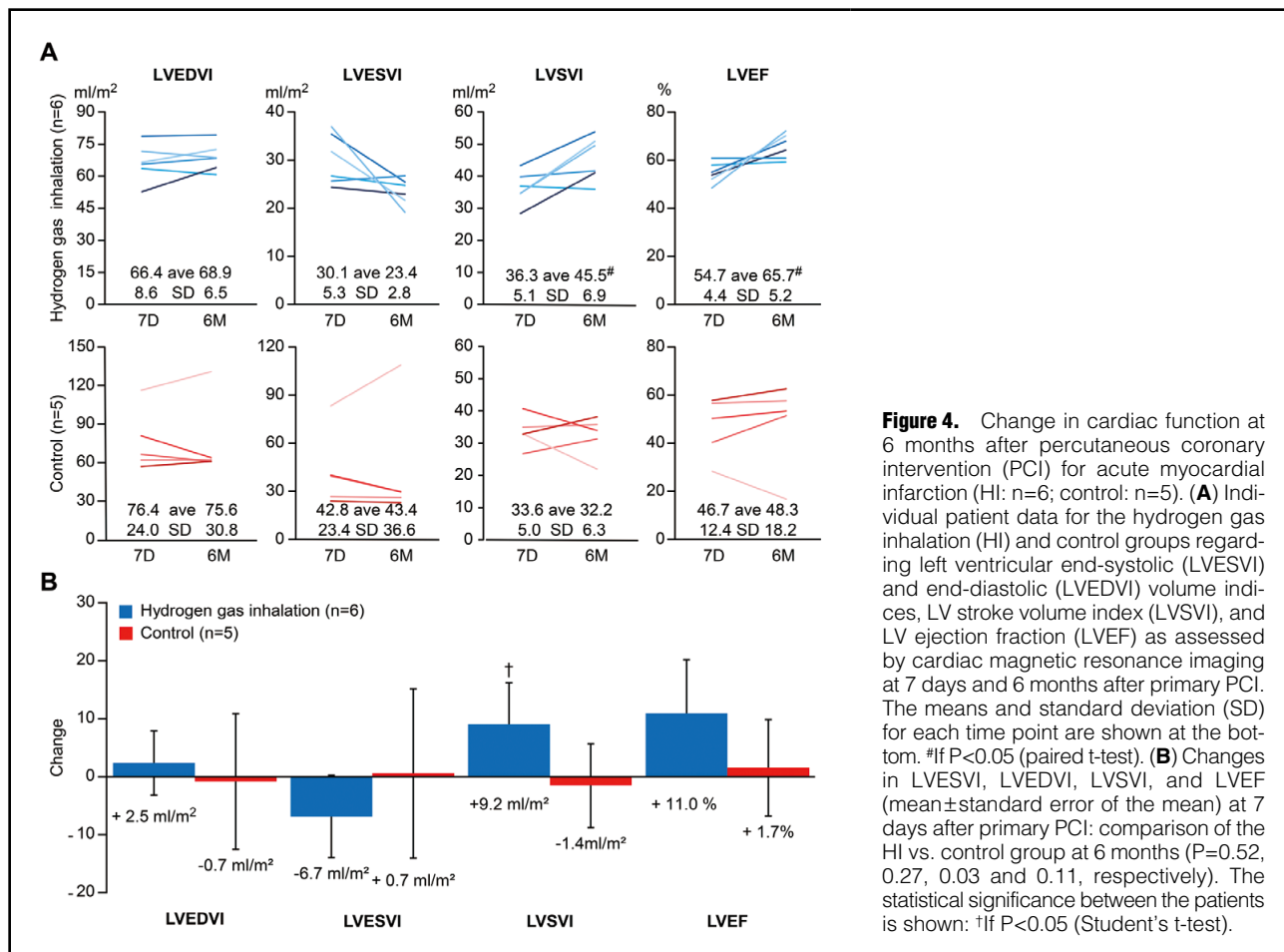
#### Treatment Safety

The baseline characteristics of all the trial participants are shown in Table S3. Two severe AEs were reported in the control group, but none in the HI group. In addition, no AEs were causally related to HI (Table 3). Although there was 1 patient with a baseline Cr value  $>1.5$  mg/dL in each group, the HI group did not have deteriorating Cr values after PCI, whereas the control group showed an elevated Cr value of 3.0 mg/dL. HI demonstrated no deterioration in kidney or liver function and there were no cases of anemia in the period of the study compared with the control group (Table S3).

#### Discussion

To the best of our knowledge, this is the first study in humans to evaluate the feasibility and efficacy of HI during routine STEMI care. No AEs related to HI were observed. Although HI therapy did not show an improvement in the primary outcome (i.e., the salvage index at the 7-day follow-up), it showed significant improvement in some of the surrogate outcomes, including LVSVI, and nonsignifi-





**Figure 4.** Change in cardiac function at 6 months after percutaneous coronary intervention (PCI) for acute myocardial infarction (HI: n=6; control: n=5). **(A)** Individual patient data for the hydrogen gas inhalation (HI) and control groups regarding left ventricular end-systolic (LVESVI) and end-diastolic (LVEDVI) volume indices, LV stroke volume index (LVSVI), and LV ejection fraction (LVEF) as assessed by cardiac magnetic resonance imaging at 7 days and 6 months after primary PCI. The means and standard deviation (SD) for each time point are shown at the bottom. <sup>#</sup>If P<0.05 (paired t-test). **(B)** Changes in LVESVI, LVEDVI, LVSVI, and LVEF (mean±standard error of the mean) at 7 days after primary PCI: comparison of the HI vs. control group at 6 months (P=0.52, 0.27, 0.03 and 0.11, respectively). The statistical significance between the patients is shown: †If P<0.05 (Student's t-test).

cant improvement in the LVEF and LVESDI at the 6-month follow-up. In the present study, we clarified that HI is a feasible and safe therapy for patients with STEMI and that it may prevent adverse LV remodeling after primary PCI for STEMI.

