those of the original FRS. The HRs of smoking and DM for females were also higher than those of the original FRS (2.59 and 3.22, respectively). The HR of a TC between 200 and 239 for females was 0.58, which was lower than that of the FRS. The HRs of other variables were similar to those of the original FRS.

Prediction Model Development and the Simplified Prediction Model for Clinical Use

Table 3 shows the best Cox model for the Suita cohort selected by a stepwise method with the total cholesterol categories. (TC Suita score) The multivariable adjusted HR for the association between CHD and Stage 3 CKD was 1.39 and that for Stage 4 and 5 CKD was 3.72, respectively. The HRs of the other predictors were similar between the with CKD and without CKD models.

Table 4 shows the best Cox model for the Suita Score with CKD according to the cut-off levels of LDL-C and HDL-C proposed in the Japan Atherosclerosis Society (JAS) Guidelines for the Prevention of Atherosclerotic Cardiovascular Diseases 2012[11, 31] (LDL Suita Score). For convenient clinical use, we developed prediction sheets based on the TC and LDL Suita Scores (**Table 5**). The beta coefficients corresponding to the Cox model were multiplied 10 times for categorical covariates and were rounded. For the age category, the midpoint of each category was multiplied by the β coefficients in **Table 4**, and then multiplied 10 times. We added all these values corresponding to each individual risk, divided the number by 10, and then the corresponding probability of CHD was calculated from the equation: P = 1 − S(t)^exp((sum of the points)/10) where S(t) is the baseline survival function of the Suita cohort.

The C-statistics of the LDL Suita Score with CKD in **Table 4**, which corresponded to the AUC of the Cox proportional hazard model, was 0.831. This was very similar to the TC Suita Score shown in **Table 3**, which had a C-index of 0.835 (**Table 6a**). The likelihood ratio test was not conducted, since the categorical variables were different and these two models were not nested. The NRI between TC Suita Score with CKD and the LDL Suita Score with CKD was not significant (P=.0.256; **Table 6b**). These findings suggest that the two models predict CHD with similar efficiency.

Validation of the Inclusion of CKD

The C-static of the Suita Score without CKD was slightly lower than the Suita Score with CKD (0.835 vs. 0.833). The comparison between the TC Suita Scores with and without CKD suggested that the addition of CKD improved the risk classification of CHD by 40%. This suggested that the inclusion of CKD in the risk prediction tool improves the prediction of the development of CHD, making it a more appropriate predictive tool.

Comparison of the Suita Score and Framingham Risk Scores

Table 7a shows the model fit, C-statistics and BIC of the Cox regression for the TC Suita Score, the original FRS and the recalibrated score for the mean value of each of the covariates. The TC Suita Score with CKD showed the best goodness-of-fit by the likelihood ratio test, and the C-statistics of the TC Suita Score with CKD were also the highest. The BIC was the lowest for the TC Suita Score with CKD, which supported its better predictive ability. The C-statistics were not changed by the recalibration of the FRS. The C-statistics of the recalibrated FRS were still smaller than the TC Suita Score with CKD.

The results of the clinical reclassification measured by the NRI are shown in **Table 7b**. The NRI for the TC Suita Score with CKD compared to the original FRS was 46.8% (P<0.001). In both the CHD and non-CHD groups, the risk categories tended to be increased by the TC Suita Score with CKD. The NRI between the TC Suita Score with CKD and the recalibrated model was lower (25.4%), but the difference remained significant (P=0.003). These associations also held for the TC Suita Score without CKD, the FRS and the recalibrated FRS.

Fig. 2 depicts the actual and predicted probabilities of the 10-year risk of cardiac events by calibration. The FRS consistently overestimated the cardiac events in all quintiles. The overall 10-year calibration of the FRS and recalibrated FRS were worse than the TC Suita Score with CKD as determined by the Hosmer-Lemeshow chi-square test (both p<0.001). The largest difference between the actual rate and the predicted rate after recalibration was 13.9% (in the fifth quintile in males), compared with the difference of 14.5% for the FRS. The difference between the actual probability and the TC Suita Score with CKD was not significant (P=0.18). The TC Suita Score with CKD model underestimated the risk of CHD in the fourth quintile, but the difference was only 2.2%. These findings consistently indicated that the FRS overestimates the CHD risk in the Japanese population.

Discussion

In this study, we demonstrated the predictive

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Table 3. The Cox regression coefficients for the Suita cohort adjusted for the original FRS variables (TC Suita Score)

3a. TC Suita Score with CKD

	β	HR	*P*-value	95% CI
Age, years	0.0766382	1.08	<0.001	1.06-1.10
Female	−0.5866078	0.56	0.001	0.39-0.78
Smoking	0.4865127	1.63	0.002	1.20-2.21
DM	0.4557071	1.59	0.042	1.02-2.45
Blood Pressure				
Optimal	−0.7183575	0.49	0.003	0.31-0.78
Normal and high normal	Referent	Reference	Reference	Reference
Stage I hypertension	0.3330895	1.40	0.055	0.99-1.96
Stage II hypertension	0.59332684	1.81	0.002	1.25-2.63
TC (mg/dl)				
<160	−1.112393	0.33	0.008	0.14-0.74
160-239	Referent	Reference	Reference	Reference
240-279	0.5110573	1.67	0.003	1.19-2.32
Over 280	0.8511397	2.34	0.002	1.36-4.04
HDL(mg/dl)				
<35	0.6173452	1.85	0.001	1.27-2.71
35-50	Referent	Reference	Reference	Reference
50-59	−0.5096169	0.60	0.008	0.41-0.87
Over 60	−0.4322771	0.65	0.022	0.45-0.94
CKD				
Stage 3	0.3278965	1.39	0.035	1.02-1.88
Stage 4 or 5	1.315004	3.72	0.005	1.48-9.38

3b. TC Suita Score without CKD

	β	HR	*P*-value	95% CI
Age, years	0.0806456	1.08	<.0001	1.07-1.10
Female	−0.7401036	0.48	<.0001	0.35-0.66
Smoking	0.4527364	1.57	0.004	1.16-2.14
DM	0.424255	1.52	0.059	0.98-2.37
Blood Pressure				
Optimal	−0.7017837	0.50	0.004	0.31-0.79
Normal and high normal	Referent	Reference	Reference	Reference
Stage I hypertension	0.3607005	1.43	0.037	1.02-2.01
Stage II hypertension	0.6305927	1.87	0.001	1.30-2.72
TC (mg/dl)				
<160	−1.073273	0.34	0.010	0.16-0.78
160-239	Referent	Reference	Reference	Reference
240-279	0.5408852	1.71	0.001	1.23-2.40
Over 280	0.8678275	2.38	0.002	1.38-4.10
HDL(mg/dl)				
<35	0.6742382	1.96	<.0001	1.35-2.86
35-50	Referent	Reference	Reference	Reference
50-59	−0.5011838	0.61	0.009	0.44-0.93
Over 60	−0.4464421	0.64	0.018	0.15-0.93

CKD, chronic kidney disease; TC, total cholesterol; LDL, low density lipoprotein; 95% CI, 95% confidence interval; CKD, chronic kidney disease; HR, hazard ratio; all other abbreviations are the same as in Table 1. The gender difference was incorporated into the model as a covariate to improve the predictability of the model.

