34. Kokubo Y, Iso H, Ishihara J, *et al.* Association of dietary intake of soy, beans, and isoflavones with risk of cerebral and myocardial infarctions in Japanese populations: the Japan Public Health Center-based (JPHC) study cohort I. Circulation 2007; 116:2553–2562.

35. Bazzano LA, He J, Ogden LG, *et al.* Dietary fiber intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. Arch Intern Med 2003; 163:1897–1904.

36. ■■ Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch Intern Med 2009; 169:659–669.

A systematic search of MEDLINE for prospective cohort studies or randomized trials showed a valid association of a limited number of dietary factors and dietary patterns with coronary heart disease.

37. Oh K, Hu FB, Cho E, *et al.* Carbohydrate intake, glycemic index, glycemic load, and dietary fiber in relation to risk of stroke in women. Am J Epidemiol 2005; 161:161–169.

38. Anderson JW, Tietyen-Clark J. Dietary fiber: hyperlipidemia, hypertension, and coronary heart disease. Am J Gastroenterol 1986; 81:907–919.

39. Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 1999; 69:30–42.

40. Marckmann P, Sandstrom B, Jespersen J. Effects of total fat content and fatty acid composition in diet on factor VII coagulant activity and blood lipids. Atherosclerosis 1990; 80:227–233.

41. Pereira MA, Pins JJ. Dietary fiber and cardiovascular disease: experimental and epidemiologic advances. Curr Atheroscler Rep 2000; 2:494–502.

42. Jenkins DJ, Axelsen M, Kendall CW, *et al.* Dietary fibre, lente carbohydrates and the insulin-resistant diseases. Br J Nutr 2000; 83 (Suppl 1):S157–S163.

43. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from National Health and Nutrition Examination Survey Data. J Nutr 2004; 134:1181–1185.

44. ■ Kokubo Y, Iso H, Saito I, *et al.* Dietary fiber intake and risk of cardiovascular disease in the Japanese population: the Japan Public Health Center-based study cohort. Eur J Clin Nutr 2011; 65:1233–1241.

A cohort study showed that higher total dietary fiber was associated with reduced risk of cardiovascular disease in Japanese nonsmokers.

45. Iso H, Kobayashi M, Ishihara J, *et al.* Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation 2006; 113:195–202.

46. Yamagishi K, Iso H, Date C, *et al.* Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women: the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. J Am Coll Cardiol 2008; 52:988–996.

47. Hooper L, Thompson RL, Harrison RA, *et al.* Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. Br Med J 2006; 332:752–760.

48. He K, Merchant A, Rimm EB, *et al.* Dietary fat intake and risk of stroke in male US healthcare professionals: 14 year prospective cohort study. Br Med J 2003; 327:777–782.

49. ■ Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, *et al.* Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. Am J Clin Nutr 2011; 93:158–171.

This dose–response meta-analysis of prospective studies indicates that milk intake is not associated with total mortality but may be inversely associated with overall cardiovascular disease risk; however, these findings are based on limited numbers.

50. Sauvaget C, Nagano J, Hayashi M, Yamada M. Animal protein, animal fat, and cholesterol intakes and risk of cerebral infarction mortality in the adult health study. Stroke 2004; 35:1531–1537.

51. Virtanen JK, Mursu J, Voutilainen S, Tuomainen TP. Serum long-chain n-3 polyunsaturated fatty acids and risk of hospital diagnosis of atrial fibrillation in men. Circulation 2009; 120:2315–2321.

52. Brouwer IA, Heeringa J, Geleijnse JM, *et al.* Intake of very long-chain n-3 fatty acids from fish and incidence of atrial fibrillation. The Rotterdam Study. Am Heart J 2006; 151:857–862.

53. Nakayama T, Date C, Yokoyama T, *et al.* A 15.5-year follow-up study of stroke in a Japanese provincial city. The Shibata Study. Stroke 1997; 28:45–52.

54. ■ Carlson SA, Fulton JE, Schoenborn CA, Loustalot F. Trend and prevalence estimates based on the 2008 Physical Activity Guidelines for Americans. Am J Prev Med 2010; 39:305–313.

In the 2008 Physical Activity Guidelines among US adults, little progress has been made during the past 10 years in increasing physical activity levels. There is much room for improvement in achieving recommended levels of physical activity among Americans, particularly among relatively inactive subgroups.

55. Physical Activity Guidelines Advisory Committee report, 2008. To the Secretary of Health and Human Services. Part A: executive summary. Nutr Rev 2009; 67:114–120.

56. Lee CD, Folsom AR, Blair SN. Physical activity and stroke risk: a meta-analysis. Stroke 2003; 34:2475–2481.

57. ■ Elsawy B, Higgins KE. Physical activity guidelines for older adults. Am Fam Physician 2010; 81:55–59.

This article provides information and guidance on the amount of physical activity recommended to maintain health and fitness for older adults in the US.

58. Nelson ME, Rejeski WJ, Blair SN, *et al.* Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007; 39:1435–1445.

59. Deplanque D, Masse I, Lefebvre C, *et al.* Prior TIA, lipid-lowering drug use, and physical activity decrease ischemic stroke severity. Neurology 2006; 67:1403–1410.

60. Noda H, Iso H, Toyoshima H, *et al.* Walking and sports participation and mortality from coronary heart disease and stroke. J Am Coll Cardiol 2005; 46:1761–1767.

61. Myint PK, Luben RN, Wareham NJ, *et al.* Combined effect of health behaviours and risk of first ever stroke in 20 040 men and women over 11 years' follow-up in Norfolk cohort of European Prospective Investigation of Cancer (EPIC Norfolk): prospective population study. Br Med J 2009; 338:b349.

Editorial Comment

The mutual exacerbation of decreased kidney function and hypertension

Yoshihiro Kokubo

See original paper on page 505

Hypertension is the strongest risk factors for cardiovascular disease worldwide [1,2]. The total population-attributable fractions of higher blood pressure for cardiovascular disease have been estimated as approximately 50% in men and 30% in women [3]. When high blood pressure levels and other risk factors, such as diabetes mellitus [4] or chronic kidney disease (CKD) [5], are combined, the risk of cardiovascular disease becomes much higher. The prevention of hypertension is the best way to prevent cardiovascular disease.

Recently, CKD has become a major public health problem and a risk factor for stroke and coronary heart disease [6]. In end-stage renal disease, the cardiovascular disease mortality rate is more than 10 times as high as that in the general population [7]. In asymptomatic general populations or outpatients, a severely or moderately decreased glomerular filtration rate (GFR) has been shown by most studies to be an independent risk factor for stroke and coronary heart disease [6]. Some studies have shown CKD as an independent risk factor for cardiovascular disease in low-risk or general population [5,8,9]. It is extremely important for the prevention of stroke that we maintain patients' renal function.

Reduced renal function is associated with increased levels of inflammatory factors [10,11], abnormal apolipoprotein levels [10], elevated plasma homocysteine [10], enhanced coagulability [11], anemia, left ventricular hypertrophy, increased arterial calcification, endothelial dysfunction, and arterial stiffness [12]. These factors may contribute to elevated blood pressure.

In prospective cohort studies, hypertension has been shown to be a risk factor for end-stage renal disease in both men and women [13,14]. The Multiple Risk Factor Intervention Trial study showed that elevations of blood pressure are a strong independent risk factor for end-stage renal disease [14]. Compared with optimal blood pressure, hypertension is a risk factor for end-stage renal disease, with adjusted relative risks (95% confidence intervals) for stages 1–4 hypertension of 3.1 (2.3–4.3), 6.0 (4.3–8.4), 11.2 (7.7–16.2), and 22.1 (14.2–34.3), respectively. Hypertensive patients (BP $\geq$ 160/95 mm Hg) have a five-fold greater decline in GFR (2.7 ml/min per 1.73 m^2/year), compared with patients with BP less than 140/90 mm Hg [15]. The Second Manifestations of Arterial Disease study shows that in the presence of albuminuria, higher blood pressure was associated with a greater GFR decrease ($\beta = 3.86$; 95% confidence intervals, 2.34–5.38 for hypertension presence) by a prospective vascular patients study [16]. In addition, the Systolic Hypertension in the Elderly Program study shows that the adjusted relative risk (95% confidence intervals) associated with the highest ($\geq$175 mmHg) compared with the lowest quartile (158–163 mm Hg) of SBP was 2.44 (1.67–3.56) for decline in kidney function [17]. Higher blood pressure is a risk factor for decline in kidney function.

There is no original article on the association between decreasing GFR and incident hypertension in general population. In this issue of the *Journal of Hypertension*, Takase *et al.* [18] studied whether GFR can predict incident hypertension in a normotensive general population ($n = 7684$). During the follow-up period (30 624 person-years), hypertension developed in 2031 participants (66.3 per 1000 person-years). The multivariable-adjusted hazard ratio of incident hypertension in the highest tertile (4.4–76.1 ml/min per 1.73 m^2) was 1.40 (95% confidence intervals 1.26–1.57) compared with the first tertile. A reduction in GFR of 10 ml/min per 1.73 m^2 was associated with an 11% increase in risk for incident hypertension. In addition, they investigated the impact of baseline GFR on yearly increases in SBP. The yearly increase in SBP significantly accelerated with a decreasing baseline GFR ($P < 0.01$). A reduction in GFR is a novel predictor of the onset of hypertension in a normotensive general population. Recently, the Suita Study has shown that CKD in the high–normal blood pressure category at the baseline survey was a risk factor for incident hypertension (multivariable-adjusted hazard ratio = 1.41) [19].

A higher level of blood pressure decreases renal function, and a decreased GFR raises blood pressure. In other

Journal of Hypertension 2012, 30:468–469

Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Suita, Japan

Correspondence to Yoshihiro Kokubo, MD, PhD, FAHA, FACC, FESC, FJSH, Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka, 565-8565 Japan. Tel: +81 6 6833 5012 x2176; fax: +81 6 6833 5300; e-mail: ykokubo@hsp.ncvc.go.jp

DOI:10.1097/HJH.0b013e328350ade8

words, increases in blood pressure and the decreases of renal function exacerbate each other. In the Suita Study, the risk of cardiovascular disease was higher in CKD patients with normal and high–normal blood pressure than in non-CKD individuals in the same blood pressure categories. In order to prevent cardiovascular disease, control of both blood pressure and renal function are important.

