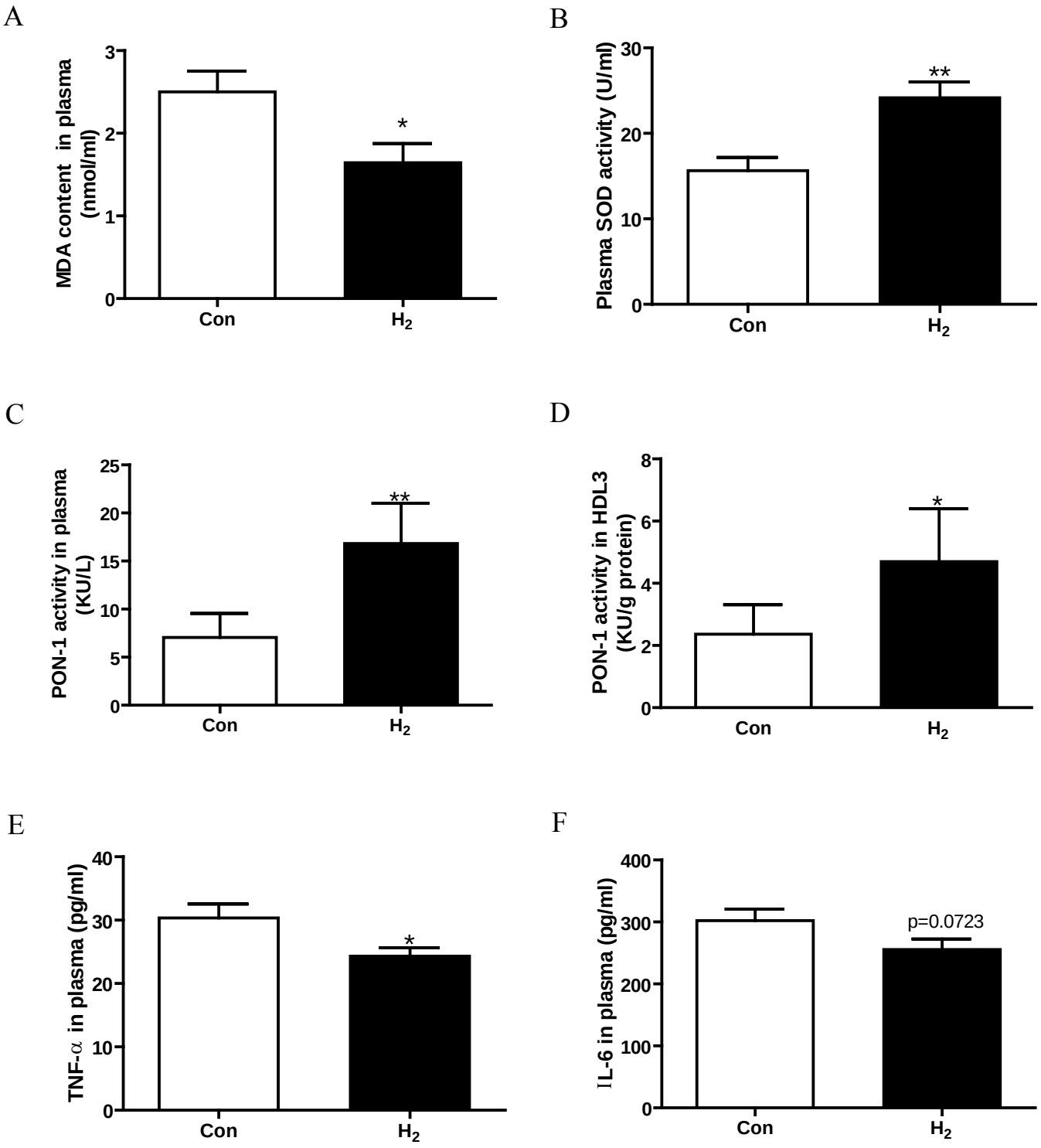
Supplemental Table 1. characteristics of subjects, biometrics, and glucose for all patients

variable	**Control group (n=34)**	**H_2 group (n=34)**
Age (years)	54.6 ± 10.6	55.8 ± 10.5
Gender-Male	16/34	17/34
Gender-Female	18/34	17/34
Mean Systolic BP (mmHg)	129.1 ± 16.7	128.7 ± 16.4
Mean Diastolic BP (mmHg)	86.7 ± 8.1	88.7 ± 9.9
Height (cm)	162.3 ± 16.8	163.8 ± 7.6
Weight (kg)	72.7 ± 11.2	72.5 ± 9.8
Waist Circumference (cm)	97.7 ± 8.4	96.5 ± 6.7
BMI (kg/m^2)	26.7 ± 3.3	26.9 ±3.6
Alcohol Use		
None	34/34	34/34
Tobacco Use		
None	34/34	34/34