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Table 4. The LDL Suita Score with CKD model according to the JAS Guideline 2012 LDL/HDL cut-off (n=5,727) (LDL Suita Score)

4a. The LDL Suita Score with CKD

	β	HR	P-value	95% CI
Age, years	0.0760078	1.08	<0.001	1.06-1.10
Female	−0.6619839	0.52	<0.001	0.36-0.74
Smoking	0.5031949	1.65	0.002	1.20-2.28
DM	0.5533678	1.74	0.031	1.05-2.88
Blood Pressure				
Optimal	−0.6763825	0.51	0.005	0.32-0.82
Normal and high normal	Reference	Reference	Reference	Reference
Stage I hypertension	0.4189501	1.52	0.019	1.07-2.16
Stage II hypertension	0.5935986	1.81	0.001	1.22-2.68
LDL (mg/dl)				
<100	Reference	Reference	Reference	Reference
100-140	0.5319015	1.70	0.039	1.03-2.81
140-160	0.6837867	1.98	0.015	1.14-3.43
160-180	1.021015	2.78	<0.001	1.57-4.91
Over 180	1.128479	3.09	<0.001	1.69-5.66
HDL(mg/dl)				
<40	Reference	Reference	Reference	Reference
40-59	−0.4730423	0.62	0.005	0.45-0.87
≥ 60	−0.5822414	0.56	0.007	0.37-0.85
CKD				
Stage 3	0.2893668	1.34	0.071	0.98-1.83
Stage 4 or 5	1.388216	4.01	0.008	1.43-11.25

4b. The LDL Suita Score without CKD

	β	HR	P-value	95% CI
Age, years	0.0795083	1.08	<0.001	1.06-1.10
Female	−0.804252	0.45	<0.001	0.32-0.62
Smoking	0.4642934	1.59	0.004	1.16-2.19
DM	0.3955565	1.48	0.106	0.91-2.40
Blood Pressure				
Optimal	−0.6602255	0.52	0.006	0.32-0.83
Normal and high normal	Reference	Reference	Reference	Reference
Stage I hypertension	0.4467296	1.56	0.012	1.10-2.21
Stage II hypertension	0.6256262	1.87	0.002	1.27-2.76
LDL (mg/dl)				
<100	Reference	Reference	Reference	Reference
100-140	0.5040579	1.66	0.049	1.00-2.74
140-160	0.664678	1.94	0.017	1.57-4.90
160-180	1.01949	2.77	<0.001	1.73-4.90
Over 180	1.151674	3.16	<0.001	1.73-5.80
HDL (mg/dl)				
<40	Reference	Reference	Reference	Reference
40-59	−0.4798994	0.62	0.004	0.45-0.86
≥ 60	−0.6092216	0.54	0.005	0.36-0.83

JAS Guideline 2012, Japan Atherosclerosis Society(JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2012; CKD, chronic kidney disease; LDL, low density lipoprotein; 95% CI, 95% confidence interval; CKD, chronic kidney disease; HR, hazard ratio; all other abbreviations are the same as in Table 1. Stage 3 and Stage 4 or 5 CKD were defined by estimated GFR levels of 30-60 ml/min/1.73 m^2 and less than 30 ml/min/1.73 m^2, respectively.

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Table 5. The prediction score sheets for the TC and LDL Suita Score

5a. The TC Suita Score

Risk Factor	
Variable	Score
Age (years)	
36-45	30
46-55	39
56-65	46
>65	58
Female	−6
Current smoker	5
DM	5
Blood pressure	
Optimal blood pressure	−7
Stage I hypertension	3
Stage II hypertension	9
TC (mg/dl)	
<160	−11
240-279	5
>280	9
HDL (mg/dl)	
<35	6
50-59	−5
≥ 60	−5
CKD	
Stage 3	3
Stage 4 or 5	15
Total Score	A

Predicted Probability of CHD in 10 years	
Total Score	Probability (%)
≤ 30	<1
31-35	1
36-40	2
41-45	4
46-50	6
51-55	10
56-60	15
≥ 61	24

5b. The LDL Suita Score

Risk Factor	
Variable	Score
Age (years)	
35-44	30
45-54	38
55-64	45
65-69	51
≥ 70	53
Female	−7
Current smoker	5
DM	6
Blood pressure	
Optimal blood pressure	−7
Stage I hypertension	4
Stage II hypertension	6
LDL (mg/dl)	
<100	0
100-139	5
140-159	7
160-179	10
≥ 180	11
HDL (mg/dl)	
<40	0
40-59	−5
≥ 60	−6
CKD	0
eGFR>60	3
Stage 3	14
Stage 4 or 5	
Total Score	A

Predicted Probability of CHD in 10 years	
Total Score	Probability (%)
≤ 35	<1
36-40	1
41-45	2
46-50	3
51-55	5
56-60	9
61-65	14
66-70	22
>71	>28

The estimated risk for CHD over a period of 10 years based on the Suita cohort experience at baseline. The summation of the risk factor category points yields the total score. JAS Guideline 2012, Japan Atherosclerosis Society (JAS) Guidelines for the Prevention of Atherosclerotic Cardiovascular Diseases 2012. LDL cholesterol was derived by Fridewald's equation. Those who had triglycerides >400 were omitted from the calculation.

ability of newly developed coronary prediction algorithms for Japanese subjects developed in the manner of the FRS. Our findings can be summarized as follows: 1) the risk profile for CHD of a Japanese population was considerably different from that of the original Framingham Heart Study cohort; 2) The prediction of CHD obtained with the risk score based on the Suita cohort with CKD variables was superior to that of the FRS or recalibration of the FRS; 3) Clinical reclassification revealed that the FRS overestimates the CHD risk in the Japanese population.

First, the risk profile of the Suita cohort proved to be considerably different from that of a Western population. The crude incidence rate of CHD in the original Framingham Cohort was 8.94 per 1000 person-years, while that of the Suita cohort was only 2.81 per 1000 person-years. The risks of hypertension, low HDL-C for males, and diabetes and smoking for females, in the Suita cohort were weighted higher than the risks in the Framingham cohort. This difference between the Suita and the Framingham cohorts constitutes a major concern for the application of the FRS in Japanese subjects, where the lower CHD incidence and different risk factor levels were observed[32-34].

Second, the discriminatory capability of the TC Suita Score with CKD is better than those of the original and recalibrated FRS. Although recalibration with the mean value of the risk factors and baseline survival

Table 6. Validation of the inclusion of CKD in the TC Suita Score and LDL Suita Score

6a.

Model	Log Likelihood	LR test, *p*-value	C-statistics	BIC
TC Suita Score with CKD	−1610.8	referent	0.835	3233.3
TC Suita Score without CKD	−1618.1	0.013	0.833	3238.5
LDL Suita Score with CKD	−1510.1	referent	0.831	3365.6
LDL Suita Score without CKD	−1513.6	0.032	0.829	3414.5

6b.