ACKNOWLEDGEMENTS

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kubo M, Hata J, Doi Y, Tanizaki Y, Iida M, Kiyohara Y. Secular trends in the incidence of and risk factors for ischemic stroke and its subtypes in Japanese population. *Circulation* 2008; 118:2672–2678.
2. Sjol A, Thomsen KK, Schroll M. Secular trends in blood pressure levels in Denmark 1964–1991. *Int J Epidemiol* 1998; 27:614–622.
3. Kokubo Y, Kamide K, Okamura T, Watanabe M, Higashiyama A, Kawanishi K, *et al.* Impact of high-normal blood pressure on the risk of cardiovascular disease in a Japanese urban cohort: the Suita study. *Hypertension* 2008; 52:652–659.
4. Kokubo Y, Okamura T, Watanabe M, Higashiyama A, Ono Y, Miyamoto Y, *et al.* The combined impact of blood pressure category and glucose abnormality on the incidence of cardiovascular diseases in a Japanese urban cohort: the Suita Study. *Hypertens Res* 2010; 33:1238–1243.
5. Kokubo Y, Nakamura S, Okamura T, Yoshimasa Y, Makino H, Watanabe M, *et al.* The relationship between blood pressure category and incidence of stroke and myocardial infarction in an urban Japanese population with and without chronic kidney disease: the Suita Study. *Stroke* 2009; 40:2674–2679.
6. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, *et al.* Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Hypertension* 2003; 42:1050–1065.
7. USRDS: the United States Renal Data System. *Am J Kidney Dis* 2003; 42(6 Suppl 5):1–230.
8. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; 351:1296–1305.
9. Ninomiya T, Kiyohara Y, Kubo M, Tanizaki Y, Doi Y, Okubo K, *et al.* Chronic kidney disease and cardiovascular disease in a general Japanese population: the Hisayama Study. *Kidney Int* 2005; 68:228–236.
10. Muntner P, Hamm LL, Kusek JW, Chen J, Whelton PK, He J. The prevalence of nontraditional risk factors for coronary heart disease in patients with chronic kidney disease. *Ann Intern Med* 2004; 140: 9–17.
11. Shlipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, *et al.* Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. *Circulation* 2003; 107:87–92.
12. Feldman HI, Appel LJ, Chertow GM, Cifelli D, Cizman B, Daugirdas J, *et al.* The Chronic Renal Insufficiency Cohort (CRIC) Study: design and methods. *J Am Soc Nephrol* 2003; 14 (7 Suppl 2):S148–S153.
13. Tozawa M, Iseki K, Iseki C, Kinjo K, Ikemiya Y, Takishita S. Blood pressure predicts risk of developing end-stage renal disease in men and women. *Hypertension* 2003; 41:1341–1345.
14. Klag MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, *et al.* Blood pressure and end-stage renal disease in men. *N Engl J Med* 1996; 334:13–18.
15. Vupputuri S, Batuman V, Muntner P, Bazzano LA, Lefante JJ, Whelton PK, *et al.* Effect of blood pressure on early decline in kidney function among hypertensive men. *Hypertension* 2003; 42:1144–1149.
16. Vlek AL, van der Graaf Y, Braam B, Moll FL, Nathoe HM, Visseren FL. Blood pressure and decline in kidney function in patients with atherosclerotic vascular disease: a cohort study. *Am J Kidney Dis* 2009; 54:820–829.
17. Young JH, Klag MJ, Muntner P, Whyte JL, Pahor M, Coresh J. Blood pressure and decline in kidney function: findings from the Systolic Hypertension in the Elderly Program (SHEP). *J Am Soc Nephrol* 2002; 13:2776–2782.
18. Takase H, Dohi Y, Toriyama T, Okado T, Tanaka S, Sonoda H, Kimura G. Evaluation of risk for incident hypertension using glomerular filtration rate in the normotensive general population. *J Hypertens* 2012; 30:505–512.
19. Kokubo Y, Nakamura S, Watanabe M, Kamide K, Kawano Y, Kawanishi K, *et al.* Cardiovascular risk factors associated with incident hypertension according to blood pressure categories in nonhypertensive population in the Suita Study: an urban cohort study. *Hypertension* 2011; 58:e132.

Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

EDITORIAL

Weight Reduction in Primary Care
– Comprehensive Dietary Counseling and the Use of Healthy Delivered "Bento (Lunch Boxes)" –

Yoshihiro Kokubo, MD, PhD

A dramatically increasing prevalence of obesity has been observed worldwide in recent decades.[1,2] Obesity is a risk factor for cardiovascular risk factors, such as hypertension, type 2 diabetes mellitus, and hyperlipidemia,[3,4] and as a consequence, obese persons have an increased risk of atherosclerosis,[5] stroke,[6] ischemic heart disease,[7] heart failure,[8] and mortality.[9] To prevent cardiovascular disease, obese persons must make lifestyle modifications to reduce their weight properly.

Article p1335

The behavior targets for obesity prevention are focused on healthy diet and physical activity.[10] The healthy diet consists of eating a lot of fruits, vegetables, whole grains, and nuts; limiting calories from added sugars, solid fats, and alcohol; and regulating energy intake rather than eating until the plate is empty. According to the Practical Guide – Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, successful weight reduction requires paying particular attention to the following topics: choosing a balanced diet comprising fats, carbohydrates, and proteins; evaluating nutrition labels to determine caloric content and food composition; giving priority to low-calorie foods; avoiding high-calorie ingredients during cooking (eg, fats and oils); avoiding overconsumption of high-calorie foods (high-fat and high-carbohydrate foods); drinking an adequate amount of water; reducing portion sizes; and limiting alcohol consumption.[11] In general, healthcare providers, especially physicians, nurses, and/or dietitians, conduct counseling for the prevention of obesity. There are 3 worthwhile reasons for talking to obese persons about lifestyle modification: (1) participants will understand that a healthy lifestyle is important; (2) an interview about current lifestyle habits opens the door to modifying these habits; and (3) patients may be more responsive to lifestyle modifications when the advice comes from a healthcare provider.[10]

Recently, a randomized controlled trial, the Practice-based Opportunities for Weight Reduction (POWER) trial, was performed to determine the effectiveness of 2 behavioral weight loss interventions. The target population consisted of obese adults (≥21 years of age) who had one or more cardiovascular risk factors. The first intervention provided patients with weight-loss support remotely by telephone, a study-specific Web site, and e-mail (remote support only group), and the other intervention provided in-person support during group and individual sessions, along with the 3 remote means of support (in-person support group). There was also a control group in which weight loss was self-directed (control group). The mean change in weight from baseline was significantly decreased by −4.6 kg and −5.1 kg in the group receiving remote support only and the in-person support group, respectively, compared with −0.8 kg in the control group.[12] Participants with obesity achieved and sustained clinically significant weight reduction over 1 year in the in-person support and in the remote support only group, where information was delivered remotely without face-to-face contact between participants and weight loss counselors for 2 years. Now that remote support coaching for weight-loss outcomes is similar to that of in-person visits, the use of mobile technologies to deliver behavioral weight-loss treatment appears to be useful in primary care. However, regardless of the amount of weight lost, it is challenging to maintain weight reduction for many years. Help with maintaining weight loss in the long term may be necessary.

There have been many previous clinical trials of calorie-restricted diets or formula food for weight loss, but there have been few original articles about the combination of delivered meals and dietary counseling in patients with hypertension and/or type 2 diabetes mellitus as an intervention study.[13,14] Troyer et al conducted a 1-year randomized controlled trial with 298 persons, among whom 50% received 7 Dietary Approach to Stop Hypertension (DASH) meals per week for 1 year. The DASH meals were found to increase compliance with dietary recommendations among noncompliant older adults with cardiovascular disease.[13] However, in that study, meals were not delivered daily to each individual's house. Individual meal delivery may be simpler and result in greater weight loss.

In a related work that appears in this issue of the Journal, Noda et al studied the effects of dietary counseling by registered dietitians and the use of delivered correctly calorie-controlled meals.[15] They recruited 200 patients with hypertension and/or type 2 diabetes mellitus who were randomly divided into 2 groups, with and without dietary counseling, and consumption of an ordinary diet for 1 month. Each group was then subdivided into 2 groups, with and without dietary counseling, and received calorie-controlled "bento (lunch boxes)" for the

Received April 25, 2012; accepted April 25, 2012; released online May 17, 2012
Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Suita, Japan
Mailing address: Yoshihiro Kokubo, MD, PhD, FACC, FAHA, FESC, FJSH, Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita 565-8565, Japan. E-mail: ykokubo@hsp.ncvc.go.jp
ISSN-1346-9843 doi:10.1253/circj.CJ-12-0554

following month. The combination of dietary counseling and delivered calorie-controlled meals was effective for weight reduction as well as alleviating cardiovascular risk factors. Comprehensive counseling that includes the delivery of healthy "bento" may be important for lifestyle modification. However, weight loss is mostly associated with weight regain and, as a result, may not be successful over a person's lifetime. Further comprehensive cross-over intervention studies are required to support the long-term prevention of obesity, along with the use of a validated nutritional survey to test interventional studies of counseling by dietitians and delivering proper calorie-controlled meals.

References

1. Caterson ID, Hubbard V, Bray GA, Grunstein R, Hansen BC, Hong Y, et al. Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: Group III: Worldwide comorbidities of obesity. *Circulation* 2004; **110:** e476–e483.
2. Kumanyika SK, Obarzanek E, Stettler N, Bell R, Field AE, Fortmann SP, et al; American Heart Association Council on Epidemiology and Prevention, Interdisciplinary Committee for Prevention. Population-based prevention of obesity: The need for comprehensive promotion of healthful eating, physical activity, and energy balance: A scientific statement from the American Heart Association Council on Epidemiology and Prevention, interdisciplinary committee for prevention (formerly the expert panel on population and prevention science). *Circulation* 2008; **118:** 428–464.
3. Saito I. Epidemiological evidence of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease in Japan. *Circ J* 2012; **76:** 1066–1073.
4. Kashihara H, Lee JS, Kawakubo K, Tamura M, Akabayashi A. Criteria of waist circumference according to computed tomography-measured visceral fat area and the clustering of cardiovascular risk factors. *Circ J* 2009; **73:** 1881–1886.
5. Lim S, Despres JP, Koh KK. Prevention of atherosclerosis in overweight/obese patients: In need of novel multi-targeted approaches. *Circ J* 2011; **75:** 1019–1027.
6. Furukawa Y, Kokubo Y, Okamura T, Watanabe M, Higashiyama A, Ono Y, et al. The relationship between waist circumference and the risk of stroke and myocardial infarction in a Japanese urban cohort: The Suita study. *Stroke* 2010; **41:** 550–553.
7. Cho E, Manson JE, Stampfer MJ, Solomon CG, Colditz GA, Speizer FE, et al. A prospective study of obesity and risk of coronary heart disease among diabetic women. *Diabetes Care* 2002; **25:** 1142–1148.
8. Komukai K, Minai K, Arase S, Ogawa T, Nakane T, Nagoshi T, et al. Impact of body mass index on clinical outcome in patients hospitalized with congestive heart failure. *Circ J* 2012; **76:** 145–151.
9. Tsugane S, Sasaki S, Tsubono Y. Under- and overweight impact on mortality among middle-aged Japanese men and women: A 10-yr follow-up of JPHC Study Cohort I. *Int J Obes Relat Disord* 2002; **26:** 529–537.
10. Cornier MA, Marshall JA, Hill JO, Maahs DM, Eckel RH. Prevention of overweight/obesity as a strategy to optimize cardiovascular health. *Circulation* 2011; **124:** 840–850.
11. National Institutes of Health, National Heart, Lung and Blood Institute, North American Association for the Study of Obesity. The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. NIH Publication Number 00-4084. October 2000. Available at: http://www.nhlbi.nih.gov/guidelines/obesity/prctgd_c.pdf (accessed May 8, 2008).
12. Appel LJ, Clark JM, Yeh HC, Wang NY, Coughlin JW, Daumit G, et al. Comparative effectiveness of weight-loss interventions in clinical practice. *N Engl J Med* 2011; **365:** 1959–1968.
13. Troyer JL, Racine EF, Ngugi GW, McAuley WJ. The effect of home-delivered Dietary Approach to Stop Hypertension (DASH) meals on the diets of older adults with cardiovascular disease. *Am J Clin Nutr* 2010; **91:** 1204–1212.
14. Imai S, Kozai H, Matsuda M, Hasegawa G, Obayashi H, Togawa C, et al. Intervention with delivery of diabetic meals improves glycemic control in patients with type 2 diabetes mellitus. *J Clin Biochem Nutr* 2008; **42:** 59–63.
15. Noda K, Zhang B, Iwata A, Nishikawa H, Ogawa M, Ninomiya T, et al on behalf of the STYLIST Study Investigators. Lifestyle changes through the use of delivered meals and dietary counseling in a single-blind study: The STYLIST Study. *Circ J* 2012; **76:** 1335–1344.

