were not investigated in relation to eGFR. As shown in eTable 3, all long-chain n-3 PUFA concentrations and ratios of long-chain n-3 PUFAs to AA showed significant associations with $eGFR_{cys}$. And although both the concentrations and the ratios did not show significant relationship with $eGFR_{cre}$, the ratio of long-chain n-3 PUFAs to AA showed higher coefficients with $eGFR_{cre}$ than n-3 PUFA concentrations. Accordingly, the present study suggests that ratios of serum long-chain n-3 PUFAs to AA could be useful when investigating the relationship between PUFA profiles and renal function.

The mechanisms by which higher (EPA+DHA):AA ratios protect renal function are still not clear. However, dietary fish oil supplementation has been shown to slow renal disease progression in patients with IgA nephropathy[19] and to suppress mesangial cell activation and proliferation in animal models.[20] Therefore, long-chain n-3 PUFAs are considered to attenuate inflammation through several pathways, including those involved in reduction of nitric oxide, downregulation of tumor necrosis factor-α, and modulation of protein kinases.[21–23] Furthermore, Minuz et al demonstrated that alterations in cytochrome P450 (CYP)-dependent AA metabolism are associated with the development of vascular and tubular abnormalities in patients with renovascular disease.[24] Arnold et al showed that EPA and DHA are efficient alternative substrates of AA-metabolizing CYP enzymes, and that dietary EPA/DHA supplementation causes a profound shift in the CYP-eicosanoid profile.[25] In addition, Cicero et al showed that long-term n-3 PUFA supplementation was associated with significant reduction in blood pressure.[26]

The hypocholesterolemic effects of fish intake have also been reported.[27] The association between (EPA+DHA):AA ratio and eGFR was independent of the presence of hypertension and cholesterol level; however, these mechanisms could be also associated with the results in the present study. Furthermore, the difference in eGFR between T_1 and T_3 was higher in men than in women. According to sex-specific characteristics of the participants (eTable 1), prevalence of smoking in men was high in T_1. Therefore, unfavorable lifestyles, such as smoking and low fish intake, might be one possible reason for poorer renal function.

Gopinath et al also showed that linolenic acid (18:3n-3; α-linolenic acid) intake was positively associated with the odds of CKD.[15] In contrast, Lauretani et al showed that a higher plasma concentration of α-linolenic acid, n-6 PUFAs, linoleic acid, and AA were associated with lower decline in Cre clearance.[1] In the present study, serum concentration of α-linolenic acid, n-6 PUFAs, linoleic acid, and AA did not show significant relationships with eGFR. A previous study reported that serum concentrations of EPA and DHA were generally higher in the Japanese population than in Western populations.[2] Therefore, in the present study, the relatively higher concentration of EPA+DHA might mask the relationship between renal function and other PUFAs, such as α-linolenic acid, n-6 PUFAs, linoleic acid, and AA.

The significant relationships between the tertile of (EPA+DHA):AA and fish intake in the present study suggest that higher fish intake could be related to higher eGFR. Indeed, the participants with higher fish intake tended to have higher $eGFR_{cys}$ in the present study. According to previous studies, fish consumption differs by country (mean daily fish intake: 71–125 g in Japan, 32 g among Caucasians in the United States [U.S.], and 85–110 g in Norway).[2,28,29] Hallen et al performed an international comparison of CKD prevalence between the participants in the third National Health and Nutrition Examination Survey (NHANES III) in the U.S. and those of a survey (HUNT II) in Norway.[30] After age standardization, the prevalence of CKD in HUNT II was 9.3%, and the prevalence in NHANES III was 11.0%. Although they did not consider fish consumption in the analyses, the difference in CKD prevalence between the two populations might be partly due to the difference in fish consumption, as well as due to the lower prevalence of diabetes and obesity in Norway. However, the prevalence of CKD is not low in the general Japanese population (12.9%), despite high fish consumption.[8] This discrepancy is most likely because average life expectancy has been extended[31]; blood pressure is relatively high[4]; and the prevalence of diabetes, hypercholesterolemia, and obesity has increased in the Japanese population.[32]

The present study had several limitations. First, because the study was cross-sectional, causality could not be determined. Second, information about corticosteroid use was not available. Third, sex-specific analyses were difficult due to the small number of participants. Fourth, although Cre clearance and insulin clearance are better markers of renal function, these data were not available in the present study. Finally, body muscle mass might be related to the results of the present study; however, these data were also not available.

In conclusion, serum long-chain n-3 and n-6 PUFA profiles, especially the (EPA+DHA):AA ratio, were significantly associated with GFR estimated by an equation using serum Cys C in Japanese community-dwellers. The results of the present study suggest that increased fish intake or supplementation with long-chain n-3 PUFAs might prevent renal dysfunction in the general population.

ONLINE ONLY MATERIALS

eTable 1. Characteristics of study participants according to serum (EPA+DHA):AA tertile in men: the Sasayama study, 2012–2013.

eTable 2. Characteristics of study participants according to serum (EPA+DHA):AA tertile in women: the Sasayama study, 2012–2013.

eTable 3. Multivariate-adjusted linear regression models between serum n-3 and n-6 PUFA profiles and estimated GFRs by 2 different equations.

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Conflicts of interest: None declared.

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PLOS ONE

Establishment of a Novel Murine Model of Ischemic Cardiomyopathy with Multiple Diffuse Coronary Lesions

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Abstract

Objectives: Atherosclerotic lesions of the coronary arteries are the pathological basis for myocardial infarction and ischemic cardiomyopathy. Progression of heart failure after myocardial infarction is associated with cardiac remodeling, which has been studied by means of coronary ligation in mice. However, this ligation model requires excellent techniques. Recently, a new murine model, HypoE mouse was reported to exhibit atherogenic Paigen diet-induced coronary atherosclerosis and myocardial infarction; however, the HypoE mice died too early to make possible investigation of cardiac remodeling. Therefore, we aimed to modify the HypoE mouse model to establish a novel model for ischemic cardiomyopathy caused by atherosclerotic lesions, which the ligation model does not exhibit.

Methods and Results: In our study, the sustained Paigen diet for the HypoE mice was shortened to 7 or 10 days, allowing the mice to survive longer. The 7-day Paigen diet intervention starting when the mice were 8 weeks old was adequate to permit the mice to survive myocardial infarction. Our murine model, called the "*modified HypoE mouse*", was maintained until 8 weeks, with a median survival period of 36 days, after the dietary intervention (male, n = 222). Echocardiography demonstrated that the fractional shortening 2 weeks after the Paigen diet (n = 14) significantly decreased compared with that just before the Paigen diet (n = 6) (31.4±11.9% vs. 54.4±2.6%, respectively, $P<0.01$). Coronary angiography revealed multiple diffuse lesions. Cardiac remodeling and fibrosis were identified by serial analyses of cardiac morphological features and mRNA expression levels in tissue factors such as MMP-2, MMP-9, TIMP-1, collagen-1, and TGF-β.

Conclusion: Modified HypoE mice are a suitable model for ischemic cardiomyopathy with multiple diffuse lesions and may be considered as a novel and convenient model for investigations of cardiac remodeling on a highly atherogenic background.

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Introduction

Heart failure is a major cause of death in developed countries. It is a common disease; more than 2% of the United States population, or almost 5 million people, are affected, and 30% to 40% of patients die within 1 year of receiving the diagnosis of heart failure. The causes of heart failure are divided into 2 main classes, ischemic and nonischemic. Coronary heart disease, the single largest cause of cardiovascular disease, is the narrowing of arteries over time caused by atherosclerotic plaques or by acute occlusion of the coronary artery by thrombosis, both of which can lead to myocardial infarction (MI) and the eventual development of heart failure [1,2]. Today, progress in the treatment of acute MI, including reperfusion therapy by balloon catheter-facilitated vessel dilatation or pharmacological thrombolysis, coronary care units, ACE inhibitors [3], and beta blockers, enables many people to survive the acute episode. However, despite the progress in acute-phase treatment, survivors often have critical heart failure. In other words, ischemic cardiomyopathy (ICM), caused by MI and subsequent cardiac remodeling, is an unsolved problem and a significant target for medical treatment.

Murine models are very useful and important in the investigation of new treatments. Permanent or temporary occlusion of the left main descending coronary artery by coronary ligation is performed in murine models of MI or ischemic injury [4,5]. However, the ligation model requires excellent techniques and anesthesia. Consequently, large-scale experiments are very difficult, and artificial effects from the operation and anesthesia cannot be avoided. Moreover, the most important problem is that the coronary ligation model does not have atherosclerotic lesions. On the other hand, the standard murine models for atherosclerosis, LDL receptor knockout (KO) [6] and apolipoprotein E (apoE) KO mice [7,8,9], which exhibit atherosclerotic lesions in the aorta, do not usually develop MI.

Braun et al. reported a murine model, HDL receptor scavenger receptor class B type I (SR-BI)-deficient and apoE-deficient double knockout mice, that exhibit coronary lesions, multiple MIs, and cardiac dysfunction. This model had very strong atherogenicity, and all of these mice died by 8 weeks of age (50% mortality: 6 weeks) [10]. Zhang et al. reported a new murine model, SRBI-deficient and hypomorphic apoE (ApoeR61$^{h/h}$) mice, called "HypoE mice" that exhibited diet-induced hypercholesterolemia, coronary atherosclerosis, MI, and premature death (50% mortality: 33±4.9 days) [11]. In the current study, we modified the period of the atherogenic diet in HypoE mice (modified HypoE mice) so that the mice would survive MI. We now report modified HypoE mice as a new murine model of ischemic cardiomyopathy that shows multiple MIs and survives with cardiac dysfunction.

Methods

Animals and diets

The study was performed under the supervision of the Animal Research Committee of Osaka University and in accordance with the Japanese Act on Welfare and Management of Animals. The protocol was approved by the Animal Care and Use Committee of the Osaka University Graduate School of Medicine (Permit Number: 21-084-2). At various time points, mice were euthanized with pentobarbital (120 mg/kg intraperitoneally [i.p.]) for collection of tissue and blood samples. For pain management during coronary angiography (CAG), mice were anesthetized with inhaled isoflurane (4–5% for induction and 2–3% for maintenance).

SR-BI KO/ApoeR61h/h mice (mixed C57BL/6×129 background) were obtained as a gift from Monty Krieger, Biology Department, Massachusetts Institute of Technology, USA. These mice were housed and fed a normal chow diet from weaning to 8 weeks of age in our breeding laboratory. Male mice were weaned at 21 to 27 days of age and 2 or 3 mice per cage were housed in a barrier facility under specific pathogen-free condition with a 12-hour light/12-hour dark cycle. Beginning at the age of 8 weeks, the mice were fed the Paigen diet for 7 or 10 days (dietary intervention); the Paigen diet was then replaced with normal chow.

The Paigen diet, which contained 7.5% cocoa butter, 1.25% cholesterol, and 0.5% sodium cholate, was prepared at Oriental Yeast Co, Ltd, Tokyo. The caloric composition of this diet was 21.4% protein, 27.4% fat, and 51.2% carbohydrate. The original Paigen diet was described previously [12,13,14]. In contrast, the caloric composition of the normal chow diet (MF diet, Oriental Yeast Co, Ltd.) was 25.6% protein, 12.8% fat, and 61.6% carbohydrate.

The findings of previous studies on SR-BI KO mice showed that, female, but not male, SR-BI KO/ApoeR61$^{h/h}$ mice are infertile. Thus, female ApoeR61$^{h/h}$ mice with heterozygous null mutations in SR-BI were mated to male SR-BI KO/ApoeR61$^{h/h}$ mice [11]. The genotypes were determined by polymerase chain reaction as previously described [15,16].