Model		TC Suita Score with CKD	LDL Suita Score with CKD	LDL Suita Score with CKD
Reference Model		TC Suita Score without CKD	LDL Suita Score without CKD	Suita cohort model with CKD
Cases	Reclassified Downward (%)	16.7	48.1	48.8
	Reclassified Upward (%)	83.3	51.9	51.2
Non Cases	Reclassified Downward (%)	36.7	26.1	43.8
	Reclassified Upward (%)	63.3	73.9	56.2
Category-free NRI(%)		40.0	43.9	10.1
P-value		<0.001	<0.001	0.256

6a, Comparison of the C-index , LR test and BIC results demonstrating the discrimination for CHD prediction models based on the Suita Score with and without CKD, the FRS and the FRS calibrated for the means of the Suita cohort. Log likelihoods were derived from the multivariate adjusted Cox proportional hazard model.; CKD, chronic kidney disease; FHS, Framingham Heart Study; LR test, likelihood ratio test; BIC, Bayesian information criteria

6b, Comparison of the FRS and Suita Scores and the corresponding reclassification rate for the prediction of CHD events during a 10-year period;. NRI, net reclassification improvement

functions for the study cohort improved the discriminatory capability for various ethnic groups in the U.S., China and the CKD population[6, 12, 16], the recalibration did not improve the discriminatory capability in Japanese subjects. We believe this is probably due to the low incidence of CHD in Japan compared to Western and Chinese populations[35]. The relative risks of various factors were similar between Suita Study cohort and the Framingham cohort. Therefore, the difference between the two prediction tools heavily depends on the difference in the absolute risks between these two cohorts. Accordingly, the clinical reclassification pointed out that the FRS overestimated the risk of CHD in Japanese subjects, especially in the non-CHD group, since the baseline survival function, which was higher than that in the original FRS, affected the estimated risk in an exponential manner and the overestimation was more severe in the high risk groups.

Furthermore, we found that CKD is an independent risk factor for CHD after adjusting for other predictors of the FRS. The cohorts in the Framingham Heart Study and the Offspring study showed no significant association between the presence of kidney disease and the incidence of CVD[36] although some collaborative analyses showed positive associations[17, 37]. Our result is essentially compatible with that of Weiner's study, which reported the HRs of CKD after adjustment of the FRS for whites and blacks[38]. No previous study has dealt with this association for Asian ethnicity as an additional covariate in the prediction tool, although many cohort studies in Japan have demonstrated a significant association between CKD and cardiovascular disease[20, 25, 39].

Finally, we developed a simple prediction sheet for the estimation of CHD based on the TC and LDL Suita Score. For the exact estimation, the beta-coefficient from the TC and LDL Suita Score are preferable. However, the calculation requires computational power, and the more simplified tool is as effective in the clinical setting as the original FRS, since both the models use beta coefficients and a simplified clinical score.

The incorporation of CKD yields limited

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Table 7. A comparison of the predicted risks in models based on the Framingham risk score and Suita Score with and without CKD

7a.

Model	Log Likelihood	LR test, *p*-value	C-statistics	BIC
TC Suita Score with CKD	−1610.8	referent	0.835	3233.3
TC Suita Score without CKD	−1618.1	0.013	0.833	3238.5
Original Framingham Score	−1678.5	<0.001	0.768	3365.6
Recalibrated Framingham Score	−1702.9	<0.001	0.740	3414.5

7b.

Model		TC Suita Score with CKD	TC Suita Score with CKD	TC Suita Score without CKD	TC Suita Score without CKD
Reference Model		Original Framingham Score	Recalibrated Framingham Score	Original Framingham Score	Recalibrated Framingham Score
Cases	Reclassified Downward (%)	42.0	50.0	42.8	49.3
	Reclassified Upward (%)	58.0	50.0	57.2	50.7
Non Cases	Reclassified Downward (%)	65.4	63.1	65.4	63.2
	Reclassified Upward (%)	34.6	36.9	34.6	37.0
Category-free NRI(%)		46.8	25.4	45.3	27.5
P-value		<0.001	0.002	<0.001	0.001

7a, Comparison of the C-index, LR test and BIC results demonstrating the discrimination for CHD prediction models based on the Suita Score with and without CKD, the FRS and the FRS calibrated for the means of the Suita cohort. The log likelihoods were derived from the multivariate adjusted Cox proportional hazard model.; CKD, chronic kidney disease; FHS, Framingham Heart Study; LR test, likelihood ratio test; BIC, Bayesian information criteria

7b, A comparison of the FRS and Suita Scores and the corresponding reclassification rate for the prediction of CHD events during a 10-year period;. NRI, net reclassification improvement

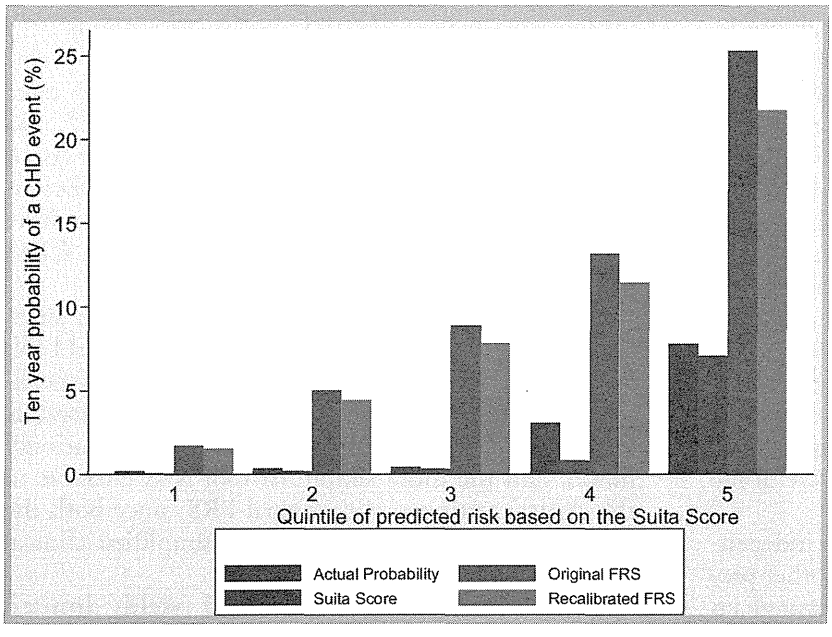

Fig. 2. The ten-year prediction of CHD events in the Suita study using the TC Suita Score with CKD

A graphical representation of the actual 10-year risk of cardiac events in the Suita cohort, along with the predicted risk and the Framingham risk function with and without recalibration for the means of the Suita cohort stratified by the quintile of predicted risk in the Suita cohort. The Suita participants were divided into quintiles of 10-year CHD risk predicted by the Suita score functions with CKD in Table 3. In each quintile, the mean predicted 10-year probabilities and actual probabilities were estimated. The Suita Score, the Suita Score with CKD shown in Table 3. FRS, Framingham risk score. CHD, coronary heart disease.

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improvement in the predictive capability in terms of C-statistics. However, the NRI and IDI showed marked improvement by the incorporation of CKD, which is a more clinically relevant index for prediction improvement. These two methods are becoming more popular and widely used in cardiovascular medicine[40, 41] For example, incorporating the homocysteine level into the FRS was evaluated by the NRI[42], since the inclusion of a new biomarker to the existing CHD risk score changed the predictability of events in a very marginal manner (less than 0.01 of the AUC)[43], and an enormously large odds ratio is needed for significant improvement[44, 45]. Clinicians currently do not have a tool for evaluating the CHD risk of patients with CKD but with relatively few other risk factors. These patients might be misjudged as having a very low risk.

Recently, an individualized risk prediction tool including more diverse risk factors increased 43% AMI and Strokes at the same cost[46]. Therefore, we believe that the inclusion of CKD in the prediction score is necessary and effective for populations at high risk for CHD. Currently, there are estimated to be more than 11 million CKD patients in Japan[47], and people have little doubt that CKD has a major impact on the population's health.