Effects of Public Education by Television on Knowledge of Early Stroke Symptoms Among a Japanese Population Aged 40 to 74 Years

A Controlled Study

Naomi Miyamatsu, RN, PhD; Kazumi Kimura, MD, PhD; Tomonori Okamura, MD, PhD; Yasuyuki Iguchi, MD, PhD; Hirofumi Nakayama, MD, PhD; Akihiro Toyota, MD, PhD; Makoto Watanabe, MD, PhD; Akiko Morimoto, RN, MSc; Miho Morinaga, RN, MSc; Takenori Yamaguchi, MD, PhD

Background and Purpose—An educational campaign by mass media has been associated with great increases in the knowledge about early symptoms of stroke. However, few studies were conducted with a controlled community intervention study.

Methods—To clarify the effects of a 1-year television campaign for the whole population on improvement of knowledge about stroke symptoms in 2 cities, a campaign area and a control area in Japan were selected. Before and after the campaign, 1960 randomly selected residents aged 40 to 74 years answered a telephone survey regarding knowledge of early stroke symptoms. We calculated the percentage and 95% CIs of participants who correctly chose all 5 early symptoms of stroke in each area and in each year.

Results—Before the campaign, 53% of participants (95% CI, 50%–55%) in the campaign area and 46% (95% CI, 44%–49%) in the control area correctly chose 5 early symptoms. After the 1-year television campaign, knowledge was significantly improved only in the campaign area (campaign area, 63%; 95% CI, 60%–66%; control area, 51%; 95% CI, 48%–54%). After sex stratification, only women showed improved knowledge of early symptoms. The audience rate for the campaign television programs was found to be higher in women than in men.

Conclusions–A 1-year stroke educational television campaign effectively improved knowledge about early stroke symptoms among Japanese women aged 40 to 74 years. No impact was found among men in this age group. Future studies should examine the impact of this approach on stroke knowledge among younger individuals and whether there are any behavioral changes that contribute to earlier presentation for treatment. **(*Stroke*. 2012;43:545-549.)**

Key Words: acute stroke ■ educational campaigns ■ knowledge ■ prevention ■ symptoms ■ warning signs

Delayed access to medical care in patients with stroke is associated with poor outcome. Knowledge of the early symptoms of stroke and the need to call an ambulance should therefore be widespread. The importance of ensuring timely treatment has grown dramatically since the introduction of thrombolytic treatment with tissue-type plasminogen activator[1–3] for cerebral infarction.

Various strategies for community education have been examined in previous studies.[4–8] Some reports have noted that television campaigns show greater efficacy for public education than other media.[4,6] However, few controlled studies have evaluated the effects of community education by television on knowledge about the early symptoms of stroke.[7] Furthermore, to our knowledge, there is no community education by television for stroke in Asian countries, where mortality due to stroke is high.[9]

The purpose of this study was to verify that television campaign could improve knowledge about early symptoms of stroke.

Methods

Study Setting

A community intervention providing information on early symptoms of stroke was conducted by television. The preintervention survey was performed in April 2009 and the postintervention survey was performed in April 2010. Because mortality of stroke varies between

Received August 8, 2011; final revision received August 26, 2011; accepted August 30, 2011.

From the Department of Clinical Nursing (N.M., A.M., M.M.), Shiga University of Medical Science, Shiga, Japan; the Department of Stroke Medicine (K.K., Y.I.), Kawasaki Medical School, Japan; the Department of Preventive Medicine and Public Health (T.O.), Keio University, Japan; the Japan Stroke Association (H.N., T.Y.), Japan; the Rehabilitation Centre (A.T.), Chugoku Rosai Hospital, Japan; the National Cerebral and Cardiovascular Center (M.W., T.Y.), Japan; and the Department of Mathematical Health Science (A.M.), Osaka University, Osaka, Japan.

Correspondence to Naomi Miyamatsu, RN, PhD, Department of Clinical Nursing, Shiga University of Medical Science, Seta Tsukinowa-cho 520-2192 Japan. E-mail miyan@belle.shiga-med.ac.jp

Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.111.634196**

Table 1. Exposure to Intervention During the Campaign Period Among Participants in the Campaign Area: Postintervention Telephone Survey 2010

	Exposure to Intervention, No. (%)			
		Sex Differential		
Educational Intervention by Television	Overall (n = 968)	Men (n = 484)	Women (n = 484)	*P**
1-min spots†	381 (39.8)	161 (33.3)	220 (45.5)	<0.001
Highlight programs‡	274 (28.3)	108 (22.3)	166 (34.3)	<0.001
Both of 1-min spots† and highlight programs‡	207 (21.4)	74 (15.3)	133 (27.5)	<0.001
At least 1 of 1-min spots† and highlight programs‡	447 (46.2)	195 (40.3)	252 (52.2)	<0.001

*P value for χ^2 test.

†One-min spots: approximately 900 times of TV spots about stroke, each airtime was 60 s.

‡Highlight programs: a total of 60 times of documentaries and reports about stroke, each airtime was 5–15 min.

western and eastern Japan, 2 cities were selected from adjoining prefectures located in western Japan: Okayama city in Okayama prefecture for the campaign area and Kure city in Hiroshima prefecture for the control area.

A local branch of Japan Broadcasting Corporation (NHK, the largest noncommercial broadcasting in Japan) produced a series of television programs for the present study and broadcast them throughout the 1-year campaign period from April 2009. Okayama city was located in the broadcasting area of this local branch (Okayama broadcasting station of NHK). Residents living in the control area had few chances to watch these educational contents, because contents of broadcasting of a local branch of NHK vary by prefectures, and 2 cities do not have a common border and are located far from each other (approximately 150 km).

Participants

Sample size was calculated based on our previous surveys without television programs.[10] The number of participants required was estimated to be 780 people for each area (α=0.05, β=0.8). We decided to recruit approximately 1000 people from both areas for each of pre- and postintervention surveys.

Potential participants were randomly selected from the telephone directory in each area in each survey. A telephone survey was then continued until 140 complete interviews had been obtained for both men and women in their 40s, 50s, and 60s; and 70 complete interviews had been obtained for both men and women at 70 to 74 years old. A total of 3920 citizens were surveyed to find 980 in the campaign area and 980 in the control area for each pre- and postintervention survey. Approximately two thirds of available contacts were nonrelevant contacts, representing contacts with individuals <40 years old or ≥75 years old. Because the population was aged 40 to 74 was 300 389 in the campaign area and 114 670 in the control area in 2009, the sampling rate was approximately 0.33% and 0.85%, respectively.

Community Education

Because television programs produced by NHK are systematically distributed, similar television programs are broadcast by all local branches of NHK. However, sometimes slots are at the discretion of the local branch, such as 1-minute spots before serial dramas or 15-minute slots for local news before national news programs. The television campaign in the present study was thus mainly performed using these time slots.

The major points of the campaign by television programs were as follows. The first point was to make broadcasting content based on accurate scientific evidence. The second point was to provide repeated audiovisual information, that is, 1-minute spots were broadcast at least twice almost everyday, whereas highlight programs were broadcast at least once a week. Both types of programs were continued throughout the study period from April 2009 to March 2010.

The Okayama broadcasting station for NHK, Kawasaki Medical School, and the Japan Stroke Association supervised the campaign programs. The 1-minute spots comprised a total of 10 versions covering stroke, both of early symptoms and risk factors, prevention, up-to-date medical treatments, and rehabilitation. Highlight programs featuring 33 topics were broadcast during the campaign period.

Main Outcome Measures

Participants were asked to choose which of 10 listed symptoms fit as early symptoms of stroke. The 10 symptoms listed consisted of 5 early symptoms of stroke[11] and 5 incorrect or atypical symptoms ("sudden nasal bleeding," "sudden hot flush," "sudden pain in the left shoulder," "numbness or palsy of both hands and/or fingers," and "sudden difficulty breathing").

At the postintervention survey in the campaign area, participants were also asked whether they had seen any of the television spots and special programs.

Statistical Analysis

We estimated 95% CIs of population proportions for those who correctly chose all 4 early symptoms of stroke in surveys according to F-distribution. Sex-specific analysis was also performed. Participants who chose all 10 symptoms (n=45) were excluded from these analyses.

Results

Response rates of telephone surveys were 31.6% and 34.7% for pre- and postintervention surveys in the campaign area and 30.3% and 35.5% in the control area, respectively. In the postintervention survey in the campaign area, approximately 40% of participants reported "I saw some of the 1-minute spots about stroke on NHK between April 2009 and March 2010," whereas 30% reported seeing the highlight programs (Table 1). These audience rates for both types of programs were significantly higher for women than for men.

Proportions of participants who correctly chose 5 early symptoms are shown in Table 2. In all groups, regardless of area or sex, we observed tendencies toward improvement in knowledge about early symptoms of stroke; however, 95% CIs of those proportions demonstrated that only the campaign area showed a significant improvement in stroke knowledge (Figure). After sex stratification, only women in the campaign area showed a significant improvement (Figure).

In addition, the participants who watched either program had better knowledge about early symptoms of stroke (age- and sex-adjusted ORs and 95% CIs, 1.41 and 1.07–1.86).