Cardiac functional analysis

Noninvasive measurements of blood pressure were performed on in the mice, using a blood pressure monitor for rats and mice (Model BP98-A, Softron Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions. To perform echocardiography on conscious mice, ultrasonography (Vevo770, VisualSonics, Inc., Toronto, Canada) was performed using a 25 MHz linear transducer (VisualSonics). The heart was imaged in the 2-dimensional parasternal long-axis view, and an M-mode echocardiogram of the midventricle was recorded at the level of the papillary muscles.

Morphological and biochemical analysis

The heart and ascending aorta of the mouse were perfused with phosphate-buffered saline (PBS) containing 1% heparin, via the left ventricular apex. Samples were isolated and fixed with formalin. Paraffin sections (10 μm) of hearts were stained with Masson's trichrome (Sigma -Aldrich, St. Louis, USA) to evaluate fibrotic areas. The distributions of fibrosis in the middle ventricle and apex were examined with respect to each compartment as described in the previous report [17]. The percent fibrosis was evaluated in 7 sections in different locations as follows: section 1, upper septum; 2, anterior left ventricular wall; 3, upper lateral left ventricular wall; 4, posterior left ventricular wall; 5, right ventricular wall; 6, lower septum; and 7, lower lateral left ventricular wall. Sections 1–5 were located in the middle ventricle and sections 6–7 in the apex.

Frozen sections of ascending aortas at the level of the aortic valve were stained with oil red O and hematoxylin. The sizes of the atherosclerotic lesions were calculated as the sum of the cross-sectional areas of oil red O positive-stained plaques, using Image J software.

Blood was collected from the *ad libitum* fed mice at the time of sacrifice with an overdose of pentobarbital. Serum was separated by centrifugation and stored at −80°C. Serum concentrations of triglyceride, insulin, creatinine, and glucose were determined using commercially available enzymatic assay kits according to the manufacturers' instructions. At each evaluation point, the lipid profile was examined using an HPLC (high performance liquid chromatography) method as previously described [18,19].

Real-time reverse transcription polymerase chain reaction

Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed according to the manufacturer's protocol (SuperScript VILO cDNA Synthesis Kit, Life Technologies Co., Carlsbad, USA). The total RNA was prepared from hearts at various time points after surgery. Total RNA was extracted from snap-frozen, homogenized tissue from the left and right ventricles. RNA was DNase-treated using SuperScript VILO and reverse-transcribed using the QuantiTect Reverse Transcription Kit (QIAGEN, Hilden, Germany). RT-PCR was performed using the Universal Probe Library (UPL) (Roche, Basel, Switzerland) and Light Cycler TaqMan Master kit (Roche). Relative levels of gene expression were normalized to the level of mouse GAPDH expression using the comparative Ct (Threshold Cycle) method according to the manufacturer's instructions [20].

Coronary angiography

Mice were anesthetized, intubationed, and heparinized. A catheter was inserted from the right carotid artery into the ascending aorta, and a solution consisting of 50% weight/volume barium sulfate suspended in 7% gelatin (weight/volume solution in water warmed in a water bath to 60°C) was injected into the ascending aorta. The heart of each mouse was then harvested and immersed in ice to solidify the contrast agent. Coronary angiography was performed using an angiographic system (MFX-80HK, Hitex Co, Ltd., Osaka, Japan) consisting of an open-type 1 μm microfocus X-ray source (L9191, Hamamatsu Photonics Co, Ltd., Hamamatsu, Japan) and a 50/100 mm (2 inch/4 inch) dual mode X-ray image intensifier (E5877JCD1-2N, Toshiba Co, Ltd., Tokyo, Japan) at 60 kV and 60 μA.

Statistical analysis

Results are shown as mean ± S.E. Paired data were evaluated using Student's *t*-test. A 1-way analysis of variance with Tukey's multiple comparison test was used for multiple comparisons. The Kaplan-Meier method with a log-rank test was used for survival analysis.

A p value <0.05 for differences was considered statistically significant.

Results

Hypo E mice survived 7 but not 10 days on the Paigen diet

Very few HypoE mice consuming the normal chow diet died during the experimental period (**Fig. 1**). Long-term observation of HypoE mice consuming a normal chow diet showed a median survival time of 192 days. We found that these HypoE mice died from MI or heart failure rather than from cerebral infarction. The atherogenic Paigen diet caused early death in HypoE mice, and most mice died within 1 month (**Fig. 1A**, blue line). It was expected that HypoE mice could survive on the Paigen diet for a short time. However, 8-week-old HypoE mice fed the Paigen diet for 10 days demonstrated a similar survival curve (**Fig. 1A**, red line) to that for those fed the Paigen diet continuously. In our breeding laboratory, HypoE mice survived after 7 days on the Paigen diet. We called these mice "modified HypoE mice." Their median survival period was 36 days after the Paigen diet intervention. The survival rate of the modified HypoE mice fell rapidly during the first 20 days and slowly thereafter (**Fig. 1A**, black line). Therefore, the observation period of the current study was 2 weeks after the end of the 7-day Paigen diet intervention. At the end of this period, 67% of the modified HypoE mice remained alive. All of the dead mice were dissected, and MI scars and enlarged hearts were found in all cases. Modified HypoE mice were examined just before beginning the Paigen diet (Pre), after 7 days on the Paigen diet (0W), 1 week after the end of the 7-day Paigen diet intervention (1W), and 2 weeks after the end of the 7-day Paigen diet intervention (2W) (**Fig. 1B**).

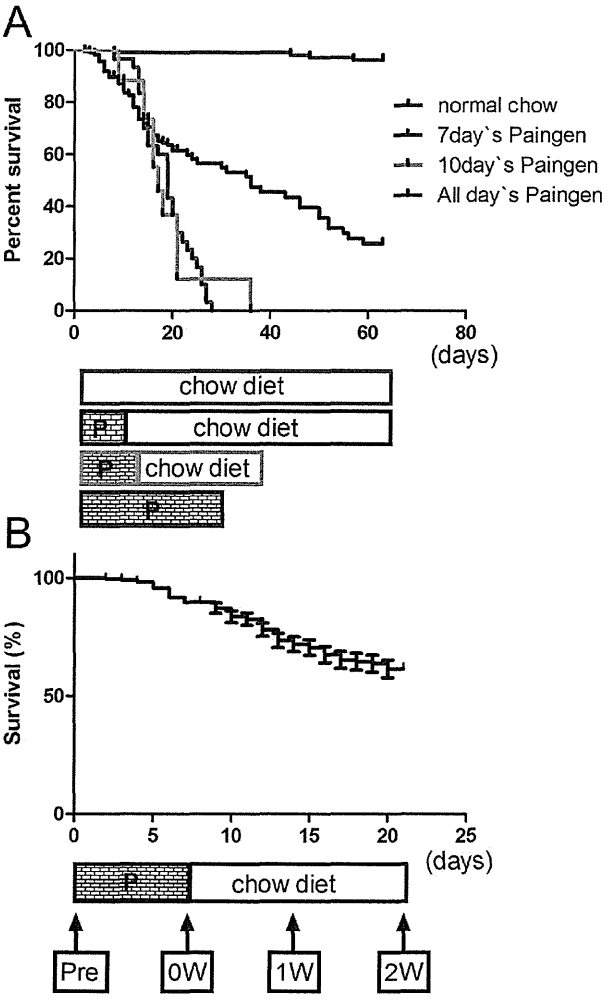

Figure 1. Effects of the Paigen diet on the survival rate of HypoE mice. Survival curves of HypoE mice observed for various periods of the Paigen diet intervention (A). Survival curve of HypoE mice after 7 days on the Paigen diet and timing of observations are shown (B). P shows the Paigen diet. Black line, 7-day Paigen diet intervention; red line, 10-day intervention; blue line, continuous feeding of Paigen diet. Pre, just before Paigen diet; 0W, at the end of the 7-day Paigen diet intervention; 1W, 1 week after the end of the 7-day Paigen diet intervention; 2W, 2 weeks after the end of the 7-day Paigen diet intervention.
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The atherogenic Paigen diet temporally increased cholesterol levels

The Paigen diet has very strong atherogenicity. The effects of the Paigen diet on the cardiovascular risk profiles of modified HypoE mice were confirmed (**Table 1**). The body weights of the mice did not change during the Paigen diet intervention but gradually increased afterward. The blood pressure and blood glucose level increased slightly but not significantly after the Paigen diet. The creatinine level and heart rate did not show clear trends. The insulin level temporally increased during the atherogenic diet. Notably, the serum levels of total cholesterol, LDL-C, Chylomicron-C and VLDL-C increased markedly after the Paigen diet intervention and rapidly returned to the pretreatment levels 1 week after the end of Paigen diet intervention. The HDL-C levels, in contrast, decreased permanently after the Paigen diet intervention.

An atherogenic diet rapidly induced atherosclerotic changes in modified HypoE mice

The total area of the atherosclerotic lesions in the aortic roots was markedly increased after the Paigen diet (**Fig. 2, A and B**). A quantitative analysis of the atherosclerotic lesions is shown in **Figure 2C**. The plaque area in modified HypoE mice 1 week after the Paigen diet was 5.4 times larger than that just before the Paigen diet. There was no significant increase in lesion area between 1 week and 2 weeks after the dietary intervention. Coronary angiography (CAG) showed multiple diffuse lesions (**Fig. 3**). Branches from the main coronary artery were rarely evaluated because of the low resolution of the images. Therefore, even successful CAG was able to detect occlusion only of the main coronary arteries. Five CAG procedures in pre-treatment mice revealed no main coronary occlusion, whereas CAG of 6 modified HypoE mice detected 2 occlusions of the RCA, 2 of the septal artery, and 1 of the LCA.

Cardiac fibrosis and cardiac function after the atherogenic diet

Cardiac fibrosis was observed in all of the HypoE mice after the atherogenic Paigen diet intervention. Fibrotic lesions were patchy and were located predominantly near the endocardium (**Fig. 4A**).

Table 1. Paigen diet-induced changes in modified HypoE mice.

	Pre	0 week	1 week	2 weeks
Body weight (g)	26.6±2.7	26.2±2.3	27.1±2.6	28.1±2.6*
SBP (mmHg)	91±6	91±6	96±4	108±24
DBP (mmHg)	52±6	59±4	59±11	58±9
Heart rate (beats/min)	637±26	620±31	645±32	595±81
Blood glucose (mg/dL)	131±27	164±8	166±34	143±44
Insulin	53.9±31.1	82.9±89.6	42.3±26.6	45.8±22.1
Creatinine (mg/dL)	0.44±0.13	0.42±0.09	0.45±0.08	0.50±0.13
Total cholesterol (mg/dL)	318±89	873±90***	347±13	361±50
Chylomicron-C(mg/dL)	56±15	293±34***	68±3	68±15
VLDL-C(mg/dL)	159±57	415±47***	191±8	185±22
LDL-C (mg/dL)	74±22	134±10***	75±8	89±15
HDL-C (mg/dL)	28±6	30±4	13±1***	20±4**

SBP, systolic blood pressure; DBP, diastolic blood pressure.
Data are mean ± SD.
*$P<0.05$, **$P<0.005$, ***$P<0.0001$. Compared with Pre-diet intervention period.
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Quantitative analysis of cardiac fibrosis was performed using Masson's trichrome staining (**Fig. 4B**). Cardiac fibrosis was particularly increased 2 weeks after the Paigen diet intervention. The extent of cardiac fibrosis in modified HypoE mice 2 weeks after the Paigen diet intervention ranged from 5.3% to 22.3%. At this time, the echocardiographic parameters LVDd and Fractional shortening (FS) ranged from 3.6 mm to 5.6 mm and from 11.7% to 45.5%, respectively. The ventricular fibrosis was not predominantly in any one location, and there was no significant difference in fibrosis between the middle ventricle and apex (**Table 2**). Areas 3 and 7 are perfused by the same main coronary artery, as are areas 1 and 6. However, we observed no significant relationship either between the percent fibrosis levels of areas 3 and 7 or between those of areas 1 and 6.