Supplemental Table 2. Effects of H_2 on the levels of plasma triglyceride and glucose in each individual patient

	Placebo		H_2		Placebo		H_2	
Patient Number	TG(mM)-before drinking	TG(mM)-after drinkin	TG(mM)-before drinking	TG(mM)-after drinking	Glucose(mM)-before drinking	Glucose(mM)-after drinking	Glucose(mM)-before drinking	Glucose(mM)-after drinking
1	1.93	1.96	1.43	1.11	5.36	5.12	6.1	6
2	1.95	5.2	2.17	2.06	5.56	5.44	5.76	5.75
3	1.4	1.11	1.63	1.83	5.3	5.05	6.38	6.77
4	2.99	3.04	2.38	1.53	5.95	5.51	5.81	5.71
5	1.4	1.34	1.55	1.18	5.95	5.98	6.06	5.43
6	0.55	0.69	1.39	1.06	5.91	5.68	9.24	6.97
7	1.69	1.68	6.12	5.18	5.99	7.15	6.42	5.73
8	2.29	2.03	1.02	1.15	6.13	5.57	7.38	7.08
9	2.55	2.26	0.9	0.61	5.59	5.57	6.1	5.46
10	1.24	1.94	2.09	1.42	9.95	11	5.74	5.72
11	5.25	2.92	3.29	3.46	6.12	6.44	5.81	5.49
12	0.65	0.76	1.43	0.61	5.97	5.74	5.61	5.39
13	2.82	2.82	3.42	3.24	22.84	12.6	5.78	5.35
14	1.86	1.5	1.37	0.95	5.69	5.76	5.57	5.88
15	3.93	1.63	3.65	1.81	6.96	5.08	5.69	6.15
16	3.76	2.09	3.08	3.11	6.46	6.86	6.45	6.23
17	1.21	2.1	2.45	1.93	8.38	7.74	6.02	7.22
18	1.93	3.69	1.25	0.7	4.57	5.38	7.49	11.2
19	0.76	0.83	2.85	1.94	4.87	3.94	5.33	5.09
20	2.1	2.31	2.33	2.33	6.25	6.12	5.63	8.66
21	2.29	1.56	1.72	1.72	9.88	9.52	6.73	6.73
22	2.4	2.4	0.87	1.03	6.28	6.2	5.45	5.46
23	2.18	2.18	1.31	2.07	6.5	8.66	6.45	7.25
24	0.72	0.57	2.63	3.55	4.73	5.53	8.62	7.97
25	1.17	1.59	2.17	1.24	6.62	5.68	5.66	5.38
26	1.79	1.23	0.84	0.66	6.49	6.19	5.6	5.24
27	3.9	3.01	2.16	3.28	7.77	7.01	8.66	7.99
28	5.52	6.45	0.54	0.49	5.61	6.81	4.98	5.34
29	1.49	0.86	1.76	3.46	8.42	8.65	9.92	5.49
30	1.93	1.22	0.62	0.69	5.14	5.5	5.27	5.62
31	0.64	0.63	1.3	4.12	8.82	8.54	11.74	12.03
32	0.5	0.86	1.52	1.75	6.26	5.91	4.6	5.24
33	3.17	3.17	2.05	1.23	6.65	9.52	5.29	5.12
34	0.45	0.58	1.2	0.55	5.36	5.26	5.56	5.22
Mean	2.070882	2.00618	1.955588	1.854412	6.892058824	6.667941176	6.438235294	6.392941176
SD	1.273981	1.28755	1.090844	1.175276	3.114336137	1.86842468	1.534481396	1.617076542

The highlighted parts show patients whose glucose level was decreased above 10% after drinking water compared with the glucose level before drinking water



Supplemental Figure 1. Effect of H2 on plasma levels of antioxidative and inflammatory biomarkers. (A) plasma concentrations of MDA. (B) SOD activity in plasma (C and D) PON1 activity in plasma and HDL3 fraction. (E) plasma concentrations of TNF-α. (F) plasma concentrations of IL-6. n = 30, * $P < 0.05$, ** $P < 0.01$.