Table 2. Proportion of Participants Who Correctly Chose 5 Early Symptoms of Stroke

	Campaign Area		Control Area	
	Preintervention 2009	Postintervention 2010	Preintervention 2009	Postintervention 2010
Overall				
No. of participants	965	968	971	971
Correct answer about stroke symptoms (%)				
Sudden numbness or weakness of the face, arm, or leg	868 (89.9)	869 (89.9)	805 (82.9)	812 (83.6)
Sudden confusion or trouble speaking or understanding others	907 (94.0)	901 (93.1)	895 (92.9)	879 (90.5)
Sudden trouble seeing with 1 or both eyes	674 (69.8)	764 (78.9)	651 (67.0)	642 (66.1)
Sudden dizziness, walking difficulties, or loss of balance or coordination	806 (83.5)	815 (84.2)	756 (77.9)	787 (81.1)
Sudden severe headache with no known cause	810 (83.9)	821 (84.8)	773 (79.6)	812 (83.6)
No. of selected correct answer about stroke symptoms (%)				
None	24 (2.5)	41 (4.2)	34 (3.5)	47 (4.8)
1	13 (1.3)	14 (1.4)	33 (3.4)	33 (3.4)
2	43 (4.5)	23 (2.4)	44 (4.5)	35 (3.6)
3	83 (8.6)	59 (6.1)	129 (13.3)	89 (9.2)
4	293 (30.4)	222 (22.9)	283 (29.1)	273 (28.1)
5*	509 (52.7)	609 (62.9)	448 (46.1)	494 (50.9)
Men				
No. of participants	478	484	484	486
Correct answer about stroke symptoms (%)				
Sudden numbness or weakness of the face, arm, or leg	422 (88.3)	421 (87.0)	389 (80.4)	388 (79.8)
Sudden confusion or trouble speaking or understanding others	444 (92.9)	437 (90.3)	437 (90.3)	429 (88.3)
Sudden trouble seeing with 1 or both eyes	342 (71.5)	365 (75.4)	328 (67.8)	325 (66.9)
Sudden dizziness, walking difficulties, or loss of balance or coordination	386 (80.8)	386 (79.8)	350 (72.3)	373 (76.7)
Sudden severe headache with no known cause	399 (83.5)	400 (82.6)	364 (75.2)	399 (82.1)
No. of selected correct answer about stroke symptoms (%)				
None	14 (2.9)	28 (5.8)	23 (4.8)	28 (5.8)
1	7 (1.5)	12 (2.5)	25 (5.2)	19 (3.9)
2	23 (4.8)	14 (2.9)	23 (4.8)	24 (4.9)
3	50 (10.5)	30 (6.2)	65 (13.4)	47 (9.7)
4	130 (27.2)	121 (25.0)	138 (28.5)	134 (27.6)
5*	254 (53.1)	279 (57.6)	210 (43.4)	234 (48.1)
Women				
No. of participants	487	484	487	485
Correct answer about stroke symptoms (%)				
Sudden numbness or weakness of the face, arm, or leg	446 (91.6)	448 (92.6)	416 (85.4)	424 (87.4)
Sudden confusion or trouble speaking or understanding others	463 (95.1)	464 (95.9)	458 (94.0)	450 (92.8)
Sudden trouble seeing with 1 or both eyes	332 (68.2)	399 (82.4)	323 (66.3)	317 (65.4)
Sudden dizziness, walking difficulties, or loss of balance or coordination	420 (86.2)	429 (88.6)	406 (83.4)	414 (85.4)
Sudden severe headache with no known cause	411 (84.4)	421 (87.0)	409 (84.0)	413 (85.2)
No. of selected correct answer about stroke symptoms (%)				
None	10 (2.1)	13 (2.7)	11 (2.3)	19 (3.9)
1	6 (1.2)	2 (0.4)	8 (1.6)	14 (2.9)
2	20 (4.1)	9 (1.9)	21 (4.3)	11 (2.3)
3	33 (6.8)	29 (6.0)	64 (13.1)	42 (8.7)
4	163 (33.5)	101 (20.9)	145 (29.8)	139 (28.7)
5*	255 (52.4)	330 (68.2)	238 (48.9)	260 (53.6)

*This proportion was defined as "participants who have knowledge about early symptoms of stroke" in the present study.

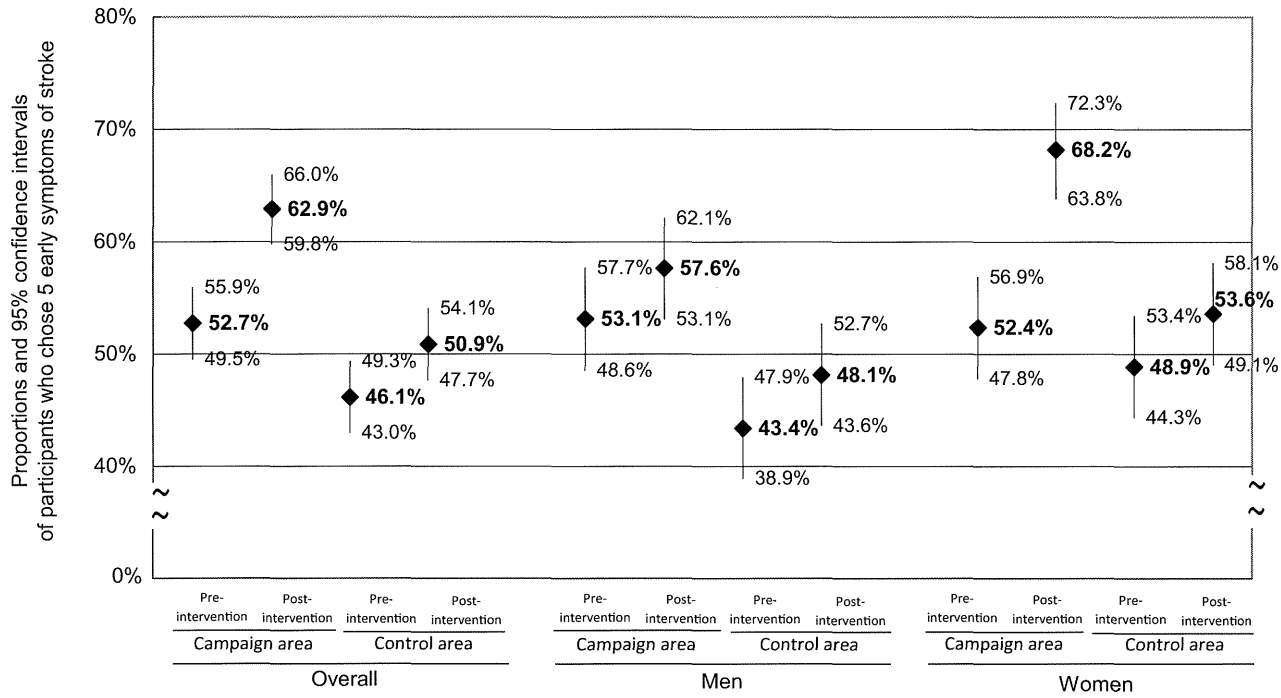

Figure. Overall and sex-stratified proportions and 95% confidence intervals (CIs) for participants who correctly chose 5 early symptoms of stroke before and after community education. Lozenge points indicate proportions of participants who correctly chose 5 early symptoms of stroke. Flickers indicate 95% CIs.

Discussion

This study is the first study of community education of stroke early symptoms in an Asian country. One advantage of the present study was the evaluation of the efficacy of television programs in the controlled trial with all participants randomly selected from the populations of the 2 areas. Another advantage was the use of a 1-year campaign, in which medically accurate contents were made by the collaboration of not only researchers and medical professionals, but also many staff from the largest noncommercial broadcasting corporation in Japan, that is, with mass media communication experts. As a result, this collaboration might have made the television programs more attractive for the audience, and many subjects reported that they had seen the 1-minute spots and the highlight programs during the campaign. In addition, our programs were repeated many times, which should have increased the likelihood of people seeing them, remembering them, and also remembering how to act if someone experiences early symptoms of stroke.

In previous studies that focused on public education about knowledge of stroke symptoms, the effectiveness of campaigns was assessed according to the ability to name ≥2 early symptoms of stroke without being shown multiple-choice items.[6,12] However, patients with stroke are unable to choose their own symptom at the time of onset, so people should be aware of all the typical early symptoms of stroke. Accordingly, the present study assessed improvements in knowledge about early symptoms of stroke based on the proportion of respondents who correctly chose all 5 early symptoms from a list of 10 symptoms.

We did not find significant improvements in knowledge about early symptoms of stroke among men. The improvement only in women may be explained by the greater exposure to television programs associated with the campaign, as suggested by the higher audience rates in women than in men. Furthermore, in previous studies of Western populations, knowledge about early symptoms of stroke was found to be better in women than in men during periods both with and without educational campaign.[12,13] Our results demonstrated not only similar sex differences to these previous studies, but also sex differences in the effects of the television campaign in a controlled trial. These results raise the possibility that men may have less general interest about health information compared with women. Therefore, it may be important to provide men various occasions to watch educational programs; for example, to increase a total number of on-air times, especially around programs that men are likely to watch such as sports, news, and action movies.

There are several limitations in the present study. First, we only evaluated the improvement in knowledge about early symptoms of stroke by broadcasting campaign; therefore, further study is necessary to assess its effectiveness in actual behaviors of patients with stroke; for example, the number of patients with stroke calling an ambulance, time from symptom onset to hospital presentation, how soon bystanders called the emergency center after having noticed early symptoms, and numbers of patients able to undergo therapy with tissue-type plasminogen activator should be evaluated. A previous cross-sectional study indicated that the knowledge about stroke symptoms was not associated with the intent to call 911 for stroke.[14] A gap may exist between the improvements in knowledge and actual changes in patient behavior. A second limitation is the lack of information about the costs involved in the campaign. The television programs were

made by NHK Okayama as its own project. Researchers thus did not need to worry about the costs of content production and broadcasting. Third, this study did not include individuals <40 years, who may be a person identifying a stroke onset of his or her family members and accessing the emergency medical services system. In addition, it is also important to distribute information about stroke to children and adolescent by television programs they are watching. They would probably advise their parents even if parents are not too interested about health information. This should be assessed in future studies.

Acknowledgments

We thank Mr Ken Goto and Ms Sakiko Yamada from NHK (Nippon Hōsō Kyōkai; Japan Broadcasting Corporation) for their excellent program production. Approval for this study was obtained from the institutional review board at Shiga University of Medical Science (No. 20-124, 2008).

Sources of Funding

This study was supported by grants from the Ministry of Health, Labor and Welfare (H19-Shinkin-Ippan-002) in association with the project prepared by the Japan Stroke Association and NHK Japan Broadcasting Corporation).

Disclosures

None.