Signs of heart failure which manifested as increases in the heart weight and lung weight were gradually evident from 1 week after the dietary intervention. Echocardiography revealed a significant decrease in cardiac function (**Fig. 5**). FS 2 weeks after the Paigen diet (n = 14) was significantly decreased as compared with that just before the Paigen diet (n = 6) (31.4±11.9% vs. 54.4±2.6%, respectively, $P<0.01$). The expression levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in the heart also showed the progression of heart failure (**Fig. 6**).

Changes in gene expression related to cardiac remodeling were evident after the atherogenic diet intervention

The changes in mRNA expression levels of genes related to cardiac remodeling are shown in **Figure 7**. The expression levels of matrix metalloproteinase (MMP)-2 and tissue inhibitor of metalloproteinase (TIMP)-1 in the heart gradually increased from pretreatment to 2 weeks after the dietary intervention. The expression levels of MMP-9 and collagen-1 also increased from their pretreatment levels, although there was no increase between 1 week and 2 weeks after the Paigen diet intervention. Transforming growth factor (TGF)-β and hypoxia-inducible factor (HIF)-1α are important molecules that regulate cardiac remodeling. The expression levels of these genes showed a tendency to increase after the dietary intervention.

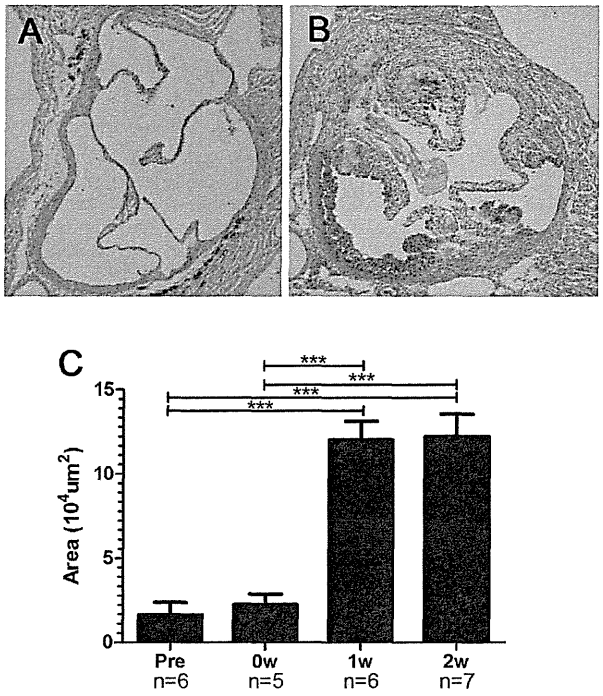

Figure 2. Atherosclerotic lesions in the aorta of modified HypoE mice. Atherosclerotic lesions at the level of the aortic valve were evaluated by oil red O staining. Representative photographs of specimens taken just before the Paigen diet (A) and 2 weeks after the end of the 7-day Paigen diet intervention (B) are shown. Area of atherosclerotic lesions markedly increased 1 week after the end of the 7-day Paigen diet intervention (C). Pre, 0W, 1W, and 2W represent the same time points as in Figure 1. ***$P<0.001$.
doi:10.1371/journal.pone.0070755.g002

Discussion

Advantages of HypoE mice as an MI model

To establish the murine model of ICM, we employed HypoE mice as an MI model, modified the period of atherogenic diet feeding, and successfully identified the characteristics of ICM. The

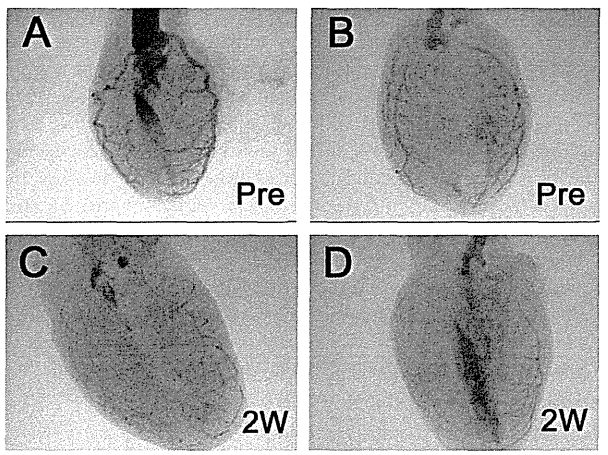

Figure 3. Coronary angiography of modified HypoE mice. The coronary arteries of HypoE mice just before the Paigen diet (A) and 2 weeks after the end of the 7-day Paigen diet intervention (B).
doi:10.1371/journal.pone.0070755.g003

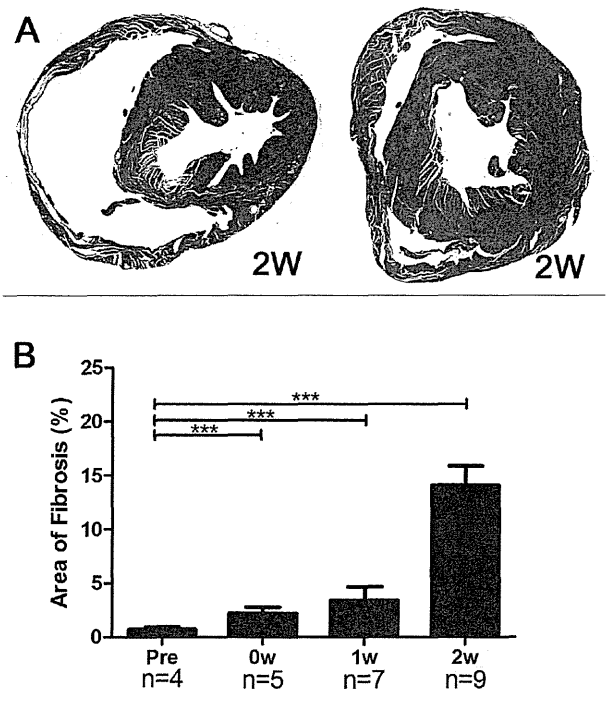

Figure 4. Cardiac fibrosis in modified HypoE mice. Representative images of cardiac fibrosis stained with Masson's trichrome 2 weeks after the end of the 7-day Paigen diet intervention (A) and the percent cardiac fibrosis as a function of time. (B). Pre, 0W, 1W, and 2W represent the same time points as in Figure 1. ***$P<0.001$.
doi:10.1371/journal.pone.0070755.g004

MI model most often used is the coronary ligation model, in which MI is induced by the ligation of the left anterior descending coronary artery (LAD) [4,5,21]. Ligation-model mice are also utilized to investigate ICM after acute MI [22]. The use of ligation-model mice has revealed many new pathological and physiological pathways involved in MI and identified proteins associated with MI [23]. The ligation model usually require anesthesia, intubation, and thoracotomy, which induce artificial injuries and inflammation at sites other than the area of MI [24]. To avoid these artificial effects, the ligation model has been improved. In the "closed chest" method, a suture or occlusion device is implanted for ligation of the LAD via thoracotomy. Using the device, the LAD is ligated from outside the body several days after the operation to prevent acute artificial effects of the surgery [25,26]. Gao et al. reported another improved ligation method in which the heart was manually exposed without intubation through a small incision [27]. This method shortens the operation time and minimizes the operative stress, but it requires excellent techniques. Despite these improvements to the ligation method, the model continues to pose substantial problems.

Human patients with MI often have risk factors such as hypertension, dyslipidemia, and glucose intolerance, which impair endothelial function and induce coronary atherosclerosis at multiple sites. These patients sometimes have multiple coronary lesions and multiple ischemic lesions. In contrast, the ligation-model mice do not have atherosclerotic lesions, and their endothelial function is intact. When MI is induced in the LAD area by coronary ligation, other intact areas fully induce the compensatory reactions and pathways that protect cardiac function. In humans, multiple ischemic lesions other than the MI lesion may influence the induction of these compensatory reactions and pathways. Therefore, it is desirable to establish other murine MI models that more closely resemble the nature of MI encountered in the clinical setting.

On the other hand, murine models for atherosclerosis have been established. ApoE-deficient mice are the mouse model most often used to investigate aortic atherosclerosis, but they do not show coronary occlusion [7,8,9]. Feeding these mice a high-fat diet, particularly the Paigen diet, induces atherosclerotic lesions in the aorta, although not in the coronary arteries [28]. MI cannot be induced in apoE-deficient mice even by feeding the Paigen diet [29]. However, some murine MI models, most of which are genetically modified apoE-deficient mice [30,31], have been

Table 2. Distribution of percent fibrosis in the various parts of middle ventricle and apex.

								Middle	Apex
	Middle ventricle				Apex			Area 1–4	Area 5–7
Mouse	area1	area2	area3	area4	area5	area6	area7	Total	Total
1	4.1	13.2	2.6	2.7	4.9	22.9	2.3	5.3	10.3
2	17.3	14.1	9.7	11.3	12.0	15.9	43.7	13.5	22.1
3	8.0	53.0	23.8	27.3	19.0	37.2	2.9	22.3	10.8
4	18.9	8.7	6.2	6.0	7.8	4.2	5.8	10.5	6.3
8	29.2	18.0	10.6	11.8	9.7	81.0	7.3	17.3	29.0
6	25.7	12.3	5.5	4.4	5.5	24.6	4.0	11.7	9.4
7	53.7	5.1	2.4	2.7	13.2	13.4	2.2	17.9	11.5
8	3.7	1.9	10.8	33.2	2.0	7.1	2.5	9.8	4.6
9	10.5	50.7	9.0	10.8	1.7	17.5	21.3	18.6	18.2
Mean	19.0	19.7	9.0	12.2	8.4	24.9	10.2	14.1	13.6
±SD	±15.8	±18.9	±6.4	±10.9	±5.6	±23.2	±13.9	±5.3	±8.0

Data are expressed as percentage of fibrosis area.
Area 1, upper septum; area2, anterior left ventricular wall; area3, upper lateral left ventricular wall; area4, posterior left ventricular wall; area5, right ventricular wall; area6, lower septum; and area7, lower lateral left ventricular wall. Areas 1–5 were located in the middle ventricle and areas 6–7 in the apex.
doi:10.1371/journal.pone.0070755.t002