Our population had higher risks for developing CHD compared to other Japanese cohorts. The Suita cohort population was selected from an urban population, in contrast to the majority of other cohorts in Japan, which have been selected mainly from rural populations. Because approximately 66% of the Japanese population lives in urban areas according to 2006 Japanese Census[48], this is an important feature of our analysis. Interestingly, the JMS cohort and JALS reported that the crude incidence of AMI was 0.68 and 0.60 per 1000 person-years, respectively[11, 14, 15, 49]. On the contrary, the crude incidence of AMI in the Suita study was 1.40[50]. These findings may suggest that there is a large difference in the incidence of CHD between rural and urban areas in Japan. Thus, our tool is more useful for predicting the risk in urbanized populations with a higher risk of CHD.

Our study is associated with several limitations. First, the single assessment of risk factors at the baseline survey may have led to a regression dilution bias[51]. Second, the response rate of the original cohort was 53.1% (6,825/ 12,200) although the participants were randomly selected from the population of Suita city. In addition, based on the urbanized nature of the study population, it may not be possible to apply this tool in the whole Japanese population. However, since the outcome of the Suita study was the development of CHD, we believe that this tool can be a complement to the NIPPON DATA 80 risk score adopted in the JAS 2012 guidelines[11], in which the outcome was CHD mortality. The external validation of our score must be evaluated in other cohort studies, although a lack of external validation is a common problem with the existing Japanese risk prediction tools, including the NIPPON DATA 80, JALS, JMS cohort and Hisayama study. Considering the increasingly Westernized lifestyle in urban areas[52], these tools should be re-evaluated using a consortium of cohort studies, which include both urban and rural areas, such as the Epoch-Japan study group[53].

Very recently, the new AHA/ACC Guideline on the Treatment of Blood Cholesterol recommended the use of the new Pooled Cohort Equations to estimate the 10-year CHD risk in both white and black males and females, aged 40-75 years, and the FRS is no longer used for risk assessment[54]. However, this guideline is known to inaccurately estimate the CHD risk for Asians, Therefore, the value of the Suita Score for Japanese subjects and other low risk Asian populations is still superior to other systems.

Third, besides CKD, new biomarkers that can predict the CHD risk are emerging[55 56 57]. However, our study could not access their importance as have other existing prediction tools for Japanese subjects. For, example, the QRISK included rheumatoid arthritis, atrial fibrillation and the BMI. These relatively common, but not classic, cardiac risk factors must also be evaluated in future studies.

In conclusion, for Japanese subjects, the Suita prediction score with the CKD category resulted in better CHD prediction than the original and recalibrated FRS.

Conflict of Interest Disclosures

None.

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III. 高トリグリセライド血症—病因・病態および管理法—
原発性高トリグリセライド血症

原発性高カイロミクロン血症

山下静也[1,2]

Primary hyperchylomicronemia

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Abstract

Primary hyperchylomicronemia is characterized by a marked hypertriglyceridemia due to an increase in chylomicrons, which may cause acute pancreatitis and eruptive xanthomas. This entity includes familial lipoprotein lipase (LPL) deficiency, familial apolipoprotein C-II deficiency, primary type V hyperlipoproteinemia, and idiopathic hyperchylomicronemia. Idiopathic hyperchylomicronemia is caused by an LPL inhibitor or autoantibody against LPL. More recently, patients with primary hyperchylomicronemia caused by mutations in the gene for glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) or lipase maturation factor 1 (LMF1). For the treatment of primary hyperchylomicronemia, a strict restriction of dietary fat is essential to avoid acute pancreatitis.

Key words: primary hyperchylomicronemia, familial lipoprotein lipase deficiency, familial apolipoprotein C-II deficiency, glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1)

はじめに

脂質異常症の中で原発性高脂血症は体質・遺伝子異常に基づいて発症するもので，他の基礎疾患を否定できるものである．これらの原発性高脂血症の中で，カイロミクロン(CM)の増加する原発性高CM血症[1]の成因には幾つかの病態がある．家族性リポタンパクリパーゼ(LPL)欠損症では乳児期から著明なCMの増加によるI型高脂血症を呈し，血清トリグリセライド(TG)値は著しく増加し，血清は乳び性で，急性膵炎や発疹性黄色腫を発症しやすい．本症の治療としては厳重な脂肪摂取の制限が重要である．家族性アポリポタンパク(アポ)C-II欠損症ではLPLが存在してもLPLによるCM・超低密度リポタンパク(VLDL)のTGの分解が起こらず，一般的にはCMとVLDLの両方が増加するV型高脂血症を呈し，急性膵炎や発疹性黄色腫を発症しやすい．そのほか，glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein (GPIHBP1)[2]やlipase maturation factor 1 (LMF1)[3]の遺伝子

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異常に起因する原発性高CM血症も報告されている.

これに対し，続発性高CM血症には①糖尿病(特に未治療の1型糖尿病，糖尿病のケトアシドーシス)，②I型糖原病，③自己免疫疾患(抗LPL抗体，抗アポC-II抗体，全身性エリテマトーデスなど)，④良性高γグロブリン血症(リポタンパク-グロブリン複合体形成，多発性骨髄腫)，⑤マクログロブリン血症，⑥ネフローゼ症候群，⑦末端肥大症，⑧肥満，⑨過食，⑩薬剤によるものなどがある．原発性高CM血症の診断は当然のことながら，上記の続発性高CM血症を除外したうえで行われる．一般的に，原発性高CM血症では動脈硬化性疾患を合併することはほとんどなく，急性膵炎の発症に最も留意する必要がある．本稿では，原発性高CM血症の成因，種々の病態と治療について紹介する.

1. CMの代謝

1) 小腸における脂質吸収の機序とCM合成

食餌として摂取された脂質は，口腔から胃に至る過程での機械的作用と，食餌中に含まれる各種の界面活性物質によりエマルジョンとなる．このエマルジョンは消化液中のリパーゼ，ホスホリパーゼA2，コレステロールエステラーゼなどの作用により，TGが加水分解され，遊離脂肪酸(FFA)2分子がはずれてモノグリセライドに，レシチンはリゾレシチンに，コレステロールエステル(CE)は遊離コレステロールにまで分解される．小腸でのコレステロール吸収にはNiemann-Pick C1-Like1(NPC1L1)が関与する．NPC1L1は主に空腸細胞の刷子縁膜に存在する．食餌由来および胆汁由来のコレステロールは，胆汁酸の働きでミセル化され，その約50％がNPC1L1によって小腸上皮で吸収される．吸収されたコレステロールはアシルCoA:コレステロールアシルトランスフェラーゼ2(ACAT2)により脂肪酸が結合してCEとなる．一方，吸収された脂肪酸とモノグリセライドは小腸粘膜上皮細胞内の滑面小胞体でTGに再合成される．細胞内のCEとTGは，ミクロソームトリグリセライド転送タンパク(MTP)の作用でアポB-48とともにCMとしてアセンブリーされ，リンパ側へと分泌される.

2) 外因性リポタンパクおよび内因性リポタンパクの代謝経路

小腸で合成され小腸上皮の腸リンパ管側へと分泌されたCMは，胸管を経て，体循環に入る．CM中のTGは，骨格筋，心筋や脂肪組織の毛細血管内皮細胞表面に存在するLPLの働きで，加水分解され，組織にFFAを供給する．FFAはβ酸化され，エネルギー源としてATP産生につながるとともに，脂肪組織では細胞内に取り込まれて，再度TGとして蓄えられる．LPLは骨格筋，心筋細胞，脂肪細胞などで合成され，細胞外にいったん分泌された後，ヘパラン硫酸の鎖によって内皮細胞の表面に結合し，その場でCMのTG加水分解を行う．更に，LPLはリポタンパクと結合することにより，細胞内へのリポタンパクの取り込みのためのリガンドとしても働く．CMはLPLの働きにより，CMレムナント(CM-R)となり，肝臓のレムナント受容体ないし，LDL受容体により取り込まれる．以上のように，食餌中の脂肪が吸収されて肝臓へと戻ってくる経路は，外因性リポタンパク代謝経路(**図1**)と呼ばれている.