References

1. The National Institute of Neurological Disorders and Stroke rtPA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* 1995;333:1581–1587.
2. Yamaguchi T, Mori E, Minematsu K, Nakagawara J, Hashi K, Saito I, et al. Alteplase at 0.6 mg/kg for acute ischemic stroke within 3 hours of onset: Japan Alteplase Clinical Trial (J-ACT). *Stroke.* 2006;37: 1810–1815.
3. Kimura K, Iguchi Y, Shibazaki K, Aoki J, Watanabe M, Matsumoto N, et al. Early stroke treatment with IV tPA associated with early recanalization. *J Neurol Sci.* 2010;295:53–57.
4. de Walle HE, van der Pal KM, de Jong-van den Berg LT, Jeeninga W, Schouten JS, de Rover CM, et al. Effect of mass media campaign to reduce socioeconomic differences in women's awareness and behaviour concerning use of folic acid: cross sectional study. *BMJ.* 1999;319: 291–292.
5. Luepker RV, Raczynski JM, Osganian S, Goldberg RJ, Finnegan JR Jr, Hedges JR, et al. Effect of a community intervention on patient delay and emergency medical service use in acute coronary heart disease: the Rapid Early Action for Coronary Treatment (REACT) Trial. *JAMA.* 2000;284: 60–67.
6. Silver FL, Rubini F, Black D, Hodgson CS. Advertising strategies to increase public knowledge of the warning signs of stroke. *Stroke.* 2003; 34:1965–1968.
7. Fogle CC, Oser CS, McNamara MJ, Helgerson SD, Gohdes D, Harwell TS. Impact of media on community awareness of stroke warning signs: a comparison study. *J Stroke Cerebrovasc Dis.* 2010;19:370–375.
8. Fortmann SP, Varady AN. Effects of a community-wide health education program on cardiovascular disease morbidity and mortality: the Stanford Five-City Project. *Am J Epidemiol.* 2000;152:316–323.
9. Ueshima H, Sekikawa A, Miura K, Turin TC, Takashima N, Kita Y, et al. Cardiovascular disease and risk factors in Asia: a selected review. *Circulation.* 2008;118:2702–2709.
10. Okamura T, Miyamatsu N, Nakayama H, Higashiyama A, Morinaga M. Effects of public education about stroke. In: *Report of Health and Labor Science Research Grants (H19-Shinkin-Ippan-002).* Kawasaki Medical School; 2009:133–154.
11. American Stroke Association. Warning Signs. Available at: www.strokeassociation.org/STROKEORG/WarningSigns/Warning-Signs_UCM_308528_SubHomePage.jsp. Accessed November 2010.
12. Hodgson C, Lindsay P, Rubini F. Can mass media influence emergency department visits for stroke? *Stroke.* 2007;38:2115–2122.
13. Marx JJ, Klawitter B, Faldum A, Eicke BM, Haertle B, Dieterich M, et al. Gender-specific differences in stroke knowledge, stroke risk perception and the effects of an educational multimedia campaign. *J Neurol.* 2010; 257:367–374.
14. Fussman C, Rafferty AP, Lyon-Callo S, Morgenstern LB, Reeves MJ. Lack of association between stroke symptom knowledge and intent to call 911: a population-based survey. *Stroke.* 2010;41:1501–1507.

Original Article

Multicenter Study to Determine the Diagnosis Criteria of Heterozygous Familial Hypercholesterolemia in Japan

Mariko Harada-Shiba[1], Hidenori Arai[2], Tomonori Okamura[3], Koutaro Yokote[4], Shinichi Oikawa[5], Atsushi Nohara[6], Tomoo Okada[7], Takao Ohta[8], Hideaki Bujo[9], Makoto Watanabe[10], Akihiko Wakatsuki[11] and Shizuya Yamashita[12]

[1]Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan
[2]Department of Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan
[3]Department of Public Health and Preventive Medicine, Keio University, Tokyo, Japan
[4]Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan
[5]Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan
[6]Department of Lipidology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan
[7]Department of Pediatrics and Child Health, Nihon University School of Medicine, Tokyo, Japan
[8]Department of Child Health and Welfare (Pediatrics), Faculty of Medicine, University of the Ryukyu, Nishihara, Japan
[9]Department of Genome Research and Clinical Application, Chiba University, Graduate School of Medicine, Chiba, Japan
[10]Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Suita, Japan
[11]Department of Obstetrics and Gynecology, School of Medicine, Aichi Medical University, Aichi, Japan
[12]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan

Aim: Heterozygous patients of familial hypercholesterolemia (FH) are known to have a high risk of coronary artery disease (CAD). Early diagnosis and prompt treatment are necessary to prevent their CAD. In this study we tried to amend the Japanese diagnostic criteria of FH for general practitioners by examining each component of the current criteria.
Methods: A multicenter study was performed, which included 1356 dyslipidemic patients at 6 centers. Pretreatment demographic information including LDL-cholesterol (LDL-C), Achilles tendon thickness (ATT), family history of FH and premature CAD and the result of genetic analysis were analyzed.
Results: Of 1356 patients, 419 were diagnosed with FH by criteria in 1988, which were used as a golden standard. We tried to define FH according to 3 conventional major items, i.e., 1) LDL-C, 2) ATT and/or cutaneous nodular xanthomas (CX), 3) family history of FH and/or family history of premature CAD. We then determined the cutoff of LDL-C using the new criteria. When we used 180 mg/dL as the cutoff of LDL-C, 94.3% of FH patients and 0.85% of non-FH satisfied 2 or more criteria. When we used 190 mg/dL, 92.1% of FH and 0.85% of non-FH satisfied 2 or more criteria; therefore, we chose 180 mg/dL for the cutoff of LDL-C in the new criteria and proposed that the diagnosis of definite FH can be made if 2 or more criteria are satisfied.
Conclusions: We examined each component for the diagnosis of heterozygous FH in a multicenter study in Japan.

J Atheroscler Thromb, 2012; 19:1019-1026.

Key words; Diagnosis criteria, Familial hypercholesterolemia, LDL cholesterol, Achilles tendon thickness, LDL receptor

Address for correspondence: Mariko Harada-Shiba, Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565, Japan
E-mail: shiba.mariko.ri@mail.ncvc.go.jp
Received: April 9, 2012
Accepted for publication: June 26, 2012

Introduction

Familial hypercholesterolemia (FH) is a genetic disease caused by a mutation in genes related to low-density lipoprotein (LDL) metabolism. Heterozygous FH patients manifest high LDL cholesterol (LDL-C)

levels, skin and/or tendon xanthomas, and increased risk of premature coronary artery disease (CAD)[1]. High LDL-C levels are the first symptom that appears even from birth, while xanthomas on the Achilles tendon usually appear during or after the late 10s and CAD that determines the prognosis of FH patients appears during or after the third decade of life in men and the fifth decade in women[2-4]. Because morbidity and mortality of CAD in heterozygous FH are much higher than in the general population[1, 5-7], special attention should be paid to screen these patients and to prevent their atherosclerotic complications. For the diagnosis of FH, several criteria have been published throughout the world, including ours, reported in 1988[8]; however, appropriate diagnosis of FH by primary care physicians is not performed in general practice in Japan[9]. Therefore, it is very important to establish useful diagnostic criteria for primary care physicians to diagnose FH with high specificity and sensitivity.

Because FH patients are estimated to be more than 250,000, primary care physicians need to take care of most of them; therefore, the criteria should be as simple as possible for clinical usefulness and have high sensitivity and specificity. We have used diagnosis criteria for FH published in 1988 in Japan[8], which include hypercholesterolemia, presence of skin/tendon xanthoma and reduced LDL receptor activity as major items; however, it is difficult to measure LDL receptor activity in routine clinical practice and even lipid specialists do not measure its activity. Furthermore, it is not covered by Japan's health insurance system; therefore, it is necessary to make the current diagnostic criteria easy to use for general practitioners. Toward this end, we performed a multicenter collaborative study of 1397 patients with dyslipidemia.

Methods

Subjects

A total of 1397 patients with dyslipidemia, referred to the outpatient clinic of 6 hospitals (Kyoto University Hospital, Osaka University Hospital, Nippon Medical School Hospital, Chiba University Hospital, Kanazawa University Hospital, and National Cerebral and Cardiovascular Center Hospital), were the subjects to this study. Among these patients, 41 were excluded due to missing data. Consequently, 1356 patients with dyslipidemia were eligible for the present study. Most had been diagnosed with or without FH by lipid specialists at each hospital according to the criteria for FH reported in 1988, and genetic analysis was performed in 223 patients, some of whom were diagnosed with FH based only on mutations of the LDL receptor or PCSK9. The criteria were as follows: Major items included 3 items, (1) IIa or IIb phenotype at serum cholesterol level of 260 mg/dL or above; (2) Tendinous xanthoma or xanthoma tuberosum is present; (3) Reduced or abnormal receptor activity. Minor items included 3 items: (1) Xanthoma palpebratum; (2) Arcus juvenalis (<50 years); (3) Juvenile (<50 years) ischemic heart disease.

Table 1. Clinical characteristics of non-FH and FH patients in this cohort

	non-FH	FH	*p*
N	937	419	
Male (n, %)	453 (48.3%)	170 (42.7%)	<0.01
Age (y.o.)	58.3 ± 16.3	52.9 ± 18.6	<0.01
TC (mg/dL)	236 ± 53	339 ± 72	<0.01
LDL-C (mg/dL)	146 ± 46	257 ± 67	<0.01

Determination of Conventional Criteria for FH

In this study we tried to amend the current criteria. For the primary care setting, three major items, i.e. serum level of LDL-C, family history and specific physical findings of FH, were chosen as diagnostic items because all are easily assessed by general practitioners. Family history and specific physical findings were also separated in more detail. Finally, we set 5 items, (1) LDL-C, (2) specific physical findings: a) ATT, b) cutaneous nodular xanthomas (CX), (3) family history: a) family history of FH in 1st or 2nd degree relatives, b) family history of premature CAD in 1st or 2nd degree relatives. A family history of premature CAD was defined as having CAD before the age of 55 in males and 65 in females. First, we assessed the prediction for FH by the combination of physical findings and family history, and then we determined the cutoff point of LDL-C with the combination of the above-mentioned two items. LDL-C levels were calculated by the Friedewald formula. ATT levels were measured by X-ray according to the method previously described and determined as positive with 9 mm or more[10].

The data in the medical records of the patients were sent to the National Cerebral and Cardiovascular Center and examined. The study protocol was approved by the ethics committee of the National Cerebral and Cardiovascular Center (D#M20-25-2 for the multicenter trial and ID#M17-56-4 for the genetic analysis). The ethics committee of each hospital also approved the study protocol.