Plasma Analysis

a) Plasma lipids

Blood samples were obtained in the morning after an overnight fast. Plasma TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, and triacylglycerols (TG) were measured by enzymatic methods on a chemical autoanalyzer (Hitachi Co, Tokyo, Japan). Patient eligibility for enrollment and randomization and treatment decisions were based on plasma TC values.

Plasma glucose levels were measured by the glucose oxidase method. Pre-β1-HDL was measured by ELISA (American Diagonostica GmbH, product No. 289194) according to the manufacturer's instructions as reported previously [1-2]. Endogenous lecithin-cholesterol acyltransferase (LCAT) activity was measured as the utilization rate of free cholesterol (FC) in native plasma according to the method by Ly et al. [3]. The FC content was measured colorimetrically by using a kit (Biovision, product No. K623-100) in pentuplicate by a microplate reader (Tecan) at zero time and after 1 hr at 37℃. LCAT activity was expressed as nanomoles FC consumed per hr per ml plasma. Plasma oxidized LDL levels were measured by using a commercially available kit (Oxidized LDL competitive ELISA, Mercodia, Salem, NC, USA).

b) Measurement of plasma oxidative stress and oxidizability

Plasma levels of malondialdehyde (MDA), a marker for oxidative stress, were determined by a spectrophotometric measurement of thiobarbituric acid-reactive substances (TBARS) according to the manufacturer's instructions (Nanjing Jiancheng Biochemistry, China, product No. A003-1). The activity of superoxide dismutase (SOD), which acts as antioxidant and protect cellular components from being oxidized by reactive oxygen species, was measured by a commercial kit (Beyotime, China, product No. S0105) according to the manufacturer's instructions. The activity of paraoxonase-1 (PON1), an antioxidant enzyme associated with HDL, was measured by adding plasma to 1 ml of Tris-HCl buffer (100 mM, pH 8.0) containing 1 mM $CaCl_2$ and 1 mM of phenylacetate (Sigma) as described

previously [4]. The rate of phenyl acetate hydrolysis was determined spectrophotometrically (Uvikon 930 spectrophotometer, Kontron) at 270 nm. PON1 activity was expressed in international units (U) per milliliter of plasma. When measuring PON-1 activity in lipoproteins, the activity was expressed in international U per gram of protein.

c) Measurement of plasma inflammatory factors

Plasma concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), were determined by ELISA kits (Bluegene, Shanghai, China) according to the manufacturer's instructions.

HDL Induced Ex Vivo Cholesterol Efflux from Bone Marrow Macrophage and RAW264.7 Macrophage

We measured plasma HDL-C-mediated efflux in bone marrow macrophage (BMM) with or without glyburide, a commonly used ABCA1 chemical inhibitor [5-6]. BMM were isolated from mice by standard procedures [7]. Acetyl LDL (AcLDL) was prepared according to the methods of Basu et al [8]. Isolated macrophages were suspended and cultured in 12-well plates (0.25×10^6 /ml) in DMEM with 10 % FBS plus antibiotics at 37°C. After 4 days, macrophages were labeled for 24 h at 37°C with 2 μCi/ml [1, 2-^{3}H]cholesterol and 50 μg protein/ml acLDL in the presence of 1.0 % FBS and antibiotics in DMEM. BMM were washed extensively with PBS with 1% BSA and equilibrated overnight with or without 2.5 μM glyburide (Sigma-Aldrich, Cat. No. G2569) in medium containing 1% BSA. At the end of the equilibration period, cell monolayers were washed twice with PBS containing 1 % BSA. Subsequently, cholesterol efflux was induced by incubation for 4 h at 37°C with 200 μg protein/ml of HDL in DMEM containing 1% BSA. Cholesterol efflux was obtained by measuring the release of radiolabeled cholesterol into the medium as described previously [9].