いったん，肝臓へ戻ったTGやコレステロールなどの脂質は，加水分解された後に，再度TG，CEになり，VLDLの中にアポB-100とともに組み込まれて，VLDLとして肝細胞から合成・分泌される．一部のコレステロールは胆汁酸へと分解され，胆汁として排泄される．VLDLの合成・分泌はミクロソームで起こるが，この際のTGやコレステロールの転送とVLDL分泌に関与するのがMTPである．MTPは小腸上皮細胞内でも，CMの合成・分泌に関与する．MTPの遺伝子欠損により，小腸でのCM，肝臓でのVLDL合成・分泌が障害され，先天性無βリポタンパク血症を発症する．肝臓から分泌されたVLDLはLPLの働きでTG部分が加水分解され，よりTG-poor，コレステロール-richとなり，IDLに変換される．IDLのTGは更に肝性リパーゼ(HTGL)の働きによって分解され，IDL

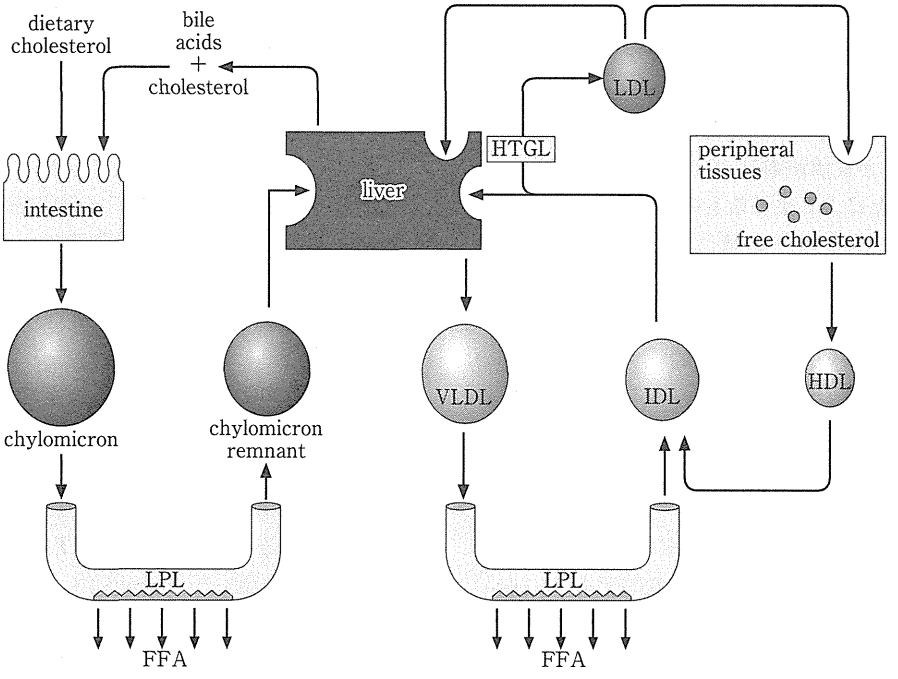

図1 外因性および内因性リポタンパク代謝経路

はLDLとなる．IDLやLDLは肝臓のLDL受容体に取り込まれ，異化を受ける．また，LDLはLDL受容体を介して末梢細胞に取り込まれ，末梢細胞はコレステロールを受け取ることになる．これらの，肝臓から末梢細胞にリポタンパクが運ばれる経路は，内因性リポタンパク代謝経路と呼ばれる．

2. 家族性LPL欠損症

1) 病　態

LPL欠損症はCMやVLDL中のTGを加水分解するLPL遺伝子の異常によって生じ，一般的にI型の高脂血症表現型を呈する，著明な高CM血症，高TG血症を基本病変とする高脂血症である．遺伝形式としては常染色体性劣性遺伝形式をとる．高CM血症はホモ接合体のみ発症し，その頻度は50万～100万人に1例とされる．我が国でも30家系以上が既に報告され，血族結婚が多いとされている．しかし，ヘテロ接合体においても，脂肪摂取や大量飲酒などの後天的な影響によって，IV型高脂血症を呈しやすいと考えられている．ミルクを主食とする乳児期に重篤となることが多い．女性の場合には妊娠中に高CM血症で発見される場合もある．

本症はLPL遺伝の異常に起因し，その遺伝子変異の種類としては，

(1) クラスI変異：LPL酵素タンパクの合成障害のタイプ
(2) クラスII変異：酵素活性が不活性型のLPLが産生されるタイプ
(3) クラスIII変異：ヘパラン硫酸への結合異常を示すLPL変異

に分類される．クラスI変異では遺伝子異常として大きな欠失・挿入，スプライシング異常，ナンセンス変異，フレームシフト変異による早期の停止コドン形成などによる酵素欠損が報告されている．クラスII，III変異の遺伝子異常としてはエクソン2-9のミスセンス変異が報告されており，タンパク構造の変化により酵素活性やヘパリン結合能が失われるものと考えられる．

2) 臨 床 像

病態の中心をなすのは血中でのCMの著明な蓄積と，それに伴って発症する臨床症状である．

最も重篤なものは急性膵炎で，時に劇症で死亡することもある．また，著明な高CM血症が持続すると，皮膚に発疹性黄色腫が出現したり，肝脾腫を呈したりすることがあるが，これらはCMのTGが皮膚組織球や肝脾の網内系へ取り込まれることにより生じるものである．血液はCMによって白色クリーム状になり，眼底検査で眼底血管を通じて直接観察できることがある(網膜脂血症(lipemia retinalis))．本症と動脈硬化との関連について，従来動脈硬化は合併しないと考えられていたが，最近の研究では動脈硬化性疾患の合併例も存在することが報告されている[4]．

3) 診 断 法

検査所見としては，血清TGの著明な上昇(通常1,000-20,000 mg/dL)が特徴的であり，血清を4℃で24時間以上放置するか軽度の遠心を加えるとCMのクリーム層が浮くことでI型高脂血症と診断できる．血清コレステロールは上昇しても軽度である．HDLコレステロールは低下するが，一般的に行われている沈殿法による測定は困難なことが多い．血中CMの増加と血清TGの上昇は食餌中の脂肪量に依存するところが大きいため(したがって本疾患を脂肪起因性高脂血症と呼ぶ)，ミルクを飲む乳児期に発疹性黄色腫で発症することが多い．

LPL欠損症の診断であるが，まず著明な高TG血症(1,000 mg/dL以上)があれば高CM血症が存在すると考えてよい．CMの増加は，血清を4℃で24時間以上静置すると，透明な血清上にCMがクリーム層として浮上することから確認できる．超遠心分析やアガロース電気泳動，ポリアクリルアミドディスク電気泳動によるリポタンパク分析でI型高脂血症であることも診断の一助となる．LPLの基質にはCMと同様にVLDLもなりうるにもかかわらず，LPL欠損症でVLDL増加がなくI型の表現型を呈する理由は不明である．LPL欠損症の確定診断はヘパリン静注後の血清LPL活性の欠損を証明することである．ヘパリン静注後の血清にはLPLのみならず肝性リパーゼ(hepatic lipase: HL)も増加してくるため，活性測定にあたってはLPLとHLを分別測定する必要がある．このため，高塩濃度(通常1 M NaClの添加：LPLのみ活性阻害される)で活性を測定したり，LPLもしくはHLに対する特異抗体の添加後に活性を測定したりする．また，両酵素に対するモノクローナル抗体を用いた酵素イムノアッセイ(EIA)による酵素タンパクの定量法も開発され，タンパク量によっても診断可能である．後述するアポC-II欠損がないことも確認する必要がある．