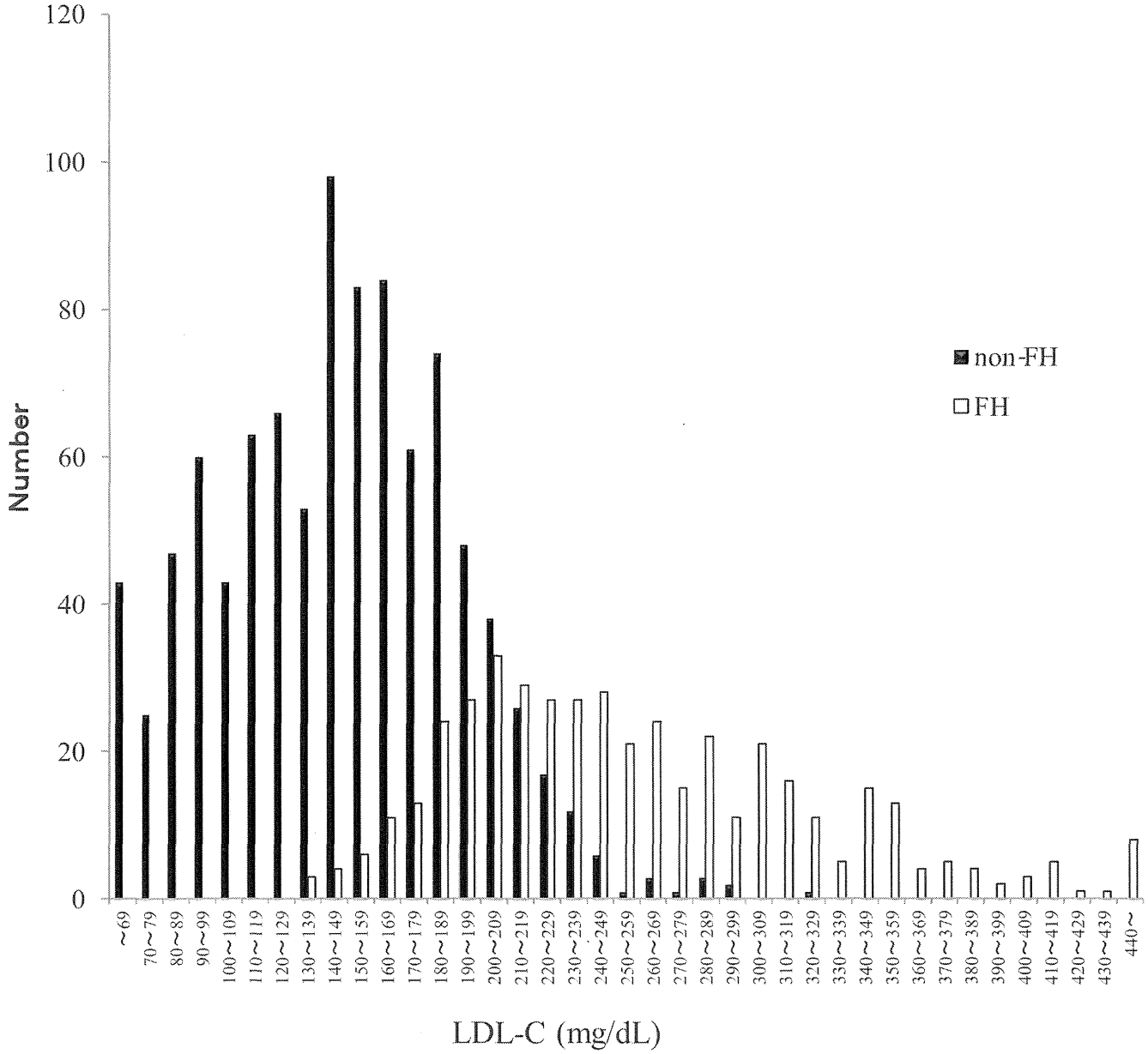

Fig. 1. Distribution of LDL-C levels before treatment in FH and non-FH patients. LDL-C levels were calculated by the Freidewald formula in patients with dyslipidemia diagnosed with FH or non-FH by specialists.

Statistical Analyses

Continuous variables are presented as the means ± SD. Categorical data are presented as numbers and percentages. Unpaired Student's *t*-test and one-way analysis of variance (ANOVA) were used to assess differences between groups in continuous variables. Differences in categorical variables were assessed by the χ^2 test.

Results

Among 1356 patients, 419 had been diagnosed with FH, while 937 with non-FH. Patient demographic data are shown in **Table 1**. FH patients were younger than non-FH patients. TC and LDL-C levels were 339 and 257 mg/dL in FH patients, respectively, and were significantly higher than in non-FH patients. The distribution of LDL-C levels in both groups is shown in **Fig. 1**. FH patients were divided into 3

Table 2. LDL-C levels in FH patients with or without genetic data

LDL-C (mg/dL)	FH (Total)	FH (Mut+)	FH (Mut−)	FH (no genetic data)	*p*-value
N	419	224	41	173	
Mean	257.4	266.2*	229.0*	252.9	
SD	67.39	69.85	60.14	63.70	
MEDIAN	244	253	216	241	0.003
IQ					
25%	205	213	189	203	
75%	300	308	244	295	

FH (Mut+): mutations in the LDL receptor or PCSK9, FH (Mut−): no mutations found, FH (no genetic data): no genetic analysis
*$p<0.005$ by Bonferroni

Table 3. Sensitivity and specificity in screening FH by physical findings and family history

	Specificity	Sensitivity
Physical findings		
ATT (+) (%)	98.6	64.1
CX (+) (%)	99.6	9.4
ATT (+) or CX (+) (%)	98.6	64.6
ATT(+) and CX(−)	99.6	11.7
Family history		
Family history of FH (+) (%)	93.6	98.2
Family history of CAD (+) (%)	96.3	28.3
Family history of FH (+) or CAD (+) (%)	91.7	98.7
Family history of FH (+) and CAD (+) (%)	98.2	27.4

ATT: Achilles tendon thickness, CX: Cutaneous nodular xanthomas
FH ($n=224$) was diagnosed by mutations in the LDL receptor and/or PCSK9. Non-FH ($n=937$) was diagnosed by specialists.

groups depending on their genetic data: FH with mutation(s) in LDL receptor or PCSK9, FH with no mutation(s) and FH with no genetic data. The mean and median of LDL-C along with SD and interquartile range of each group are shown in **Table 2**. LDL-C levels in FH with mutations were higher than those in FH without mutations.

We tried to define FH according to the screening standards as 3 major items, i.e., 1) LDL-C, 2) ATT and/or cutaneous nodular xanthomas (CX), 3) family history of FH and/or family history of premature CAD. We used LDL-C instead of total cholesterol, because LDL-C should better reflect the activity of the LDL receptor and is used for the goal of lipid management in the current Japanese guideline[8]. We incorporated "family history" as a major item because general practitioners were able to find FH by a family history of FH and/or premature CAD instead of LDL receptor activity. Sensitivity and specificity in screening FH by physical findings and family history are listed in **Table 3**. Based on these data, we decided to use 1) ATT or CX, and 2) family history of FH or CAD as 2 major items in addition to high LDL-C levels.

Next we tried to determine the cutoff levels of LDL-C. The percentage of the patients who satisfied each criterion according to LDL-C levels is listed in **Table 4**. Levels of 180 or 190 mg/dL are suggested as candidate cutoff levels. Therefore, the criteria for model 1 were set as those who satisfy 2 or more of the 3 criteria: 1) LDL-C 180 mg/dL or higher, 2) ATT (+) or CX (+), 3) Family history of FH or CAD, and for model 2, for which the cutoff point of LDL-C was changed to 190 mg/dl or higher, their sensitivity, specificity, and false positive and false negative rates were compared (**Table 5**). When we compared model 1 with model 2, higher sensitivity in model 1 than model 2 was obtained without any change in specificity, suggesting that 180 mg/dL is a better cutoff for LDL-C. The percentages were quite similar in FH with mutation (s) in LDL receptor or PCSK9, FH with no mutation (s) and FH with no genetic data. The diagnostic criteria of FH were then determined

Table 4. Percent satisfying each LDL-C level in non-FH and FH patients

	non FH	FH (All)	FH (Mut+)	FH (Mut−)	FH (No genetic data)
N	937	419	223	41	155
LDL-C ≥ 170 mg/dL (%)	30.5	94.5	96.0	85.4	94.8
LDL-C ≥ 180 mg/dL (%)	24.3	94.3	94.6	82.9	92.9
LDL-C ≥ 190 mg/dL (%)	16.6	92.1	93.7	75.6	89.7
LDL-C ≥ 200 mg/dL (%)	11.6	80.0	84.3	63.4	78.1

FH(Mut+): mutations in the LDL receptor or PCSK9, FH(−): no mutations found, FH (no genetic data): no genetic analysis

Table 5. Accuracy metrics of FH criteria using LDL-C cutoff levels of 180 or 190 mg/dL

	Sensitivity (%)	Specificity (%)	False positive (%)	False negative (%)
Model 1: Satisfying 2 or more of the following criteria: 1) LDL-C ≥ 180 mg/dL, 2) ATT(+) or CX(+), 3) Family history of FH or CAD	94.5	99.1	0.85	5.5
Model 2: Satisfying 2 or more of the following criteria: 1) LDL-C ≥ 190 mg/dL, 2) ATT(+) or CX(+), 3) Family history of FH or CAD	92.1	99.1	0.85	7.9

Table 6. Diagnostic criteria for adult (15 years or older) heterozygous FH

1	Hyper-LDL-cholesterolemia (LDL-C level before treatment: 180 mg/dL or more)
2	Tendon xanthoma (tendon xanthoma of the dorsal hands, elbows, and knees, or Achilles tendon thickening) or nodular xanthoma of the skin
3	Family history (relatives in the second degree): FH or premature CAD

-A diagnosis should be made after ruling out the possibility of secondary hyperlipidemia.
-Patients meeting 2 criteria should be regarded as having FH. Concerning those meeting 1 criterion, refer to Fig. 4. When FH is suspected, gene tests should be conducted to make a diagnosis.
-Nodular xanthoma of the skin does not include palpebral xanthoma.
-Patients with Achilles tendon thickening (9 mm or more) on radiography should be regarded as having xanthoma.
-When the LDL-C level is 250 mg/dL or more, FH should be strongly suspected.
-During drug therapy, the pretreatment lipid level should be employed as a reference value.
- CAD in males younger than 55 years old and females younger than 65 years old is defined as premature CAD.
-When a diagnosis of FH is made, the patient's family should also be investigated.
- LDL-C may be decreased after surgery, myocardial infarction, severe inflammation and so on. In these cases, LDL-C values before the diseases should be requested to give a diagnosis.
- To diagnose patients who have already been treated with statins, pretreatment levels of LDL-C should be requested; however, termination of statin treatment is not recommended to obtain pretreatment levels of LDL-C, even if the data are not available.

and are shown in **Table 6.**

Discussion

FH has the highest prevalence in genetic metabolic diseases, being heterozygous in one in 500 of the general population[1, 11]. Most young heterozygous FH patients have no symptoms other than high LDL-C levels, and those who have Achilles tendon thickness have no symptoms. The reason for undiagnosed FH patients to go to a clinic may be mainly divided into the following 4 situations: 1) a chance visit to a primary care physician due to flu or gastritis, etc., 2) recommendation of further medical examination due to high cholesterol at a health checkup, 3) transportation to the emergency room due to the development of acute coronary syndrome, 4) recommendation of medical consultation due to the presence of FH in his/

her family. The diagnostic criteria should be applied to these patients. Accordingly, conventional criteria are needed for the primary care setting.