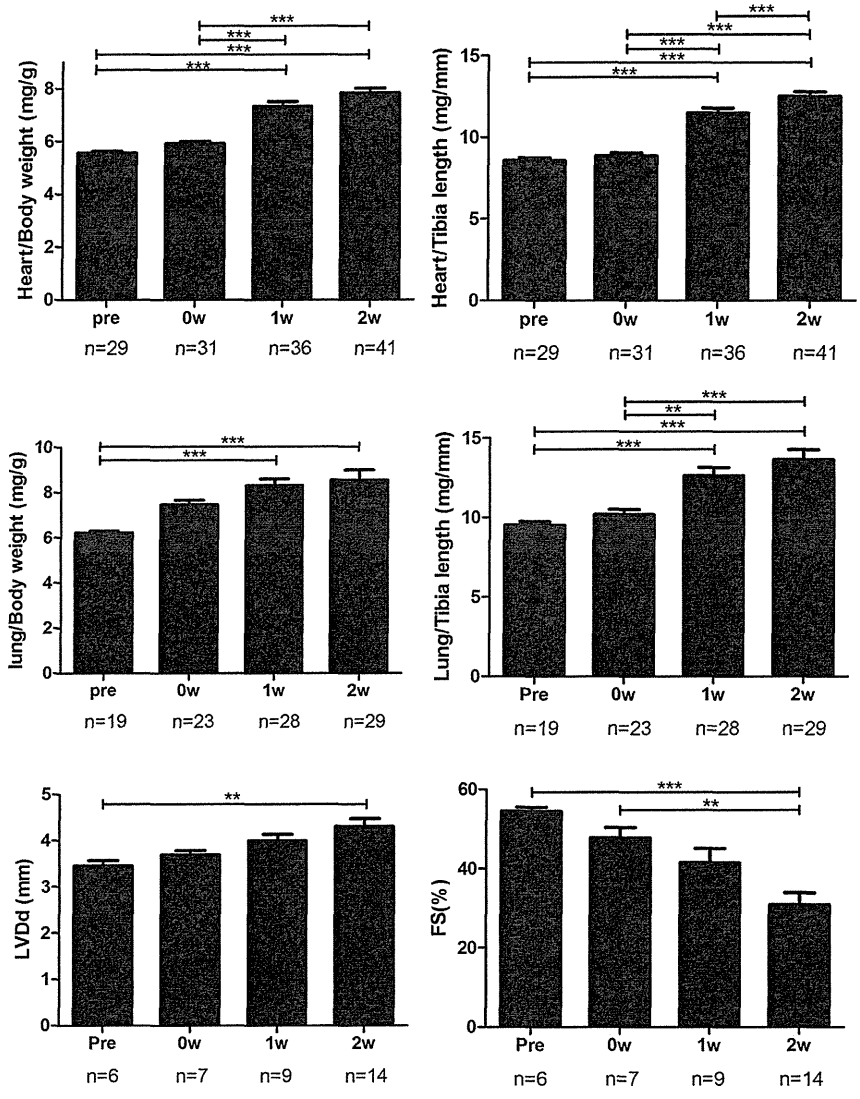

Figure 5. Signs of heart failure and cardiac function. Signs of heart failure, which manifested as increases in the heart and lung weights adjusted by body weight or tibia length, were observed. Echocardiography demonstrated an increase in the left ventricular end-diastolic dimension (LVDd) and a decrease in fractional shortening (FS) after the Paigen diet intervention. Pre, 0W, 1W, and 2W represent the same time points as in Figure 1. *$P<0.05$, **$P<0.01$, ***$P<0.001$.
doi:10.1371/journal.pone.0070755.g005

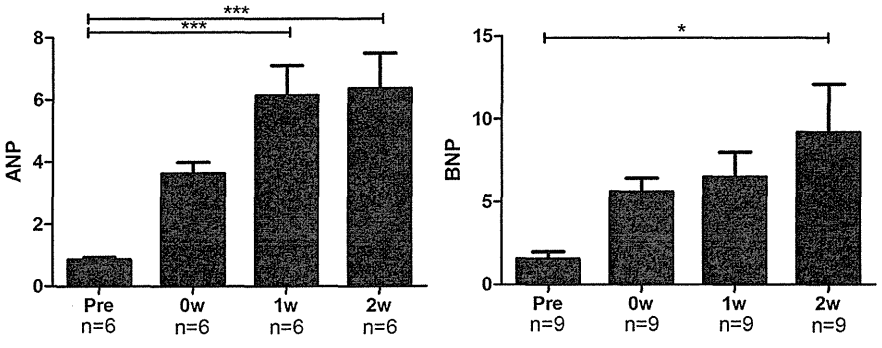

Figure 6. Expressions of ANP and BNP in the hearts of modified HypoE mice. The expression levels of the markers of heart failure, ANP and BNP, gradually increased from the end of the 7-day Paigen diet intervention. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide. Pre, 0W, 1W, and 2W represent the same time points as in Figure 1. *$P<0.05$, ***$P<0.001$.
doi:10.1371/journal.pone.0070755.g006

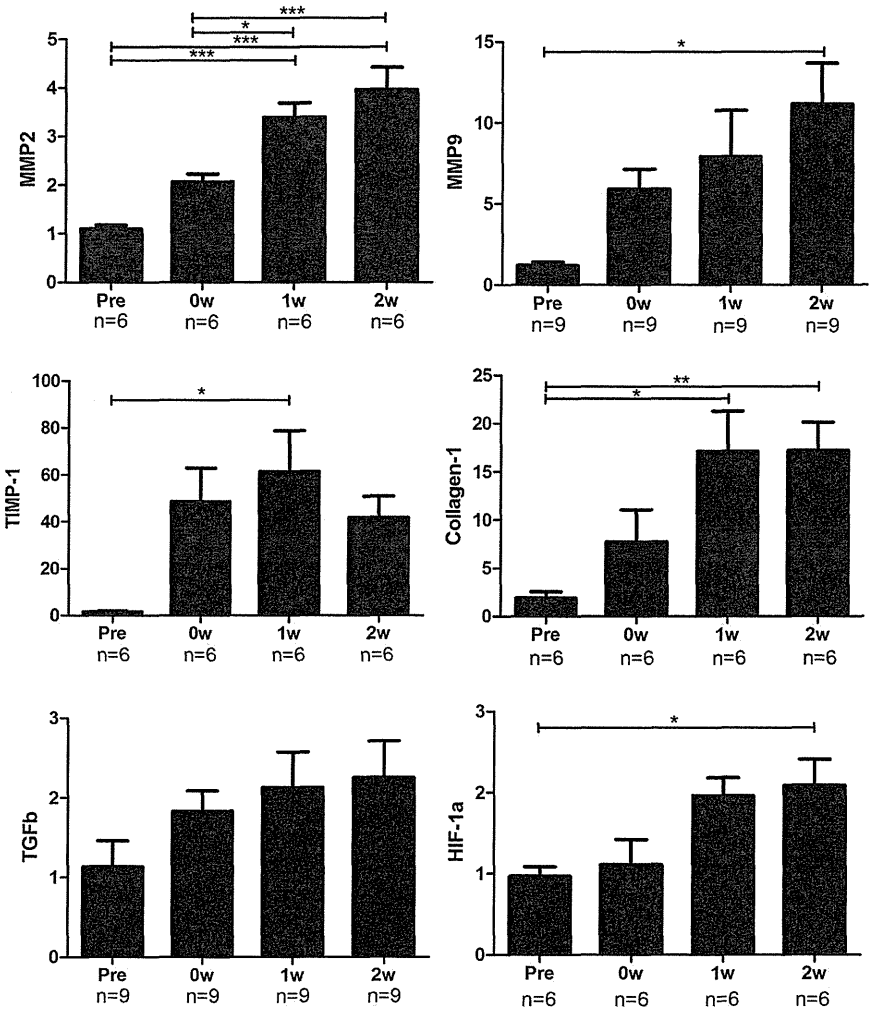

Figure 7. mRNA expression levels of remodeling-related genes and HIF-1αin the hearts of modified HypoE mice. The expression levels of MMP-2, MMP-9, TIMP-1, Collagen-1, and TGF-β show that cardiac remodeling was induced. HIF-1α was also induced along with these mRNA expressions. MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TGF, transforming growth factor; HIF, hypoxia-inducible factor. Pre, 0W, 1W, and 2W represent the same time points as in Figure 1. *$P<0.05$, **$P<0.01$, ***$P<0.001$.
doi:10.1371/journal.pone.0070755.g007

reported. Gene modification in mice can induce MI. Akt1 and apoE deficient mice exhibit reduced migration of vascular smooth muscle cells [30]. Mice with these deficiencies are useful for investigate specific mechanisms of MI, but these generally differ from MI as encountered in the clinical settings.

HypoE mice showed high-fat diet-induced MI lesions. High-fat-fed HypoE mice demonstrate marked hypercholesterolemia and increased levels of remnant lipoproteins [11], that resemble familial hypercholesterolemia and the dyslipidemia involved with diabetes or metabolic syndrome, respectively. Further investigations using synchrotron radiation [32] may reveal impairment of coronary endothelial function in HypoE mice. We believe that the high-fat-fed HypoE mice can be a useful model to investigate MI and cardiac remodeling.

Modification of the atherogenic Paigen diet

Recently, Toyama and Zhang reported the effects of dietary manipulation and social isolation on HypoE mice [33]. They also investigated the survival rates of HypoE mice that were fed the Paigen diet for 10, 12, or 14 days. HypoE mice survived 10 days of the Paigen diet. The authors proposed that HypoE mice might be a promising novel model for the study of heart remodeling, but they did not demonstrate other evidence of cardiac remodeling. We revealed histological changes, cardiac function, and the expression levels of cardiac remodeling-related genes for the first time in this mouse model.

In our laboratory, HypoE mice did not survive 10 days of the Paigen diet. Total cholesterol levels did not contribute to the observed difference in survival, because total cholesterol levels were higher in the previously described Paigen diet-fed HypoE mice (1600 mg/dL) than in our modified HypoE mice [13]. One possible explanation for the difference in survival is that the mice do not tolerant environmental changes. For example, social isolation reduced the survival rate of Paigen diet-fed HypoE mice [33]. These findings indicate that the appropriate duration of the Paigen diet should be determined each time HypoE mice are imported into a new breeding laboratory.

Multiple diffuse coronary lesions in the modified HypoE mice

Histological examination of modified HypoE mice showed that the lesions of cardiac fibrosis were patchy and were predominantly located near the endocardium. This finding implies the presence of multiple distal occlusions of the coronary arteries. Moreover, the cardiac fibrosis was not distributed predominantly in any one region of the heart. The data in Table 2 suggest that the fibrosis in the ventricle was attributable not only to occlusions of the main coronary arteries but also to distal microvascular lesions. Another possible explanation is that diffuse fibrosis developed in response to repeated episodes of occlusion and reperfusion at many different sites in the coronary arteries.

CAG clearly demonstrated occlusions of the main coronary arteries 2 weeks after the Paigen diet treatment. However, these occlusions alone cannot explain the diffuse distribution of cardiac fibrosis. Although we cannot provide quantitative data, CAG seemed to show multiple and diffuse stenosis as well as a decreased number of branched small coronary arteries in modified HypoE mice. These observations suggest that distal microvascular lesions can also contribute to diffuse cardiac fibrosis.

ICM was originally defined as chronic heart failure with severe multiple coronary lesions, but it is now often defined as chronic heart failure with significant coronary lesions [34,35]. CAD patients with diabetes or those who are elderly often demonstrate multiple diffuse lesions of the coronary arteries [36,37]. Familial hypercholesterolemia patients with visceral fat accumulation sometimes exhibit multiple diffuse lesions [38]. Modified HypoE mice can be a useful model of ICM for investigating MI and cardiac remodeling in these clinical syndromes.

Histological examination revealed no obvious plaque rupture. Massive accumulation of foam cells in the coronary lumen was often present, and coronary occlusion by foam cells and thrombus was sometimes observed in this model (**Fig. S1**). These findings are compatible with those from previous reports on HypoE mice [11]. The thrombus formation observed was likely due to the extreme hyperlipidemia and accumulation of foam cells causing injury to the endothelial cells of the coronary arteries and thus attenuating their anti-thrombotic effects.

However, SR-BI/apoE dKO mice developed coronary artery lesions that more closely resembled human coronary lesions, with more severe and complex pathological features such as fibrin deposition [10]. Our modified HypoE mice did not develop complex lesions. However, long-term observation of modified HypoE mice might also reveal the development of complex lesions with fibrin deposition as seen in SR-BI/apoE dKO mice.