4) 治 療 法

LPL欠損症の治療であるが，厳重な脂肪制限(1日20g以下)により，外因性リポタンパクであるCM上昇を抑制することが必須である．乳児期には，CMに取り込まれずに門脈中から直接肝臓に運ばれる中鎖脂肪酸(MCT)で構成された人工ミルクを用いて栄養補給する．LPL欠損症の治療の目的は主に膵炎，黄色腫の発生防止である．一般に，TGが1,000 mg/dL以下であれば膵炎は発症しないことが多いといわれている．

3. アポC-II欠損症

1) 病　　態

アポC-IIは，CM，VLDL，HDL粒子に存在する分子量8,800の比較的小さなアポリポタンパクであるが，LPLの活性化に必須である．このアポC-IIが欠損すると，LPLの機能が障害され，家族性LPL欠損症と類似した高脂血症が発症する．本疾患はまれであり，我が国でも数家系の報告があるのみである．アポC-IIの遺伝的欠損に起因するものであるが，遺伝子変異の型として，アポC-IIタンパク合成が認められないタイプと，タンパク合成は認められるもののLPL活性化能のない変異タンパクが存在するタイプとが報告されており，それぞれの遺伝子変異も明らかにされている．

2) 臨 床 像

膵炎の合併も認められる．しかし，LPL欠損症と異なりV型の表現型(CMとVLDLの両方が増加)を呈することが多く，また高TG血症の程度も比較的軽度で，発疹性黄色腫もまれである．

3) 診 断 法

アポC-IIの欠損を証明することで診断がつ

くが，アポC-IIが日常の臨床検査で測定されている現在では，比較的容易に診断可能である．

4）治療法

治療はLPL欠損症に準じて行う．

4. 原発性V型高脂血症

1）病　態

本症は誘因となる疾患もなくV型の表現型(CMとVLDLの増加)を呈する高脂血症で，家系内でも同じタイプが認められることが多い．原発性V型高脂血症の病因はいまだに不明であるが，本症が食餌中の炭水化物，脂肪のいずれの過剰摂取でも増悪することから，VLDLの合成亢進，CM，VLDLの異化障害の組み合わされた異常であることが推察されている．

2）臨床像

症候としては，著明な高TG血症，高CM血症を呈するが，過栄養や飲酒などの負荷をかけなければLPL欠損症よりも比較的軽度である．糖尿病の合併や，上記の食餌性の負荷で悪化し，膵炎を発症することもある．

3）診断法

診断としてはV型高脂血症を呈した症例で，LPL欠損症，アポC-II欠損症でないことを証明する．家族性IV型高脂血症でも，糖尿病の合併や過栄養などによってV型の表現型を呈することがあるが，原発性V型高脂血症ではこれらの増悪因子のない場合でもCMの上昇があることで鑑別することができる．

4）治療法

治療としては，脂肪および炭水化物の過剰摂取を控え，節酒を指示する．

5. 特発性高CM血症

上記の2.-4.と続発性高CM血症を除く高CM血症が含まれる．

1）LPL阻害因子やLPL自己抗体による高CM血症

Brunzellら[5]によって，LPL阻害因子が血中に存在し，しかも家族性に常染色体優性遺伝形式で高CM血症が遺伝する極めてまれな家系が報告されている．このような患者では血漿中のLPL活性は欠損しているものの，組織中のLPL活性は正常である．

一方，著者らは特発性血小板減少性紫斑病とバセドウ病を有する若い女性患者で，LPLおよびHTGLに対するIgA自己抗体が生じたため，I型の高CM血症を発症した症例を報告し，新しい疾患概念であるautoimmune hyperchylomicronemiaを提唱した[6]．

2）GPIHBP1の遺伝子異常による高CM血症

GPIHBP1は毛細血管内皮細胞に発現し，LPLと強く結合する．また，GPIHBP1欠損マウスでは著明な高CM血症，高TG血症が生じるため，LPLは毛細血管内腔でLPLと結合し，TG分解のためのplatformとして機能すると考えられている(**図2**)[7]．最近，GPIHBP1が血管内皮下腔において，LPLをpick upしてLPLを内皮細胞経由で血管内腔側へ輸送するトランスポーターとして働く可能性も指摘されている．高CM血症の家系で，LPLと結合できないGPIHBP1の遺伝子変異も発見されており，逆にGPIHBP1と結合できないLPLの遺伝子変異も見いだされている[2]．

3）LMF1の遺伝子異常による高CM血症

LMF1は小胞体の膜に結合したタンパクであり，LPL，HTGLや内皮リパーゼなどの一連のリパーゼのfoldingやアセンブリー(成熟化)に必須である．最近，LMF1の遺伝的欠損によりLPL欠損と高TG血症を示す家系が報告されている[3]．これらのLMF1の遺伝子変異はリパーゼとの相互作用や活性維持に重要で，進化的に保持されている大きなドメイン(DUF1222)の短縮を起こし，リパーゼの成熟化が阻害される．

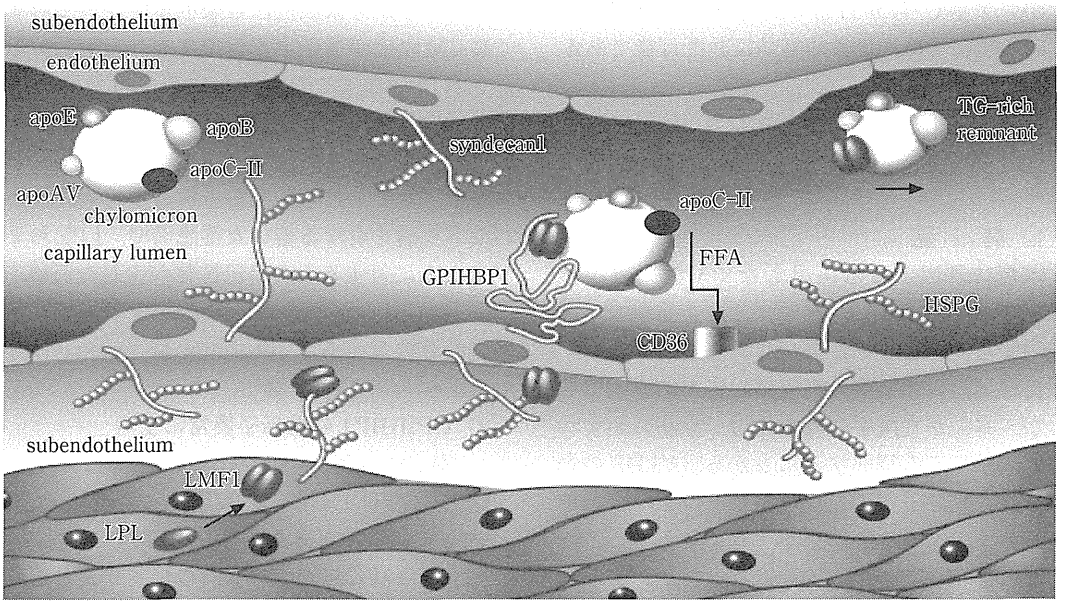

図2 末梢組織におけるTGの加水分解の機序(文献[7]より改変)