Heterozygous FH patients show high levels of LDL-C, cutaneous and tendon xanthomas, and are complicated with myocardial infarction at young age by atherosclerosis due to intravascular exposure to high levels of LDL-C for many years. Because early diagnosis and treatment are recommended for these patients, the diagnostic criteria for FH have been reported in many countries including Japan[8, 12-17]. While some criteria give a satisfactory diagnosis of FH using specific items, others are adopting a scoring system. The Japanese criteria reported in 1988[8] were as follows. Major items included the following 3 items: (1) the patient shows the IIa or IIb phenotype at a serum cholesterol level of 260 mg/dL or above, in principle; (2) Tendinous xanthoma or xanthoma tuberosum is present; (3) Reduced or abnormal receptor activity is noted by LDL receptor analysis; however, for LDL receptor activity, even lipid specialists do not routinely measure activity. It would be even more difficult for primary care physicians to measure activity for the diagnosis of FH.

The cutoff level of serum cholesterol used in the first criterion in the criteria published in 1988 was 260 mg/dL; however, LDL-C is directly affected by dysfunction of the LDL receptor and is routinely measured in clinics by the direct method or Friedewald formula; therefore, we tried to use LDL-C as a cutoff level instead of total cholesterol. The presence of tendon and/or cutaneous nodular xanthomas was also used because of its convenience, high sensitivity and specificity. A family history of FH or premature CAD in 1st or 2nd degree relatives was proposed for the third criterion instead of measuring LDL receptor activity in the new diagnostic criteria. A family history of FH showed high sensitivity and specificity; however, primary care physicians may have difficulty obtaining this because it was not easy for them to reach a diagnosis of FH with the previous criteria. In the present study, accurate diagnosis of a family history of FH seemed to have been given because lipid specialists made the diagnosis at all the hospitals; however, the same result may not be applied to primary care physicians. Therefore, a family history of CAD, which may be easier to obtain, was added to the criteria. It should be noted that the sensitivity of a "family history of FH or CAD" was slightly higher than that of a "family history of FH". Accordingly, we chose a "family history of FH or premature CAD in 1st or 2nd degree relatives" as the third criterion.

The cutoff level of LDL-C for the diagnosis of FH should be set by its sensitivity and specificity in different cutoff points. The cutoff level of LDL-C for the diagnosis of FH was reported to be 190 mg/dL in Simon Broome[17], NICE[15] and 205 mg/dL in MEDPED[16]. In this study, 180 mg/dL was selected as the cutoff level together with the presence of xanthoma and the family history as the criteria for the diagnosis of FH because of its high sensitivity and specificity.

Reduced LDL receptor activity is direct evidence of FH and was used as one of the criteria in the previous version. Usually, LDL receptor activity is determined by the binding of fluorescent-labeled LDL to lymphocytes. The procedure of measuring LDLR activity is cumbersome and it is difficult to measure in routine clinical settings. Further, few companies can measure LDLR activity. Indeed, the specialists involved in this study measured LDLR activity only in 7 of 419 patients of FH, showing the sensitivity of the previous criteria as 60.9%. Therefore, in order to determine criteria sensitive enough to give a diagnosis of FH, the third item was changed from LDLR activity to family history.

There are some limitations in the present study. First, the patients analyzed in this study may have different characteristics from those followed by primary care physicians, because the physicians in this study are taking care of many FH patients and information about family history can be obtained more easily than by primary care physicians. Second, it is sometimes difficult for primary care physicians to take a complete family history, especially FH, and to diagnose ATT and/or the presence of CX, about which information can be missed in the primary care setting. Third, FH has been reported to have mutations in LDL receptor, PCSK9 and apolipoprotein B. Because mutations in PCSK9 may cause milder forms of FH, the sensitivity of the criteria may be reduced in these patients. Further study is required to address the applicability of the criteria for the primary care setting.

In conclusion, we have determined the cutoff of LDL-C for the diagnosis of FH by a multicenter study and proposed conventional diagnostic criteria by using high LDL-C, ATT and/or the presence of CX, and a family history of FH and/or CAD for primary care settings.

Acknowledgments

We thank Dr. Daisaku Masuda, Miyako Yuasa-Kawase (Osaka University), Mihoko Ogita (Kyoto University), Masa-aki Kawashiri (Kanazawa University) Minoru Takemoto (Chiba University), Taro Harada (Nippon Medical School) and Noriyuki Iwamoto

(National Cerebral and Cardiovascular Center) for their help with data acquisition and analysis. This work was supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Health, Labor and Welfare (H23-seisakutansaku-ippan-004 and Research on Measures for Intractable Diseases) and the Cardiovascular Research Foundation (Suita, Japan).

Disclosures

COI

Dr. Harada-Shiba
- Kowa Co. Ltd. – Honoraria
- MSD – Research Grants
- Kaneka Medix Co. Ltd. – Research Grants

Dr. Arai
- MSD – Honoraria
- Daiichi-Sankyo Co. Ltd. – Honoraria and Research Grants
- Kowa Co. Ltd. – Honoraria
- Otsuka Co. Ltd. – Research Grants

Dr. Okamura
- None

Dr. Yokote
- MSD K.K. – Honoraria
- Ono Pharmaceutical Co. Ltd. – Honoraria
- Pfizer Co. Ltd. – Honoraria
- Astellas Pharma Inc. – Honoraria
- Takeda Pharmaceutical Co. Ltd. – Honoraria
- Kowa Pharmaceutical Co. Ltd. – Honoraria
- Novartis Pharma K.K. – Research Grants
- MSD K.K. – Research Grants
- Takeda Pharmaceutical Co. Ltd. – Research Grants
- Daiichi-Sankyo Co. Ltd. – Research Grants
- Eli Lilly Japan K.K. – Research Grants
- Sanofi-Aventis K.K. – Research Grants
- Novo Nordisk Pharma Ltd. – Research Grants
- Pfizer Co. Ltd. – Research Grants
- Dainippon Sumitomo Pharma Co. Ltd. – Research Grants
- Kyowa Hakko Kirin Co. Ltd. – Research Grants
- Astellas Pharma Inc. – Research Grants
- Shionogi & Co. Ltd. – Research Grants

Dr. Nohara
- MSD – Research Grants
- Kaneka Medix Co. Ltd. – Research Grants
- Daiichi-Sankyo Co. Ltd. – Research Grants
- Shionogi & Co. Ltd. – Research Grants
- Kowa Co. Ltd. – Research Grants

Dr. Okada
- None

Dr. Ohta
- None

Dr. Bujo
- None

Dr. Watanabe
- None

Dr. Wakatsuki
- None

Dr. Oikawa
- Daiichi-Sankyo Co. Ltd. – Research Grants
- MSD – Research Grants

Dr. Yamashita
- MSD – Honoraria
- Kowa Co. Ltd. – Honoraria, Advisory Board
- Skylight Biotech Inc. – Advisory Board
- Astellas Pharma Inc. – Research Grants
- MSD – Research Grants
- Kissei Co. Ltd. – Research Grants
- Otsuka Co. Ltd. – Research Grants
- Shionogi & Co. Ltd. – Research Grants

References

1) Goldstein JL HH, Brown MS: Familial hypercholesterolemia. Edited by Scriver CR BA, Sly WS, Valle D, pp2863-2913, McGraw-Hill, New York, 2001
2) Bujo H, Takahashi K, Saito Y, Maruyama T, Yamashita S, Matsuzawa Y, Ishibashi S, Shionoiri F, Yamada N, Kita T: Clinical features of familial hypercholesterolemia in Japan in a database from 1996-1998 by the research committee of the ministry of health, labour and welfare of Japan. J Atheroscler Thromb, 2004; 11: 146-151
3) Mabuchi H, Koizumi J, Shimizu M, Takeda R: Development of coronary heart disease in familial hypercholesterolemia. Circulation, 1989; 79: 225-232
4) Harada-Shiba M, Sugisawa T, Makino H, Abe M, Tsushima M, Yoshimasa Y, Yamashita T, Miyamoto Y, Yamamoto A, Tomoike H, Yokoyama S: Impact of statin treatment on the clinical fate of heterozygous familial hypercholesterolemia. J Atheroscler Thromb, 17: 667-674
5) Mabuchi H, Miyamoto S, Ueda K, Oota M, Takegoshi T, Wakasugi T, Takeda R: Causes of death in patients with familial hypercholesterolemia. Atherosclerosis, 1986; 61: 1-6
6) Jensen J, Blankenhorn DH, Kornerup V: Coronary disease in familial hypercholesterolemia. Circulation, 1967; 36: 77-82
7) Stone NJ, Levy RI, Fredrickson DS, Verter J: Coronary artery disease in 116 kindred with familial type II hyperlipoproteinemia. Circulation, 1974; 49: 476-488
8) Teramoto T, Sasaki J, Ueshima H, Egusa G, Kinoshita M, Shimamoto K, Daida H, Biro S, Hirobe K, Funahashi T, Yokote K, Yokode M: Diagnostic criteria for dyslipidemia. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. Journal of atherosclerosis and thrombosis, 2007; 14: 155-158

9) Teramoto T: Status of lipid-lowering therapy prescribed on recommendations in the 2002 report of the Japan Atherosclerosis Society Guideline for Diagnosis and Treatment of Hyperlipidemia in Japanese Adults: A study of the Japan Lipid Assessment Program (J-LAP). Current Therapeutic Research, 2005; 66: 80-95
10) Mabuchi H, Ito S, Haba T, Ueda K, Ueda R: Discrimination of familial hypercholesterolemia and secondary hypercholesterolemia by Achilles' tendon thickness. Atherosclerosis, 1977; 28: 61-68
11) Mabuchi H, Nohara A, Noguchi T, Kobayashi J, Kawashiri MA, Tada H, Nakanishi C, Mori M, Yamagishi M, Inazu A, Koizumi J: Molecular genetic epidemiology of homozygous familial hypercholesterolemia in the Hokuriku district of Japan. Atherosclerosis, 2011; 214: 404-407
12) Watts GF, Sullivan DR, Poplawski N, van Bockxmeer F, Hamilton-Craig I, Clifton PM, O'Brien R, Bishop W, George P, Barter PJ, Bates T, Burnett JR, Coakley J, Davidson P, Emery J, Martin A, Farid W, Freeman L, Geelhoed E, Juniper A, Kidd A, Kostner K, Krass I, Livingston M, Maxwell S, O'Leary P, Owaimrin A, Redgrave TG, Reid N, Southwell L, Suthers G, Tonkin A, Towler S, Trent R: Familial hypercholesterolaemia: a model of care for Australasia. Atheroscler Suppl, 2011; 12: 221-263
13) Catapano AL, Reiner Z, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman MJ, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Perrone Filardi P, Riccardi G, Storey RF, Wood D: ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis, 2011; 217 Suppl 1: S1-44
14) Damgaard D, Larsen ML, Nissen PH, Jensen JM, Jensen HK, Soerensen VR, Jensen LG, Faergeman O: The relationship of molecular genetic to clinical diagnosis of familial hypercholesterolemia in a Danish population. Atherosclerosis, 2005; 180: 155-160
15) Wierzbicki AS, Humphries SE, Minhas R: Familial hypercholesterolaemia: summary of NICE guidance. BMJ, 2008; 337: a1095
16) Williams RR, Hunt SC, Schumacher MC, Hegele RA, Leppert MF, Ludwig EH, Hopkins PN: Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol, 1993; 72: 171-176
17) Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ, 1991; 303: 893-896