Nextly, we measured ABCA1 stimulated efflux in RAW264.7 macrophages with or without cAMP, which can induce ABCA1 activity. Cholesterol efflux experiments were performed as described by Smith et al [10]. RAW264.7 macrophages at 50 % confluence were cholesterol loaded and labeled in 1 ml of RGGB (RPMI 1640 supplemented with 50 mM

glucose, 2 mM glutamine, and 0.1 % BSA) containing [^{3}H]cholesterol (1 μCi/ml) and AcLDL (100 μg protein/ml) for 30 min. Then, macrophages were washed twice with 0.1% BSA-PBS, and equilibrated with RGGB for 24 h with or without cAMP. On the following day, the medium was then replaced with RGGB containing 200 μg protein/ml of HDL. After 6 hr of incubation, the culture was centrifuged to remove cell debris and 100 μl of the medium was removed for determination of radioactivity. At the end of the chase period, the macrophages were dissolved in 0.4 ml of 0.1 M sodium hydroxide, and the radioactivity per aliquot was measured. The percentage cholesterol efflux was calculated by dividing the media-derived radioactivity by the sum of the radioactivity in the media and the macrophages.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Human umbilical vein endothelial cells (HUVECs) were grown to confluence in 96-well plates and pretreated with or without HDL (100 μg protein/ml) for 2 hours and stimulated with oxidized LDL (ox-LDL, 100 μg protein/ml) for 12 hours. Cells treated with medium only served as a negative control group. After removing the supernatant of each well and washing twice by PBS, 10 μl of MTT solution (5 mg/ml in PBS) and 100 μl of medium were then introduced. After incubation for another 4 h, the resultant formazan crystals were dissolved in dimethyl sulfoxide (150 μl) and the absorbance intensity measured by a microplate reader (Tecan, Sweden) at 490 nm with a reference wavelength of 620 nm. All experiments were performed in quadruplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

Detection of apoptosis by flow cytometry analysis

The Annexin V-FITC/PI double-staining assay was used to quantify apoptosis according to the manufacturer's protocol (BD Biosciences, Cat. #556547). Following treatment, cells were centrifuged, washed with PBS and resuspended in binding buffer, and incubated with Annexin V-FITC and propidium iodide (PI) solution for 10 min at room temperature in the dark. The samples were analyzed on a FACScan flow cytometer using CellQuest software (Becton Dickinson, San Jose, CA, USA). Double staining of cells with Annexin V-FITC and

PI allowed the identification of different cell populations based on their staining patterns as follows: live cells ($FITC^-PI^-$), early apoptotic ($FITC^+PI^-$), late apoptotic ($FITC^+PI^+$) and necrotic cells ($FITC^-PI^+$).

Endothelial Cell - Monocyte Adhesion Assay

Monocyte adhesion assays were performed under static conditions as previously described [11] with minor modification. Human umbilical vein endothelial cells (HUVECs) were grown to confluence in 24-well plates and pretreated with or without HDL (100 μg protein/ml) for 2 hours and stimulated with ox-LDL (100 μg protein/ml) for 6 hours. THP-1 cells were labeled with a fluorescent dye, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM), by incubation with 10 μmol/L BCECF-AM at 37°C for 1 hour in RPMI-1640 medium and were subsequently washed with EBM-2.
Confluent HUVECs in 24-well plates were washed 3 times, and labeled THP-1 cells ($2x10^5$ cells per 200 μL) were added to each well of HUVECs. THP-1 cells were allowed to adhere to HUVECs by incubation at 37°C for 60 minutes, and unbound THP-1 cells were removed by washing (3 times, 5 minutes). THP-1 cells bound to HUVECs were counted under fluorescent microscope. The numbers of adherent leukocytes were determined by counting 4 fields per ×100 high-power-field well using fluorescent microscopy (Nikon, Japan) and photographed. Four randomly chosen high-power fields were counted per well. Experiments were performed in duplicate or triplicate and were repeated at least 3 times. The person counting the adherent monocytes was unaware of the treatment.

Western Blots

Cells were harvested and protein extracts prepared as previously described [12]. They were then subjected to western blot analyses using intercellular adhesion molecule 1 (ICAM-1, Santa Cruz, Cat. #sc-8439), vascular cell adhesion molecule 1 (VCAM-1, ABclonal, Cat. #A0279), and β-actin (Sigma, Cat. #A2228) antibodies. For plasma apolipoprotein measurement, 0.2 μl of plasma was denatured at 90℃ for 10 min and then subjected to

western blot analyses using apolipoprotein AI (apoAI, Santa Cruz, Cat. #sc-23605), apoB100 (Novus Biologicals, Cat. #NB200-527) and apoM (Cell Signaling Technology, Cat. #5709) antibodies. The proteins were visualized and quantified using an enhanced chemiluminescence method (Pierce) and quantified using a Chemiluminescence imaging system (Bioshine ChemiQ 4800mini, Shanghai, China).

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