LPLは骨格筋，心筋細胞，脂肪細胞などで合成され，LMF1により成熟し，内皮下腔に分泌される．ここでLPLはヘパラン硫酸プロテオグリカン(HSPGs)と結合する．LPLはその後，内皮細胞の中を通過してGPIHBP1が存在する内腔側の細胞表面に到達する．GPIHBP1は恐らくsyndecan1やCD36のような長鎖脂肪酸トランスポーターとともに脂質ラフトに存在すると推定される．更に，LPLはGPIHBP1と結合し，LPLの働きでレムナントリポタンパクが形成され，これが肝臓へ流入する．

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Original Article

Reference Interval for the Apolipoprotein B-48 Concentration in Healthy Japanese Individuals

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Aim: Small intestine-derived chylomicrons and chylomicron remnants, which are predominant in patients with postprandial hypertriglyceridemia, chylomicron syndrome and/or familial dyslipidemia, carry one molecule of apolipoprotein B-48 (apo B-48) per lipoprotein particle. We investigated the reference interval for the apo B-48 concentration.
Methods: We studied 516 individuals who provided written informed consent and confirmed that they were not taking any medications. BMI, waist circumference, blood pressure and the fasting serum concentrations of LDL-C, triglyceride (TG), HDL-C and apo B-48 were measured. The Apo B-48 concentrations were compared according to sex, a pre- or postmenopausal status, dyslipidemia (LDL-C ≥ 140 mg/dL, TG ≥ 150 mg/dL, HDL-C < 40 mg/dL), metabolic syndrome (MetS) and the number of risk factors.
Results: The fasting apo B-48 concentrations (mean ± SD) were significantly higher in men than in women (3.8 ± 3.3 μg/mL vs 2.4 ± 1.9 μg/mL, $p < 0.001$), subjects with a BMI of ≥ 25 kg/m^2 versus a BMI of < 25 kg/m^2 (4.4 ± 3.7 μg/mL vs 2.8 ± 2.4 μg/mL, $p < 0.001$) and those with versus without MetS (6.5 ± 4.3 μg/mL vs 3.0 ± 2.6 μg/mL, $p < 0.001$). High apo B-48 concentrations were also observed in correlation with the number of risk factors for the MetS. The upper reference limit of apo B-48 was estimated to be 5.7 μg/mL among the 332 patients with normolipidemia, excluding those exhibiting a mean value above ± 2.58 standard deviations (SDs), as the mean and range of mean ± 1.96 SD were calculated to be 2.04 μg/mL (reference value) and 0.74 to 5.65 μg/mL (reference interval), respectively.
Conclusions: Based on our study of normolipidemic patients, the upper reference limit for the fasting apo B-48 concentration is estimated to be 5.7 μg/mL.

J Atheroscler Thromb, 2014; 21:000-000.

Key words: Apolipoprotein B-48 (apo B-48), Chylomicrons, Chylomicron remnants, Reference interval

Introduction

Fasting and postprandial hypertriglyceridemia are serious causative factors of cardiovascular events and sudden cardiac death[1]. An increased serum triglyceride (TG) concentration results from the accumulation of TG-rich lipoproteins (TRLs), particularly after a meal. Postprandial hyperlipidemia refers to the occurrence of a high TG concentration after a meal, which is known to be significantly associated with the development of atherosclerotic cardiovascular disease[2, 3]. TRL contain two types of apolipoprotein

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(apo) B, apo B-100 derived from the liver and apo B-48 derived from the small intestine[4].

Chylomicrons (CMs) are synthesized from apo B-48, TG and cholesterol ester in small intestinal cells following the ingestion of lipid-rich foods. After being released into the peripheral blood, CMs are metabolized into smaller remnant particles, CM-remnants, by lipoprotein lipase (LPL) attached to the peripheral vascular wall and taken up by the liver. Apo B-100, a major component of very-low-density lipoprotein (VLDL) is produced in the liver. VLDL is also reduced to smaller VLDL-remnants (or intermediate-density lipoprotein, IDL) by the actions of LPL in the peripheral blood. These remnant particles (CM-remnants and VLDL-remnants) directly infiltrate the vascular wall, subsequently triggering the development of atherosclerotic disease via accelerated macrophage foam cell formation, platelet coagulation and small dense LDL accumulation, as well as the induction of a low concentration of high-density lipoprotein (HDL) cholesterol (HDL-C)[5].

A number of remnant cholesterol assays have been developed and are currently being used to evaluate the risks of atherosclerotic diseases, such as cardiovascular disease (CAD)[6-8]. However, these methods cannot be used to accurately discriminate small intestine-derived CM-remnants from liver-derived VLDL-remnants; therefore, the development of a new assay system is required in order to quantitatively measure the CM-remnant concentration independently. Since one CM-remnant particle contains one apo B-48 molecule and the concentration of apo B-48 is equivalent to that of CM-remnants, we developed a new assay system for measuring the apo B-48 concentration. First, we prepared an enzyme-linked immunosorbent assay (ELISA)[9] for use on a fully automated analyzer system based on the chemiluminescent enzyme immunoassay (CLEIA)[10]. Remnants are usually metabolized immediately; however, the apo B-48 concentration remains elevated due to increased food-derived lipid intake, accelerated TRL synthesis and/or delayed TRL catabolism.

The half-life of the CM particles produced following the ingestion of fat is approximately 30 minutes in the peripheral blood, although the measurable concentration of apo B-48 proteins remains under a fasting condition due to the large amount of lipid absorption and CM production in the small intestine. Therefore, the fasting apoB-48 concentration is correlated with an increase in the TG level following the consumption of a high-fat meal, implying that the fasting apo B-48 concentration is a marker of postprandial hyperlipidemia[11]. High apo B-48 concentrations are usually observed in patients with type Ⅲ hyperlipidemia[9], metabolic syndrome (MetS)[12], type Ⅱb hyperlipidemia[13] or CD36 deficiency[14]. However, the reference interval for the apo B-48 concentration in healthy fasting individuals has not yet been established.

Aim

In this study, we attempted to establish the upper reference limit and reference interval for the fasting apo B-48 concentration in individuals with normolipidemia.

Subjects and Methods

Subjects

The subjects of this study included 516 individuals who received their annual health checkup and were not taking any medications. The study was carried out under the approval of the Osaka University Health Care Center and Saint (St.) Marguerite Hospital, and all participants provided their written informed consent. The institutional ethics committees of both facilities approved the research protocol. After confirming the lack of a significant adverse medical history known to affect lipoprotein or carbohydrate metabolism, various anthropometric parameters, including height, body weight and waist circumference were obtained and the body mass index (BMI, body weight [kg]/height [m]^2) was calculated. Blood samples were collected in the morning after overnight fasting. The serum samples were then separated via low-speed centrifugation and stocked at −80℃ until the analyses. All specimens were handled according to the protocols of the Helsinki Declaration.