Atherosclerosis 225 (2012) 208–215

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

A multicenter study on the precision and accuracy of homogeneous assays for LDL-cholesterol: Comparison with a beta-quantification method using fresh serum obtained from non-diseased and diseased subjects

Takashi Miida [a,*], Kunihiro Nishimura [b], Tomonori Okamura [c], Satoshi Hirayama [a], Hirotoshi Ohmura [d], Hiroshi Yoshida [e], Yoh Miyashita [f], Masumi Ai [g], Akira Tanaka [h], Hiroyuki Sumino [i], Masami Murakami [i], Ikuo Inoue [j], Yuzo Kayamori [k], Masakazu Nakamura [l], Tsutomu Nobori [m], Yukihisa Miyazawa [n], Tamio Teramoto [o], Shinji Yokoyama [p]

[a] Department of Clinical Laboratory Medicine, Juntendo University Graduate School of Medicine, Tokyo, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
[b] National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan
[c] Department of Preventive Medicine and Public Health, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
[d] Department of Cardiology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
[e] Department of Laboratory Medicine, Jikei University Kashiwa Hospital, 163-1 Kashiwashita, Kashiwa, Chiba 277-8567, Japan
[f] Center of Diabetes, Endocrine and Metabolism, Toho University Sakura Hospital Medical Center, 564-1 Shimoshizu, Sakura, Chiba 285-0841, Japan
[g] Department of Life Sciences and Bioethics, Tokyo Medical and Dental University Graduate School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
[h] Laboratory of Clinical Nutrition and Medicine, Kagawa Nutrition University, 3-9-21 Chiyoda, Sakado, Saitama 350-0288, Japan
[i] Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan
[j] Department of Endcrinology and Diabetics, Saitama Medical University, 38 Morohongo Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[k] Department of Clinical Laboratory and Laboratory Medicine, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan
[l] Osaka Medical Center for Health Science and Promotion, CRMLN Lipid Reference Laboratory, 1-3-2 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan
[m] Department of Molecular and Laboratory Medicine, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan
[n] Department of Laboratory Science, Faculty of Medical Technology, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan
[o] Department of Internal Medicine, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan
[p] Nutritional Health Science Research Center and Food and Nutritional Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan

ARTICLE INFO

Article history:
Received 22 March 2012
Received in revised form
9 August 2012
Accepted 16 August 2012
Available online 24 August 2012

Keywords:
Direct LDL-C assay
Beta-quantification
Friedewald equation
Hypertriglyceridemia
Standardization
Lipoprotein assay

ABSTRACT

Background: Homogeneous assays for low-density lipoprotein-cholesterol (LDL-C) have good precision and are pretreatment-free procedures. However, their accuracies have been questioned, especially in diseased subjects. In this study, we aimed to verify whether LDL-C levels determined by homogeneous assays [LDL-C (H)] agree with those determined by a beta-quantification method [LDL-C (BQ)] in fresh clinical samples.

Methods: We determined LDL-C levels in 49 non-diseased and 124 diseased subjects whose triglyceride (TG) levels were less than 11.29 mmol/L (1000 mg/dL) using 12 homogeneous assays and a BQ method simultaneously.

Results: In total, 30.6% of non-diseased subjects and 46.0% of diseased subjects were in the postprandial state. The maximum inter- and intra-assay CVs were 1.8% and 1.5%, and 8 reagents had a CV of 1.0% or less. The mean bias ranged from −0.5% to 1.8% for non-diseased subjects and from −0.7% to 1.6% for diseased subjects. For non-diseased subjects, all but one reagent achieved the National Cholesterol Education Program (NCEP) total error requirement in more than 90% of samples. However, for diseased subjects, the number of reagents that met this requirement was low. With some reagents, LDL-C (H) was higher than LDL-C (BQ), especially in subjects with hypertriglyceridemia. While for other reagents, the difference between the two methods was not associated with hypertriglyceridemia except for type I ($n = 2$) and type III hyperlipidemia ($n = 1$). Postprandial sampling was not the main factor for discordant results.

Conclusions: LDL-C (H) agrees with LDL-C (BQ) in non-diseased subjects, but exhibits positive bias for subjects with hypertriglyceridemia in diseased subjects for some reagents.

* Corresponding author. Tel.: +81 3 5802 1104; fax: +81 3 5684 1609.
E-mail address: tmiida@juntendo.ac.jp (T. Miida).

0021-9150/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2012.08.022

1. Introduction

Epidemiological studies indicate that low-density lipoprotein-cholesterol (LDL-C) is an important risk factor for cardiovascular diseases (CVD) [1–3]. Furthermore, clinical trials have confirmed that LDL-C-lowering therapies reduce CVD [4,5]. Since guidelines use LDL-C levels as the cut-off values in managing hypercholesterolemia [6–8], LDL-C levels should be standardized. However, LDL-C measurement is more difficult than cholesterol measurement because LDL is converted from its precursor lipoproteins in the circulation. The Center for Disease Control and Prevention (CDC) adopted beta-quantification (BQ) as a reference method [9] for the international standardization program for LDL-C. This method separates lipoproteins using ultracentrifugation and precipitation of apolipoprotein B-containing lipoproteins. In clinical practice, physicians calculate LDL-C levels using fasting levels of total cholesterol, triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C) [10]. In 1997, a Japanese manufacturer developed an LDL-C homogeneous assay which requires no pretreatment [11]. Currently, 12 homogeneous assays are available in Japan. However, their accuracies were reported to be unsatisfactory, especially in diseased subjects including those with rare lipoprotein disorders who had very low LDL-C concentrations [12]. Since compositions and/or metabolism of LDL in such subjects are different from those in subjects with common dyslipidemia and normolipidemia [13], the accuracy of LDL-C homogeneous assays needs to be further evaluated using common clinical samples.

We aimed to clarify whether LDL-C levels determined by homogeneous assays [LDL-C (H)] agree with those determined by the BQ method [LDL-C (BQ)] in non-diseased and diseased subjects. We examined fresh blood samples from non-diseased and diseased subjects, who are commonly observed during a medical checkup and in clinical practice.

2. Methods

2.1. Study subjects

We enrolled Japanese men and women with and without disease at 7 departments across 6 institutions. We excluded subjects with rare lipoprotein disorders, severe systemic infections, and decompensated liver cirrhosis. Since reactivity to lipoprotein-X and lipoprotein-Y varies among reagents, we also excluded patients with cholestatic liver disease [14]. Furthermore, patients with TG levels >11.29 mmol/L (1000 mg/dL) were excluded because the chylomicron layer makes it hard to reproducibly aspirate serum samples through probes of automated analyzers.

We first recruited 178 subjects, and excluded 5 subjects due to severe hypertriglyceridemia. As a result, 173 subjects participated in this study. Written informed consent was obtained from all subjects. The study protocol was reviewed and approved by the ethics committees of all participating institutions.

2.2. Blood sampling and transport

We collected and processed fasting and non-fasting blood samples in a similar manner as the CDC LDL-C standardization program [15]. Serum was separated within an hour and pooled into a plastic tube. Aliquots were divided into screw-capped tubes and transported to SRL and Osaka Medical Center for Health Science and Promotion (OMC-HSP) using a cooling box with a refrigerant (Supplemental Fig. S1). Temperature was maintained at <4 °C for at least 24 h.

2.3. LDL-C determination

Within 24 h of blood collection, LDL-C levels were determined simultaneously by homogeneous assays and the BQ method. For homogeneous assays, the reagents, calibrators, and controls were provided by the manufacturers and distributors (Reagent-A, Denka Seiken; Reagent-B, Wako; Reagent-C, Sysmex; Reagent-D, Serotec; Reagent-E, Fureiya; Reagent-F, Kyowa Medex; Reagent-G, Toyobo; Reagent-H, Shino-Test; Reagent-I, Sekisui Medical; Reagent-J, Ortho Clinical Diagnostics; Reagent-K, Siemens Healthcare, and Reagent-L, Beckman Coulter). Nine reagents were used on Hitachi 917 (Tokyo, Japan). The other 3 reagents were provided along with analyzers made by their distributors because these reagents were permitted to be sold only with these analyzers (Supplemental Table S1). The operators were blinded to sample information.

LDL-C (H) levels were determined in triplicate. In Hitachi 917, LDL-C levels were determined after one cycle in a predetermined order, and this was repeated over 3 cycles. To avoid carryover effects, we used alkaline solution for washing cells, and acidic solution for washing the probes (Table S2). There were no condensation effects over the 3 cycles (21 min) (Table S3).

The BQ method was performed at OMC-HSP which has been a member of the Cholesterol Reference Method Laboratory Network (CRMLN: organized by CDC) for nearly 20 years. In principle, each serum was divided into 2 centrifuge tubes. After ultracentrifugation (18 °C, 105,000 × g, 18.5 h), d = 1.006 top fraction was removed by a tube slicer. The bottom fraction was transferred to a volumetric flask, and its volume was restored to the original volume. After precipitation of LDL with heparin-manganese solution, the supernatant was obtained by low-speed centrifugation. Cholesterol levels in the bottom and top fractions were determined in duplicate for each tube by the Abell–Kendall method [9,16].

In subjects with TG levels <4.52 mmol/L (400 mg/dL), we calculated LDL-C levels using the Friedewald's equation [LDL-C(F)] [10]. For this purpose, HDL-C level was determined by the homogeneous assay obtained from each manufacturer or distributor of LDL-C homogeneous assays.

2.4. Statistical analysis

A statistician received the entire data for LDL-C (H) and LDL-C (BQ), and analyzed them using the same methods of Miller et al. [12] and van Deventer et al. [17]. Briefly, total error (TE) was calculated using the first value of triplicate determinations for each sample [TE = (%bias) + 1.96 × (%CV)]. National Cholesterol Education Program (NCEP) requires that %bias, %CV, and TE are <4%, <4%, and <12%, respectively. Error component analysis is based on the hypothesis that analytical errors are derived from 3 components: inter-assay CVs, intra-assay CVs, and patient-specific errors. In the Bland-Altman plots [18,19], we used the absolute difference between LDL-C (H) and LDL-C (BQ). We give more detailed explanations of statistical procedures in Table S4.

3. Results

3.1. Subject characteristics

We collected 173 fresh blood samples from 49 non-diseased and 124 diseased subjects. In the whole subjects, 41.6% were non-fasted (Table S5). Among diseased subjects, there were type I (n = 2) and type III (n = 1) hyperlipidemia. Most dyslipidemic patients received lipid-lowering agents, especially statins. About 20%–30% of diseased subjects had CVD, diabetes, or fatty liver disease.