This study showed that HI is a feasible method that does not interfere with the PCI procedure. HI had no detrimental effects on hemodynamic stability, blood test findings, or clinical symptoms during the PCI procedure, the hospitalization period, or the 6-month follow-up period after hospital discharge. There were no severe AEs with HI therapy, such as stent thrombosis or AKI. Although both groups included 1 patient with stage 3b chronic kidney disease, only the patient in the control group showed a deterioration in kidney function immediately after primary PCI. We previously reported that HI during contrast-enhanced computed tomographic scanning reduced contrast-induced AKI in a rat model,<sup>12</sup> which is consistent with our current study results. Because eruption and constipation occurred after both HI therapy and the control therapy, these AEs are believed to be unrelated to HI therapy. Furthermore, the dangers of using H<sub>2</sub>, such as explosion, were not a concern in the medical center, because we used a concentration under the explosive level.

We could not achieve proof-of-concept by demonstrating that HI mitigates myocardial IR injury. This apparent discordance between animal experimental studies and the present clinical study may be associated with genetic and

**Table 3. All AEs Including Individual Components of AEs in Both Groups**

	HI (n=10)		Control (n=10)	
	n	Rate (%)	n	Rate (%)
<b>Severe AEs</b>				
Acute renal injury	0	0.0	1	10.0
Stent restenosis	0	0.0	1	10.0
<b>Other AEs</b>				
Dizziness	0	0.0	1	10.0
Eruption	1	10.0	1	10.0
Constipation	1	10.0	2	20.0
Cystitis	0	0.0	2	20.0

AE, adverse effects; HI, hydrogen gas inhalation.

environmental variations in humans. In particular, the heterogeneity of CAG characteristics and the onset to balloon time made it difficult to compare the therapeutic effects of HI between the 2 groups in this study with small sample sizes. Nevertheless, HI upon admission resulted in a significant improvement of LV function between 7 days and 6 months based on our analysis of each individual patient's data. These findings suggest that the therapeutic effect of HI in the early phase of MI is not simply caused by a reduction in IR injury during HI but is also related to multifactorial mechanisms that continue to affect LV

remodeling after ceasing HI. MI results in LV remodeling involving the infarcted border zone and remote non-infarcted remote myocardium. The inflammatory response,<sup>23–26</sup> metabolic changes,<sup>27</sup> stimulation of the sympathetic nervous system by an impaired baroreceptor reflex, and disturbance of inter-organ communication all contribute to the pathogenesis of LV remodeling. HI during the acute phase of MI could modulate the immune response cascade, which ultimately results in myocardial healing. It could suppress sympathetic hyperactivity that has been shown to occur early after MI. It also could inhibit contrast-induced nephropathy in patients undergoing PCI for AMI. These changes are likely involved in suppression of the late phase of LV remodeling. Reverse translational research is required to answer this crucial question.

There is a growing body of evidence that indicates that with optimal treatment remodeling can be reversed, causing a gradual improvement in cardiac function and a consequently improved prognosis represented by reverse cardiac remodeling. However, not all patients experience this process.<sup>28</sup> Because prognosis is better in patients who have undergone reversed cardiac remodeling, reversal should be considered a primary treatment goal. In an era when most patients are already treated with renin-angiotensin system inhibitors,  $\beta$ -blockers, and mineralocorticoid receptor antagonists, it was challenging to develop a new treatment that shows additional beneficial effects. In this sense, it is worth noting that HI administration during PCI helped accelerate the reversal of post-MI LV remodeling.

### Study Limitations

There are several important limitations to this study. First, the statistical assessment for between-group comparisons may lack power because of the relatively small number of cases. In addition, several patients withdrew consent and the heterogeneity of patient characteristics, including CAG characteristics, in the absence or presence of a prior history of MI or occult LV dysfunction, may discredit the results despite the reasons for the exclusions appearing to be independent of efficacy evaluations. Furthermore, this was not a randomized study. Future randomized studies with a large sample size of patients with first onset of AMI with a proximal LAD occlusion are required to overcome these limitations. Additionally, 1.3% H<sub>2</sub> had to be achieved before reperfusion, requiring the treatment to begin before CAG. Patients on >10 L/min oxygen could not inhale 1.3% H<sub>2</sub> simultaneously. If a lower concentration of H<sub>2</sub> gas is effective in treating cardiac IR injury, HI therapy could be applied to more patients with MI.

### Conclusions

This study provides the first clinical data showing that HI therapy during primary PCI is a feasible and safe treatment option for patients with STEMI and may prevent adverse LV remodeling after primary PCI. The study was not powered to test efficacy and further large-scale trials are warranted.

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### Conflict of Interest Statement

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### Supplementary Files

#### Supplementary File 1

**Table S1.** Medications at discharge

**Table S2.** Medication at 6 months

**Table S3.** Baselines characteristics of the analysis for safety cohort

**Table S4.** Kidney and liver function after HI

Please find supplementary file(s);  
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