Cardiac remodeling in the modified HypoE mice

We examined the time course of cardiac remodeling in the modified HypoE mice and successfully identified changes indicative of cardiac remodeling. Serum levels of troponin I markedly increased just after the Paigen diet intervention and then quickly decreased but remained higher than the pre-intervention levels (data not shown). This result showed that MI was mainly induced during the 7 days of the Paigen diet intervention and that myocardial injury, probably due to mild ischemia, continued from 1 week to 2 weeks after the Paigen diet intervention ended. Serum levels of IL-6 demonstrated changes similar to those of troponin I (data not shown). The expression of MMP-9, which is mainly derived from macrophages [39], remained elevated until 1 week after the Paigen diet intervention. Therefore, macrophages may be recruited to repair the injured MI tissue in the acute phase of cardiac remodeling.

Heart failure, represented by ANP and BNP expression, was induced just after MI, but these changes were not significant. During 1 week and 2 weeks after MI, Masson's trichrome staining, ANP and BNP expression, and echocardiography revealed significant progression of cardiac fibrosis, heart failure, and cardiac dysfunction. The expression of collagen-1 peaked 1 week after MI. Therefore, cardiac fibrosis accelerated during 1 week and 2 weeks after MI and might show a constant increase thereafter. This appears to be the cause of the continuing steady decrease in survival 2 weeks after the Paigen diet. MMP-2 is involved in neovascularization and enlargement of the left ventricle (LV) [40,41]. The expression levels of MMP-2 and TIMP-1 seem to increase in parallel with LV enlargement. The changes in the expression levels of these genes may represent cardiac remodeling in the modified HypoE mice. To investigate the cause of fibrosis, we examined TGF-β, which is an important factor in the induction of fibrosis [42,43], and HIF-1α, which controls gene expression under ischemic conditions [44]. The expression levels of TGF-β and HIF-1α increased gradually, but did not show clear changes when compared with the expression levels of other genes. Therefore, TGF-β and HIF-1α do not appear to be the main controllers of the expression of genes related to cardiac remodeling in the modified HypoE mice. Our modified HypoE mice may be a useful model for evaluating pharmacological effects and investigating pharmacological targets in cardiac remodeling.

Reproducibility and limitations of modified HypoE mice

The reproducibility of experimental data is very important for animal models. However, the standard deviations of FS (6%) and cardiac fibrosis (11%) measured 2 weeks after the Paigen diet intervention seem large compared with those from reports using the ligation model. Previous reports found SDs of FS of 2.4% [45] and 5.5% [46], and SD of cardiac fibrosis of approximately 5–10% [47]. The relatively low reproducibility and the wide variations of measures of cardiac function and fibrosis are probably caused by the presence of multiple coronary lesions that occlude coronary arteries at various positions determined by chance.

There are major limitations to the use of this model. First, mechanical occlusion models of MI induce a highly reproducible infarct at a specific time point; this is not the case in modified HypoE mice, in which the pathology is likely to differ with the specific pattern of coronary disease produced. Second, if clinical translation is the goal, the model does not recapitulate the pathophysiologic basis of CAD in humans (which likely involves multiple factors), as it appears to induce extremely severe hypercholesterolemia in the absence of other major coronary risks.

In conclusion, we demonstrated that 7 days on the atherogenic Paigen diet induced MI and subsequent cardiac remodeling with multiple diffuse coronary lesions in HypoE mice.

This model mouse, called the modified HypoE mouse, is well suited as a model of ischemic cardiomyopathy and may be considered as a novel and convenient model for the investigation of cardiac remodeling on a highly atherogenic background. This may be a useful murine model to evaluate pharmacological effects and to investigate pharmacological targets in MI and cardiac remodeling.

Supporting Information

Figure S1
(TIF)

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Author Contributions

Conceived and designed the experiments: HN YNT MN DM T. Ohama IK SY. Performed the experiments: HN YNT MN T. Okada RK TY MYK KN TS MS. Analyzed the data: HN YNT MN SY. Contributed reagents/materials/analysis tools: HN YNT MN TS MS. Wrote the paper: HN YNT MN SY. Conception and design of this study: HN YNT MN T. Okada RK TY MYK KN DM T. Ohama TS MS IK SY. Acquisition of data, or analysis and interpretation of data: HN YNT MN T. Okada RK TY MYK KN DM T. Ohama TS MS IK SY. Drafting the article or revising it critically for important intellectual content: HN YNT MN T. Okada RK TY MYK KN DM T. Ohama TS MS IK SY. Final approval of the version to be published: HN YNT MN T. Okada RK TY MYK KN DM T. Ohama TS MS IK SY.

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新春特集―主要疾患の最新の話題 2014

脂質異常症
～食後高脂血症の病態と治療～

●血中に存在するリポタンパクとカイロミクロンの代謝・apoB-48　●食後高脂血症の病態とそのメカニズム　●食後高脂血症に対する治療

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1 血中に存在するリポタンパクとカイロミクロンの代謝・apoB－48

循環血液中において脂質はリポタンパクを形成し運搬される。リポタンパクは小腸あるいは肝臓で合成され，小腸由来のものを外因性経路（exogenous pathway，apoB-48を含むカイロミクロンが中心），肝臓由来のものを内因性経路（endogenous pathway，apoB-100を含む超低密度リポタンパク，very low density lipoprotein：VLDLが中心）と呼ぶ（図1）。小腸上皮細胞の核内において合成されたapoB-48分子を核としてコレステロール・中性脂肪（TG）が付与されることにより巨大なカイロミクロン粒子となり血管内に分泌され，血管壁に存在するリパーゼ（LPLあるいはHTGL）の作用によりTGが水解を受け，脂肪酸を末梢組織に受け渡しながら小粒子化（レムナント化）し，最終的に肝臓に再取り込みされる。apoB-48はこの経緯のすべてでカイロミクロン内に残存しており，しかも1粒子に1分子しか存在していないことから，われわれはapoB-48濃度のsandwich ELISA法による測定系を開発した[1]。カイロミクロンのクリアランスは分泌から30分で大半は血中から消失するが血中には微量で残存しており，特にカイロミクロンレムナントの蓄積を背景とした高中性脂肪血症を示す脂質代謝異常において空腹時apoB-48濃度は高値であった。現在さらに自動測定可能なCLEIA系を開発し[2]，空腹時のapoB-48が様々な病態におけるマーカーとしての有効性について検討を継続している。

2 食後高脂血症の病態とそのメカニズム

1．空腹時高トリグリセライド（TG）血症と食後高脂血症

血清TGの高値は動脈硬化性疾患の独立したリスクファクターであるが，非空腹時TG値も冠動脈疾患の発症リスクを増加させる。本邦でIsoらが日本人約1万1千人を前向きに調査したデータでは，非空腹時TG値が84mg/dLの群に比較し，166mg/dL以上で約3倍の冠動脈疾患の発症リスクを有し，空腹時TGと同様に非空腹時（食後）TG値が強い動脈硬化性疾患のリスクファクターであることが判明している[3]。食後高脂血症は，食後の高TG血症によりつけられた呼称であるが，この背景には小腸由来のカイロミクロンおよびカイロミクロンレムナントの血中への蓄積が存在している。この原因としては①小腸上皮からのカイロミクロンの過分泌，②LPL活性の低下に伴うカイロミクロンレムナントの代謝遅延，③肝臓での取り込み低下，のいずれかが考えられる。この背景には，耐糖能異常・糖尿病に伴って起こるFFA・TGの小腸での過剰産生が一因ではないかと示唆されている。インスリン抵抗性が存在する糖尿病患者群での検討において，小腸でのカイロミクロン合成亢進，LPLの活性低下，small, dense LDLが増加するなどの報告がある[4]。インスリン抵抗性動物モデルのfluctose-fed hamstersを用いて検討すると，この小腸上皮において脂肪酸の生合成および小粒子カイロミクロンの産生が増加して，血中のカイロミクロンレムナントが増加していた[5]。われわれは脂肪酸トランスポーターであるCD36の欠損状態を欠損症患者およびCD36ノックアウト（KO）マウスで検討した。欠損症患者には食後高脂血症・インスリン抵抗性・高血圧といったメタボリックシンドロームの表現型が蓄積しており，ノックアウトマウスの小腸での脂肪酸の生合成の亢進・TGの産生増加・小粒子カイロミクロンの産生亢進を発見した[6]。食後高脂血症のメカニズムについては相反するデータも多くまだ結論が得られてないが，インスリン抵抗性を背景とした小腸カイロミクロンの過分泌・血管内でのカイロミクロンレムナントの蓄積がその一因と考えられる。

2．カイロミクロンレムナントの動脈硬化惹起性

カイロミクロンレムナントの動脈硬化惹起性は，食後高脂血症の概念を提唱したZilversmidtにより1979年に予見され[7]，現在では以下のような様々な報告がなされている[8]。

レムナントの動脈硬化惹起性因子についてのシェーマを図2に示す。具体的には，カイロミクロンレムナントは病理学的な検討により動脈壁通過・内皮下層へ蓄積し，LDL受容体およびLRPなどのLDL受容体ファミリーを介してマクロファージへ取り込まれ，マクロファージの泡沫化を惹起し，培養血管平滑筋細胞から単球走化活性因子（MCP-1）を，また内皮細胞からのPAI-1産生を刺激する。これらの作用はすべて動脈硬化プラークの発症進展にかかわっており，食後のカイロミクロンレムナントの産生蓄積は動脈硬化プラー

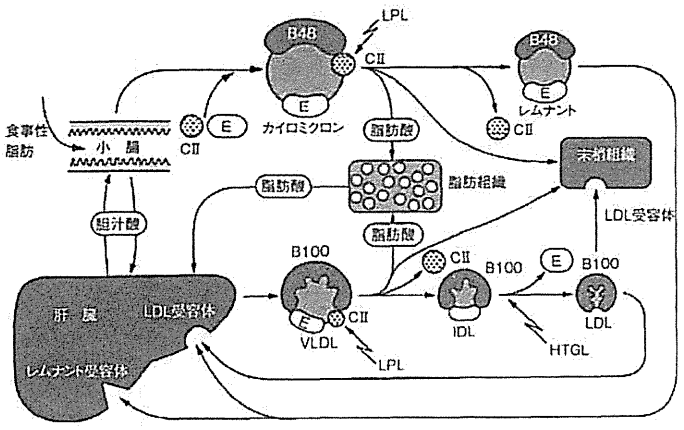
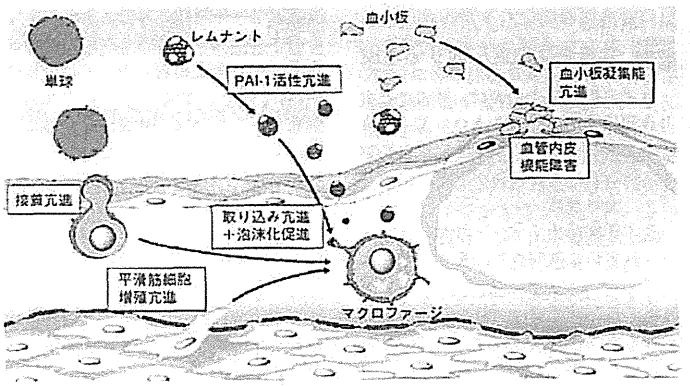


図1　リポタンパク代謝マップ



レムナントの蓄積が
・リポ蛋白代謝異常：small dense LDL増加，HDL低下
・炎症反応の亢進：Egr-1，MCP-1，IL-1β，CD40等の増加
・血管壁での変化を誘導する

図2　レムナントの動脈硬化惹起性

（Fujioka Y, et al：J Atheroscler Thromb, 2009）

クの進展に強い影響を有することが考えられる[8]。さらに，カイロミクロンレムナントの蓄積はインスリン抵抗性や2型糖尿病と関連し[9]，糖尿病患者の頸動脈の内膜中膜壁厚を悪化させ[10]，small dense LDLを増加させる[11]。さらに，メタボリックシンドローム患者において問題となる血中アディポネクチン・レプチン濃度は直接血中apoB-48，RLP-CおよびTG値と相関し，またapoB-48濃度は血中インスリン濃度，HOMA指数および内臓脂肪，皮下脂肪および総脂肪面積ときわめて明確な相関を有している[12]。

これらのことから，カイロミクロンレムナントの代謝障害とその結果起こるカイロミクロンレムナントの蓄積は，他の代謝障害，特にインスリン抵抗性と相互に関係してさらに病態を悪化させていると考えられる。われわれの空腹時apoB-48を用いた検討では，TG値が正常範囲でやや高値(100≦TG＜150 mg/dL)の集団において空腹時apoB-48濃度と頸動脈エコーにおける中膜肥厚度(IMT)と強い相関を示していることを発見した[13]。さらに空腹時apoB-48濃度は心臓カテーテル検査実施例の冠動脈狭窄有病率と有意な相関があり独立した危険因子であること，さらに他の冠動脈危険因子(低HDL-C値，高HbA1c値，高TG値，低アディポネクチン値)と併存した場合に罹患率が有意に上昇することが判明した[14]。このように，空腹時apoB-48濃度測定はカイロミクロンレムナントによる動脈硬化惹起性を極めて効果的に示すマーカーであり，今後も様々な病態において検討を続けて行く予定である。

3 食後高脂血症に対する治療

食後高脂血症の治療は，前述のとおり食事由来のカイロミクロンおよびカイロミクロンレムナントの産生を抑制することにある。

1．食事療法

近年動脈硬化イベントの抑制効果も示されている魚油などに多く含まれるエイコサペンタエン酸(EPA)などの多価不飽和脂肪酸は，他の脂肪酸より吸収が低下するため食後のTG値の上昇を抑える働きがあり，食後高脂血症に有効である。また炭素数10以下の中鎖脂肪酸はTGの再合成に関わらず遊離脂肪酸のまま血中へ放出されカイロミクロンが形成されず，またジアシルグリセロールはTG再合成過程が遅延するため食後高脂血症の抑制に有効である[15]。そのほか，食事中に食物繊維(オートブラン，米糠，小麦繊維，麦芽)を添加することで食後高脂血症を抑制することが示されている[16]。

2．薬物療法

現在，食後高脂血症に効果のある脂質代謝異常治療薬として，スタチンおよびフィブラートが検討されて[17,18]，さらに近年コレステロール吸収抑制薬の有効性も示されてきている。

アトルバスタチンはフルバスタチン，ロバスタチン，プラバスタチン，シンバスタチンと比較して最も有意に食後高TG血症の抑制およびリポタンパク組成改善に有効であった[19]。Parhoferらはアトルバスタチン4週間投与が高TG血症患者のTG値を43%低下させ，さらに脂肪負荷後血清中の小粒子TRLを有意に改善させていたことから，カイロミクロンレムナントの改善に有効であることを示している[20]。さらに，われわれがⅢ型高脂血症患者に対してアトルバスタチンを使用したところ空腹時apoB-48が約43%，apoB-100が約52%有意に低下し食後高脂血症を改善した[21]。

フィブラートは核内受容体PPARαに結合することにより脂肪酸合成の低下，肝臓での脂肪酸酸化促進，LPLの発現増加やapoC-Ⅲの発現抑制をきたしTG値を低下させる。Ooiらはフェノフィブラートを複合型高脂血症(高LDL-Cおよび高TG血症合併)患者に投与した結果，空腹時TG，LDL，RLP-CおよびRLP-TGを低下させ，さらに食後のこれらのパラメーターの曲線下面積(area under the curve，AUC)はAUC-TGで68%減少させることを確認した[22]。われわれはこのメカニズムについてCD36KOマウスで検討したところ，フェノフィブラート投与はapoB-48濃度およびカイロミクロンを抑制し，小腸でのapoB-48の産生抑制を中心としたカイロミクロン合成・分泌を抑制することを見いだした[23]。

小腸でのコレステロール吸収はトランスポーターであるNPC1L1 (Niemann-Pick C1 Like 1 protein)を介して行われ，NPC1L1のinternalizationを阻害することでコレステロールの吸収を阻害するエゼチミブはLDLコレステロールを約20%低下させ，マウスでの検討では動脈硬化抑制効果がある[24]。このエゼチミブは高TG血症を合併する高LDL-C血症患者においては空腹時TG値も低下させることから，食後高脂血症を有するⅡb型高脂血症患者10名にエゼチミブ10mg/日を経口投与したところ，空腹時LDL-C・TG・apoB-48が有意に低下し，脂肪負荷後のTG，RemL-C，apoB-48さらに比較的小粒子のカイロミクロン分画の上昇は有意に抑制された[25]。この背景には小腸における脂肪酸の吸収が考えられる[26]。このような効果のほかにも，エゼチミブには脂肪肝を改善させインスリン抵抗性を改善させるなど多面的な効果も示されており，今後の研究の進展が望まれる。

おわりに

既に動脈硬化性疾患の発症リスクとして確立している高LDLコレステロール血症と異なり，高TG血症あるいは高レムナント血症はその発症要因も表現型も多彩であり，そのリスク程度が極めて捉えにくい。しかしapoB-48など定量性のあるマーカーも最近検討され始めており，脂質異常症に伴う動脈硬化性疾患において残されたリスク(residual risk)に対する介入も徐々に開始されつつある。今後もレムナントの蓄積とそのメカニズムと有効な治療介入への検討を続ける必要がある。

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Review

Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation

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ABSTRACT

Familial hypercholesterolaemia (FH) is a dominantly inherited disorder present from birth that markedly elevates plasma low-density lipoprotein (LDL) cholesterol and causes premature coronary heart disease. There are at least 20 million people with FH worldwide, but the majority remain undetected and current treatment is often suboptimal. To address this major gap in coronary prevention we present, from an international perspective, consensus-based guidance on the care of FH. The guidance was generated from seminars and workshops held at an international symposium. The recommendations focus on the detection, diagnosis, assessment and management of FH in adults and children, and set guidelines for clinical purposes. They also refer to best practice for cascade screening and risk notifying and testing families for FH, including use of genetic testing. Guidance on treatment is based on risk stratification, management of non-cholesterol risk factors, and safe and effective use of LDL lowering therapies. Recommendations are given on lipoprotein apheresis. The use of emerging therapies for FH is also foreshadowed.

This international guidance acknowledges evidence gaps, but aims to make the best use of contemporary practice and technology to achieve the best outcomes for the care of FH. It should accordingly be employed to inform clinical judgement and be adjusted for country-specific and local health care needs and resources.

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Table 1
Summary of Recommendations.

1. Detection of Index cases: Screening and Phenotypic Diagnosis

1.1 Targeted, opportunistic and universal screening strategies should be employed to detect index cases [**2B**].
1.2 Index cases should be sought by targeted screening of adults with premature cardiovascular disease (CVD), primarily coronary heart disease (CHD) and a personal and/or family history of hypercholesterolaemia. [**1A**]
1.3 Opportunistic screening of adults and children in primary care, based on age- and gender-specific plasma LDL-cholesterol levels, should be routinely adopted. [**2B**]
1.4 Universal screening based on age- and gender- specific plasma LDL-cholesterol levels should be considered prior to age 20 years and ideally before puberty. [**2C**]
1.5 In adults, country-specific clinical tools, such as the Dutch Lipid Clinic Network, Simon Broome, MED-PED or Japanese FH criteria, may be used to make a phenotypic diagnosis. [**1A**]
1.6 The effect of acute illness and concurrent use of statins in lowering plasma LDL-cholesterol must be considered: testing for FH should not be carried out during acute illness; LDL-cholesterol level should be appropriately adjusted in people on statins, particularly if a reliable pre-treatment value is not available [**2A**]
1.7 All patients with suspected FH should be referred to a clinic specialising in lipidology and/or metabolic disorders for further assessment, if such a service is available. [**3A**]

2. Diagnosis and Assessment of Adults

2.1 Secondary causes of hypercholesterolaemia should first be excluded. [**1A**]
2.2 The most reliable diagnosis of FH can be made using both phenotypic (see **1.5** above and **4.8** below) criteria and genetic testing, but when genetic testing is not available the diagnosis can be made phenotypically. [**1A**]
2.3 DNA testing increases the accuracy of detecting FH and, if resources permit, should be considered to confirm the diagnosis, especially if cascade screening is planned; a fully accredited laboratory should be used. [**1A**]
2.4 Although FH is a life-time coronary risk equivalent, patients should be assessed for additional major cardiovascular risk factors, including lipoprotein(a) [Lp(a)], the level of hypercholesterolaemia at diagnosis and the prematurity of the family (especially first-degree relatives) or personal history of CVD. Framingham or other cardiovascular risk equations should not be used. [**2A**]
2.5 The presence of additional cardiovascular risk factors should guide the intensity of medical management. [**2A**]
2.6 Cardiovascular imaging (eg. cardiac computed tomography and carotid ultrasonography) may be useful for assessing asymptomatic patients, but its value is not fully established. [**2C**]

3. Diagnosis and Assessment of Children and Adolescents

3.1 Secondary causes of hypercholesterolaemia should first be excluded. [**1A**]
3.2 With the exceptions noted in 3.3, children should be genetically tested for FH only after a pathogenic variant (mutation) has been identified in a parent or first degree relative. [**1A**]
3.3 Children may initially be genetically tested for FH when parents or first degree relatives are unknown or deceased, or as an accepted screening practice in certain countries, such as the Netherlands [**3B**]
3.4 Age-, gender- and country -specific plasma LDL-cholesterol concentration thresholds should be used to make the phenotypic diagnosis; because of biological variation, two fasting LDL-cholesterol values are recommended.[**1B**]
3.5 A plasma LDL-cholesterol of 5.0 mmol/L or above indicates high probability of FH in the absence of a positive parental history of hypercholesterolaemia or premature CHD; an LDL-cholesterol of 4.0 mmol/L or above indicates high probability of FH in the presence of a positive parental history of hypercholesterolaemia or premature CHD [**1B**]
3.6 Patients should be risk stratified according to age, presence of other cardiovascular risk factors, family history of early onset CVD (especially in first-degree relatives) and the level of LDL-cholesterol at diagnosis. [**2A**]
3.7 The presence of additional cardiovascular risk factors, and hence risk stratification, should guide the intensity of medical management. [**3A**]
3.8 Carotid ultrasonography may be employed to assess risk, but its value is not fully established; it should only be carried out in centres with specific expertise. [**2C**]
3.9 Cardiac CT should not be used routinely to assess patients with heterozygous FH. [**3A**]

4. Cascade Screening: Testing and Risk Notification of Families

4.1 Notification of relatives at risk of FH should generally not be carried out without the consent of the index case. [**3A**]
4.2 Relatives should only be directly notified of their risk without consent of the index case if there is specific legislative provision for breach of confidentiality in the relevant jurisdiction. [**3C**]
4.3 A proactive approach that respects the principles of privacy, justice and autonomy is required. [**3A**]
4.4 Pre-testing counselling should be offered to at risk family members of an index case prior to any form of testing. [**1A**]
4.5 Systematic cascade screening should ideally be co-ordinated by a dedicated centre and should not be carried out in primary care without central co-ordination, particularly if employing DNA testing. [**1B**]
4.6 Cascade screening of families should be carried out using both a phenotypic and genotypic strategy, but if DNA testing is not available a phenotypic strategy alone should be used [**1A**]
4.7 Cascade screening should initially be carried out as a priority in first-degree relatives and then extended to second- and third-degree relatives. [**1A**]
4.8 In the absence of genetic testing, the diagnosis of FH should be made in close relatives using age- , gender- and country- specific plasma LDL-cholesterol levels. Diagnostic clinical tools for index cases, such as the Dutch Lipid Clinic Network and Simon Broome criteria, should not be employed to make the diagnosis of FH in relatives [**1A**]
4.9 DNA testing makes cascade screening more cost-effective and should be employed to screen family members after the mutation is identified in the index case. [**1A**]
4.10 Children with xanthomata or other physical findings of homozygous FH, or at risk of homozygous FH should be screened as early as possible and definitely by 2 years of age. [**2A**]
4.11 Children with suspected heterozygous FH should be screened between the ages of 5 and 10 years; age at screening should be similar in boys and girls. [**2B**]

5. Genetic Testing

5.1 Genetic testing for FH should ideally be offered to all 'index cases' who have a phenotypic diagnosis of FH. [**3A**]
5.2 When the phenotypic diagnosis of FH is unlikely (e.g. by Dutch Lipid Clinic Network Criteria), genetic testing of the 'index case' need not be carried out. [**1C**]
5.3 Genetic testing for FH must be carried out in an accredited laboratory using standardised methods that target specific mutations and/or by exon-by-exon sequencing. [**1A**]
5.4 If genetic testing detects a variant, its significance as a pathogenic mutation, a previously reported variant of uncertain significance, a novel variant of uncertain significance or a benign (normal) variant needs to be assessed and recorded. [**1A**]
5.5 If genetic testing does not detect a variant, FH due to undetected mutations or mutations in untested genes cannot be excluded, particularly if the clinical phenotype is strongly suggestive of FH. [**1A**]

6. Management of Adults

6.1 All adult patients with FH must receive advice on lifestyle modifications and advice to correct all non-cholesterol risk factors should be provided according to expert recommendations. [**2A**]
6.2 Therapy should ideally aim for at least a 50% reduction in plasma LDL-cholesterol, followed by an LDL-cholesterol < 2.5 mmol/L (absence of CHD or other major risk factors) and < 1.8 mmol/L (presence of CHD or other major risk factors). [**2C**]
6.3 Achieving these targets will require a fat-modified, heart-healthy diet and statin therapy with or without ezetimibe. [**1A**]
6.4 Drug combinations including bile acid sequestrants, niacin, probucol or fibrates, may be required with more intensive strategies to further reduce LDL-cholesterol. [**1B**]
6.5 Plasma levels of hepatic aminotransferases, creatine kinase, glucose and creatinine should be measured before starting drug therapy. All patients on statins should have hepatic aminotransferases monitored; creatine kinase should be measured when musculoskeletal symptoms are reported; glucose should be monitored when there are risk factors for diabetes. [**2A**]

Table 1 (*continued*)

6. Management of Adults

6.6 All women of child-bearing age should receive pre-pregnancy counselling, with appropriate advice on contraception, before starting a statin and this should be reinforced at annual review. [**2A**]
6.7 Statins and other systemically absorbed lipid regulating drugs should be discontinued 3 months before planned conception, as well as during pregnancy and breast feeding. [**2A**]
6.8 Although carotid ultrasonography has been used in clinical trials, its role in monitoring therapy as part of the clinical care for FH has not been established and it should therefore not be used at present for this purpose. [**3C**]
6.9 Lomitapide and Mipomersen should be considered as adjunctive treatments to diet and cholesterol lowering drugs in adults with homozygous FH to further reduce plasma LDL-cholesterol, particularly if lipoprotein apheresis is not available. [**1C**]
6.10 Well controlled and low complexity patients should be followed-up in primary care, whereas higher complexity patients will need regular review by a specialist, with the option of shared care. Review intervals should vary according to clinical context. Opportunities should be created for integrating the primary and specialist care of FH. [**3B**]

7. Management of Children and Adolescents

7.1 Patients must receive advice on lifestyle modifications and on correcting non-cholesterol risk factors; primordial prevention (counselling to inhibit the development of risk factors) is particularly important. [**2A**]
7.2 To lower elevated plasma LDL-cholesterol in this age group generally requires a fat-modified, heart-healthy diet and a statin, with the possible addition of ezetimibe or a bile acid sequestrant. [**1A**]
7.3 All patients should be treated with diet, with statins considered at age 8 to 10 years and ideally started before age of 18 years; plasma LDL-cholesterol targets in this age group need not be as intense as for adults [**2B**]
7.4 Boys and girls should generally be treated at similar ages, although with a particularly adverse family history of CHD and other major risk factors, boys with heterozygous FH could be considered for earlier treatment with statins. [**2B**]
7.5 Children, between the ages of 8 and 10 years, with proven FH on a suitable diet and LDL-cholesterol > 4.0 mmol/L on two occasions should be started on low-dose statin mono therapy, aiming for an LDL-cholesterol < 4.0 mmol/L.[**3C**]
7.6 After the age of 10 years, children with proven FH on a suitable diet and LDL-cholesterol > 3.5 mmol/L on two occasions should be started on statin monotherapy, aiming for an LDL-cholesterol < 3.5 mmol/L, with the addition of ezetimibe or a bile acid sequestrant if required. [**3C**]
7.7 The preferred statins for initiating therapy are those that are licensed for clinical use in this age group in specific countries; other statins may be prescribed according to clinical indications, higher doses of potent statins being required in homozygotes. [**1C**]
7.8 Although statins can be safely used in children, weight, growth, physical and sexual development, and well-being should be monitored in this age group. [**1A**]
7.9 Plasma levels of hepatic aminotransferases, creatine kinase, glucose and creatinine should be measured before starting drug therapy. All patients on statins should have hepatic aminotransferases monitored; creatine kinase should be measured and compared with pre-treatment levels when musculoskeletal symptoms are reported; glucose should be monitored if there are risk factors for diabetes. [**2A**]
7.10 All adolescent girls should receive pre-pregnancy counselling, with appropriate advice on contraception before starting a statin and this should be reinforced at annual review. [**3A**].
7.11 Although carotid ultrasonography has been used in clinical trials, its role in monitoring therapy in patients with heterozygous FH has not been established and it should therefore not be used for this purpose. [**3C**]
7.12 Well controlled and lower complexity patients should be followed up in primary care, whereas higher complexity patients will need regular review by a paediatrician. Opportunities should be created for integrated care between GPs and paediatricians. Family based and transitional care clinics should be considered by adult and paediatric services. [**3B**]
7.13 Children with homozygous FH should be referred on diagnosis to a specialist centre and drug and/or apheresis treatment commenced as soon as possible. [**2A**]
7.14 In children with homozygous FH and rapidly progressive atherosclerosis, Lomitapide and Mipomersen, although not yet tested in children, should be considered, employing special access or compassionate use schemes, as adjunctive treatments to diet and conventional drugs to further reduce plasma LDL-cholesterol, particularly if apheresis is not available or declined by the patient/family. [**3C**]

8. Lipoprotein apheresis and related treatments

8.1 Lipoprotein apheresis (LA) should be considered in all patients with homozygous or compound heterozygous FH (i.e. homozygous FH phenotype) and carried out in a dedicated centre with the relevant expertise. [**1A**]
8.2 LA should be considered in patients with heterozygous FH with CHD who cannot achieve LDL-cholesterol targets despite maximal drug therapy or because they cannot tolerate statins. [**2A**]
8.3 LA should be considered in children with homozygous FH by the age of 5 and no later than 8 years. [**2A**]
8.4 Diet and drug therapy to lower LDL-cholesterol should be continued during treatment with LA [**2A**].
8.5 The efficacy, tolerability and safety of LA must be regularly reviewed. [**3A**]
8.6 The effect of LA on progression of atherosclerosis should be monitored according to clinical indications in FH patients with echocardiography (aortic valve and root), carotid ultrasonography and exercise stress testing. [**3B**]
8.7 Lomitapide should be considered as an adjunctive to standard diet and drug therapy to further lower plasma LDL-cholesterol in adults with homozygous FH on LA. [**1C**]
8.8 Lomitapide should be considered, via a special access scheme, as an adjunctive treatment to further lower plasma LDL-cholesterol in children and adolescents with homozygous FH on LA with rapidly progressive atherosclerosis. [**3C**]
8.9 Mipomersen should be considered as an adjunctive to standard diet and drug therapy to further lower plasma LDL-cholesterol in adults, children and adolescents with homozygous FH on LA who cannot tolerate lomitapide. [**3C**]
8.10 If available, orthotopic liver transplantation should be considered for younger patients with homozygous FH who have rapid progression of atherosclerosis or aortic stenosis, cannot tolerate LA or when plasma LDL-cholesterol cannot be adequately lowered with LA, diet and drug treatment. [**3B**]

9. Organization and Development of Care

9.1 Care pathways for FH should be developed for country-specific and local needs. [**3A**]
9.2 Specialist services should be multidisciplinary based and integrated with primary care. [**3B**]
9.3 Specialist care of FH should ideally be supported by cardiology, paediatric, genetic, imaging, transfusion medicine, nursing, dietetic, psychology, pharmacy and pathology laboratory services. [**3A**]
9.4 Cascade screening should ideally be centrally co-ordinated by a dedicated centre. [**1A**]
9.5 Low complexity patients should be managed in primary care, with the option of annual specialist review. [**3A**]
9.6 Higher complexity patients should be managed principally in specialist centres. [**3A**]
9.7 Medical, nursing and allied health staff managing patients with FH should be accredited in cardiovascular prevention. [**3A**]
9.8 Services should establish partnerships with academic and professional organizations to enhance teaching, training and research. [**3A**]
9.9 A registry of patients and families should be established for clinical, research and audit purposes. [**3A**]
9.10 A support group of patients and families should be established as a major priority for enhancing public, government and health care provider awareness, as well as the total quality of care of FH. [**3A**]

Endorsement

The recommendations contained in this document have been fully endorsed by The National Lipid Association, 6816 Southpoint Parkway (Suite 1000), Jacksonville, FL3316, US.

Box

Levels of evidence and grades of recommendation.

Levels of evidence
1 = systematic review/meta-analysis/at least one randomized control trial/good quality diagnostic tests.
2 = good quality clinical or observational studies.
3 = expert opinion or clinical experience/argument from first principles.
(The evidence for therapeutic interventions was considered principally in respect of effects on plasma LDL-cholesterol concentrations, but where available was also based on data on subclinical atherosclerosis or cardiovascular outcomes.)

Grades of recommendation

A = can be trusted to guide practice.
B = can be trusted to guide practice in most situations.
C = can be used to guide practice, but care should be taken in application.

Abbreviations and conversion factors

Abbreviations

ApoB, apolipoprotein B-100; CAC, coronary artery calcification; CHD, coronary heart disease; CK, creatine kinase; CIMT, carotid intima-medial thickness; CT, computerized tomography; CVD, cardiovascular disease; DLCNC, Dutch Lipid Clinic Network Criteria; DLCNS, Dutch Lipid Clinic Network Score; DRG, Diagnosis-related Groups; FDA, Food and Drug Administration; FH, familial hypercholesterolaemia: GP, general practitioner; HDL, high-density lipoprotein; ICD, International Classification of Diseases; LA, lipoprotein apheresis; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; Lp(a), Lipoprotein (a); Mabs, monoclonal antibodies; MEDPED, make early diagnosis to prevent early death; MHT, menopausal hormone therapy; MPLA, multiplex ligation probe amplification; MTP, microsomal triglyceride transfer protein; PCSK9, proprotein convertase subtilisin-like kexin Type 9; REMS, risk evaluation mitigation strategy; VLDL, very low-density lipoprotein.

Conversion factors

mg/dL cholesterol = mmol/L × 38.7; mg/dL triglyceride = mmol/L × 88.6; mg/dL lipoprotein(a) = 0.0357 μmol/L.

1. Introduction

Familial hypercholesterolaemia (FH) is the most common dominantly inherited disorder in man [1]. FH is most frequently due to dominant, loss-of-function mutations in genes affecting the low-density lipoprotein (LDL) receptor that clears LDL particles from plasma [2], and therefore LDL-cholesterol levels are markedly elevated from birth. FH accelerates atherosclerotic cardiovascular disease (CVD), especially coronary heart disease (CHD), with clinical manifestations often occurring after one to four decades of life [3,4]. Screening allows early detection of individuals [5–9], thereby allowing use of preventive interventions including lifestyle measures, cholesterol-lowering medications and management of other CVD risk factors [6–16]. There are probably more than 15 million people with FH worldwide, but less than 10% have been detected and only 5% adequately treated [3,4,17,18].

To address this major gap in coronary prevention, the International FH Foundation facilitated a series of discussions with experts to develop harmonized guidance for the care of FH. Formal presentations and informal discussions took place at the XVI International Symposium on Atherosclerosis (Sydney, 2012) in workshops that addressed evidence for treatment, screening and DNA testing, paediatric management, novel therapies, health economics, regional diversity in management, and models of care. Workshop moderators identified and collated consensus based on published research, clinical experience, common themes, expert opinion, and other international guidelines on FH [6–9,19–22]. To supplement this process a brief questionnaire on potentially contentious issues in FH (screening options, DNA testing, risk stratification, testing and treatment of children, use of imaging, and therapeutic targets) was then completed by a selected group of international experts, with the majority view employed to inform further consensus. This international guidance presents a standard of care for FH patients within a framework that can be adjusted for country-specific, regional and local requirements, and within which future evidence and consensus may be developed [23].

2. Detection of index cases: screening for FH

A systematic strategy for detecting index cases (i.e. first individuals diagnosed in families) of FH is essential [6–9]. The index case is the trigger for cascade screening, whereby new cases can be efficiently discovered [3,8,9]. Both screening methods need to be well integrated in models of care [8]. Universal screening prior to age 20 years and ideally before puberty, based on age- and gender-specific plasma LDL-cholesterol levels, should also be considered if feasible [9,21]. However, experience concerning its use and implementation is very limited. From a population perspective, universal and cascade screening methods for FH should be closely integrated [24]. The success of all detection strategies will depend on adequately addressing several barriers, including population awareness of FH and family, physician and societal concerns of the value of screening for FH.

Potential index cases of FH should be sought amongst patients aged less than 60 years with CVD presenting to coronary care, stroke, cardiothoracic and vascular units [8,25], as well as amongst similar patients attending cardiac rehabilitation programmes. The greatest yield will be from screening younger adult patients with CHD [25,26]. A coronary event in a family member can increase the perception of risk and the willingness of relatives to be subsequently tested for FH. Screening in primary care should employ an initial non-fasting lipid profile [8], which should be undertaken opportunistically, based on family history of hypercholesterolaemia and premature CVD (age < 60 years) [27,28]. FH screening should also be offered to all patients with tendon xanthomata and premature arcus cornealis [6–9,22]. All forms of opportunistic screening should account for the effect of any acute illness in lowering plasma total and LDL-cholesterol [29], in which case lipid testing should be delayed or repeated at least 8 weeks after recovery. There is a role for community laboratories alerting primary care physicians about FH on the basis of a high plasma LDL-cholesterol (e.g. >5 mmol/L) or high total cholesterol (e.g. >7 mmol/L) [30]. When feasible, all patients with suspected FH should be referred to a specialist with expertise in FH for confirmation of the diagnosis [26,28,31]. All patients with homozygous FH (untreated LDL-cholesterol > 13 mmol/L; treated LDL-cholesterol > 7.5 mmol/L) [32] must be referred to the nearest specialist centre for management [8,31,33].

3. Diagnosis and assessment of adults

There are a number of criteria for diagnosing FH phenotypically in adults, but none are internationally agreed [6–9,22]. The Dutch Lipid

Clinic Network Criteria (DLCNC) are used to calculate a numerical score predicting the probability of the diagnosis of FH [34]. These criteria are increasingly accepted as simple and comprehensive [8,13,34]; the numerical score is not highly dependent on the plasma level of LDL-cholesterol and can be more sensitive in detecting index cases, with a score >5 making the diagnosis highly probable. The Simon Broome system is comparable to the DLCNC in predicting an FH mutation, but does not employ arcus cornealis and may also overlook patients with true FH who are not overtly hypercholesterolaemic. The MEDPED System, which relies on plasma total and LDL-cholesterol and strictly requires that cholesterol measurements be first known in other family members [35], may also be less specific in predicting mutations than other methods. The Japanese criteria, which are comparable to the Simon Broome system, employ a population-specific LDL-cholesterol >4.7 mmol/L and allow for a radiographic diagnosis of Achilles tendon xanthomata [22]. Further international research is required to establish simple and harmonized criteria for the clinical diagnosis of FH.

The diagnosis of FH should be based on at least two fasting measures of plasma LDL-cholesterol [8,10,13]. Obtaining the family history of CHD and hypercholesterolaemia is important to enhance the phenotypic diagnosis [6,26,34–36], but this information is often neglected or may not be available in practice [25,37]. The presence of tendon xanthomata in early life with marked elevation in plasma LDL-cholesterol establishes the diagnosis of severe FH [32], but sitosterolaemia should also be excluded with plasma phytosterol and DNA testing [38]; in adults, tendon xanthomata with normal plasma cholesterol may be seen in sitosterolaemia and cerebrotendinous xanthomatosis. Secondary causes of hypercholesterolaemia (e.g. hypothyroidism, proteinuria, medications) must be excluded [8,13,22], but the clinical stigmata of FH do not occur in these conditions. LDL-cholesterol is underestimated by the Friedewald equation if the plasma triglyceride level is >4.5 mmol/L [28], when a direct LDL-cholesterol assay should be employed. The levels of apoB and non-HDL-cholesterol (i.e. total cholesterol minus HDL-cholesterol) for diagnosing FH have not been defined. FH must be distinguished from familial combined hyperlipidaemia [22,39], a multigenic disorder with a variable lipid phenotype that co-expresses with insulin resistance and does not exhibit tendon xanthomata. Significant hypertriglyceridaemia makes the diagnosis of FH less likely, but may rarely be seen with co-existing genetic defects in lipoprotein metabolism [40,41]. For patients on cholesterol lowering medication, pre-treatment LDL-cholesterol values must be obtained or interpolated from the drug type and regimen used [42].

All patients diagnosed with FH should be investigated for other CVD risk factors [8,22,43,44] and the presence of symptomatic or subclinical atherosclerosis [7,8,45]. Assessment must take account of the psychological, intellectual, social and ethnic status of the patient [46,47]. Detailed and culturally appropriate exploration of the individual's family history of CVD, particularly amongst first-degree relatives, is essential. Risk of CVD amongst patients with FH can vary widely [43,44]. This may relate to the pre-treatment plasma level of cholesterol, genetic causes affecting lipid metabolism or arterial biology, and the presence of other major risk factors, in particular smoking, obesity, low HDL-cholesterol, hypertension and diabetes [43,44]. Mutations that very markedly elevate plasma LDL-cholesterol [48] and independently increase plasma Lp(a) concentrations also enhance the risk of CVD in FH [49]. Elevated Lp(a) may also contribute to the development of aortic stenosis [50]. Framingham Risk Scores, or scores derived from other cardiovascular risk engines, are not reliable to guide management in FH and should not be used [8,10,11,51], particularly in younger patients, in whom a measure of long-term risk based on imaging of subclinical atherosclerosis may be more appropriate [14,52,53]. An assessment of cholesterol-life years, or cumulative cholesterol burden, may also be useful in risk assessment [54], but its value in managing individual patients requires evaluation.

FH is associated with early onset and increased burden of subclinical atherosclerosis [53,54]. Certain measures of subclinical atherosclerosis have been independently associated with the onset of CHD in the general population [55,56], but have been generally applied to FH in research settings alone and require further evaluation. Increased carotid intima-medial thickness (CIMT) and the presence of plaques may be assessed by carotid ultrasonography [53,55,57]. Coronary artery calcification (CAC) and luminal obstruction can be assessed with cardiac computed tomography/angiography [45,55,58]. In asymptomatic individuals, CAC score may be superior to CT coronary angiography in risk prediction [59], and more clinically useful than CIMT [60]. Non-invasive testing for atherosclerosis could be useful in the assessment and management of FH, but this is not established. Testing could be individualized to specific clinical situations [58,61], being particularly useful when the family history of CVD is unclear [62]. Subclinical atherosclerosis should be defined according to recognized criteria [55]. Reference intervals for common CIMT have recently been reported [63], but have not been validated in FH. Echocardiography for aortic stenosis may be indicated for heterozygous individuals with marked elevation in plasma Lp(a) concentration [49,50], and as a routine investigation in homozygous FH to diagnose both aortic and/or supra-aortic valve disease [8,32,43–45,64].

Given that CVD risk and the clinical expression is variable in FH, it is reasonable to employ some form of risk stratification. Subdivision into lower (absence of CHD, subclinical atherosclerosis and major risk factors) and higher (presence of CHD, subclinical alterations or major risk factors) risk can guide the intensity of medical management [7,8]. The cost-effectiveness of this therapeutic approach requires evaluation, however. Patients considered to have a homozygous FH phenotype should be classified as exceptionally high risk [32], and be referred to a specialist centre for consideration of lipoprotein apheresis [65] or trials of new therapies [8].

4. Diagnosis and assessment of children and adolescents

FH should be identified in youth, certainly before 18 years of age and younger if indicated and feasible [19–21,54,66,67]. This is particularly important for those at risk for homozygous FH, where recognition at about 2 years or even earlier is considered optimal [19–21,54,66,67]. Sitosterolaemia may masquerade as homozygous FH in childhood and the diagnosis should be considered [38]. Boys and girls with potential heterozygous FH should also be screened before the age of 10 years, preferably between ages of 5 and 10 years. However, earlier screening may be justified with a family history of CHD prior to age 55 years, especially in first-degree relatives [64,68,69], or at the specific request of parents wishing to embed healthy lifestyle measures at a very early age. Detection of FH in childhood employing three strategies can be considered: cascade screening, universal screening, or selective screening based on family history [21,27,67]. Different countries will utilize different strategies based on health care resources and recommendations by local expert groups. In several European countries and Australia, cascade screening based on genetic testing has been advocated [5,6,8,19], whereas in the United States selective screening beginning at age 2 years and universal screening at age 9–11 years has been advocated [9,20]. Universal screening by age 20 years, and ideally before puberty, is recommended; universal screening has been practised in Slovenia [21] and tested in some universities in Japan, but the yield and cost-effectiveness remain unclear. Universal screening of children, followed by child–parent testing may be a more effective approach to detecting FH in the population than cascade screening alone [24]. Importantly, none of the aforementioned paediatric screening strategies for FH have hitherto been validated for efficacy, risk-benefit and cost-effectiveness [70].

Age- and gender-specific plasma LDL-cholesterol concentration thresholds should be used to make an initial diagnosis of FH [68,71]. Plasma LDL-cholesterol level alone has excellent discrimination between those with and without FH below the age of 10 years [68,71,72]. However, because of biological variation [29,73], the average of at least two fasting LDL-cholesterol levels should be used to make the diagnosis of