Measurements

Blood pressure (BP) was measured in the sitting position. Hypertension was diagnosed based on a systolic BP of ≥140 mmHg and/or a diastolic BP of ≥90 mmHg. A high BP status was determined based on a systolic BP of ≥130 mmHg and/or a diastolic BP of ≥85 mmHg (according to the guidelines for the management of hypertension issued by the Japanese Society of Hypertension). The serum TG concentration was measured according to an enzymatic method, and the LDL-cholesterol (LDL-C) and HDL-C levels were measured using direct methods. We identified cases of dyslipidemia and normolipidemia based on the diagnostic criteria for dyslipidemia of the Japan Atherosclerosis Society: (a) an LDL-C level of ≥140 mg/dL, (b) a TG level of ≥150 mg/dL, (c) an HDL-C level of

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<40 mg/dL (according to the guidelines for the diagnosis and prevention of atherosclerotic cardiovascular disease for the Japanese)[15]. Abnormal factors were summarized in the patients with dyslipidemia. The fasting plasma glucose (FPG) concentration was measured according to the hexokinase UV method, and the hemoglobin A1c (HbA1c) (JDS) level was measured according to the latex agglutination method. A high fasting glucose level was defined as an FPG of ≥110 mg/dL, according to the criteria of the Japan Diabetes Society. MetS was diagnosed based on the criteria of the Japanese Society of Internal Medicine[16], namely, a waist circumference of ≥85 cm in men and ≥90 cm in women combined with at least two of the following factors: (a) a high BP status and hypertension (a systolic BP of ≥130 mmHg and/or a diastolic BP of ≥85 mmHg), (b) abnormal lipid metabolism (a TG level of ≥150 mg/dL and/or an HDL-C level of <40 mg/dL), (c) high fasting glucose (an FPG level of ≥110 mg/dL). Cardiac risk factors were summarized in cases of MetS.

The serum apo B-48 concentration was determined using the CLEIA system (Fujirebio, Inc., Tokyo, Japan)[10]. Briefly, serum samples were incubated with treatment buffer solution supplemented with surfactant in order to separate apo B-48 from CMs and CM-remnants. The pre-treated samples were incubated with ferrite particles coupled with murine monoclonal antibodies against apo B-48 in the solid phase. After washing, further incubation was carried out with alkaline phosphatase-conjugated anti-apo B monoclonal antibodies as a second antibody. After further washing, a chemiluminescent substrate was added to the test cartridge, after which the relative chemiluminescent intensity was measured and the serum apo B-48 concentration was calculated according to a standard curve.

Statistical Analysis

The statistical analysis was performed using the non-parametric Mann-Whitney *U* test according to F-study with the Stat Flex software program (ver. 6, Artec Inc., Osaka, Japan) after confirming the distribution. The level of significance was assumed to be 95%. The upper reference limit and reference interval for the apo B-48 concentration were estimated according to the methods recommended by CLSI (Clinical and Laboratory Standards Institute). Briefly, after normalizing all data using logarithm conversion, the mean and standard deviation (SD) were calculated and patients exhibiting a mean value above ±2.58 SD were eliminated. This process was repeated until no exception data were calculated. Subsequently, the value was returned to the integer, and the upper reference limit and reference interval were defined.

Table 1. Clinical and Laboratory Data

	Mean ± SD	95% Confidence Interval
Men/Women	284/232	
Age (year)	42 ± 10/42 ± 11	
Post-menopausal	48/232	
BMI (kg/m^2)	22.4 ± 3.3	22.0-22.7
Waist circ. (cm)	91.1 ± 5.6	90.0-92.2
sBP (mmHg)	115.9 ± 14.6	114.6-117.2
dBP (mmHg)	73.2 ± 11.3	72.2-74.2
TC (mg/dL)	199 ± 31	196.0-201.6
TG (mg/dL)	94 ± 69	87.7-99.7
HDL-C (mg/dL)	65 ± 15	63.1-65.9
LDL-C (mg/dL)	121 ± 29	118.1-123.3
FPG (mg/dL)	87 ± 13	86.2-88.5
HbA1c (JDS) (%)	5.0 ± 0.5	4.9-5.1
	Number of Patients	
BMI ≥25 kg/m^2	111 (21.5%)	
BMI <25 kg/m^2	405 (78.5%)	
Hypertension	47 (9.1%)	
High BP status	103 (20.0%)	
High FPG	10 (1.9%)	
Number of abnormal factors for dyslipidemia		
0	337 (65.3%)	
1	138 (26.7%)	
2	37 (7.2%)	
3	4 (0.8%)	
Number of risk factors for metabolic syndrome (MetS)		
0	303 (58.7%)	
1	135 (26.2%)	
2	53 (10.3%)	
3	24 (4.6%)	
4	1 (0.2%)	

The abbreviations used in this Table are as follows.
dBP: diastolic blood pressure, sBP: systolic blood pressure, BMI: body mass index, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides

Results

Background Characteristics of the Subjects

The total number of registered subjects was 516 (284 men and 232 women: 183 premenopausal patients, 48 postmenopausal patients and one unknown patient) at two hospitals. The assay data and classification of the subjects are summarized in **Table**

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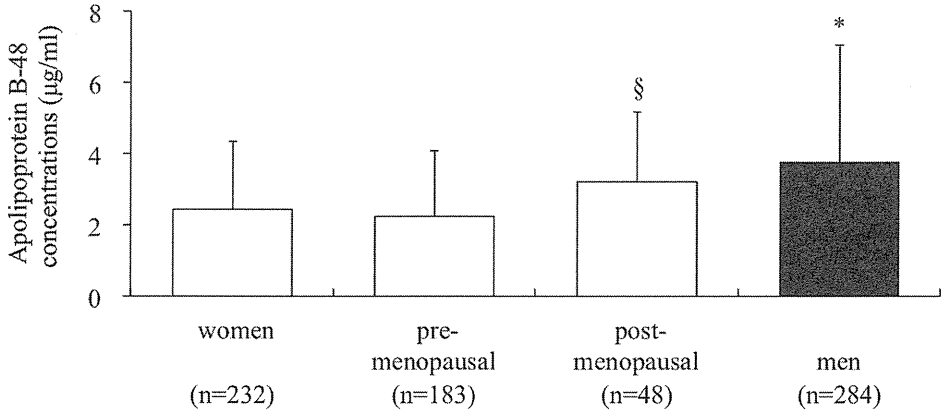

Fig. 1A. Comparison of the apolipoprotein B-48 concentrations in all cases.

The apolipoprotein B-48 concentrations in 284 men and 232 women (183 premenopausal patients, 48 postmenopausal patients and one unknown patient) were compared. The values indicate the mean ± standard deviation as follows: women = 2.4 ± 1.9 μg/mL, premenopausal women = 2.2 ± 1.8 μg/mL, menopausal women= 3.2 ± 2.0 μg/mL, men = 3.8 ± 3.3 μg/mL. Statistical significance was assessed using the Mann-Whitney *U* test. $^{*}p<0.001$ against women, $^{\S}p<0.001$ against premenopausal women.

1. A total of 111 patients had a BMI of ≥25 kg/m^2, while a waist circumference beyond the standard range (indicating abdominal obesity) was observed in 114 cases (one-fifth of all cases). Regarding abnormal factors related to dyslipidemia (a high LDL-C concentration, high TG concentration or low HDL-C concentration), two-thirds of the subjects (337 patients, 161 men and 176 women: 152 premenopausal patients and 24 postmenopausal patients) were classified as having no abnormal factors for dyslipidemia; these patients were classified into the normolipidemic group. One-third of the patients exhibited more than one abnormal factor for dyslipidemia. Twenty-four patients, or one-fifth of those with a high BMI (≥25 kg/m^2), were diagnosed with MetS, as their waist circumference was beyond the standard range and they exhibited two of three risk factors, including BP, FPG and abnormal lipid metabolism. Most of the patients exhibited either no risk factors (58.7%, 303 patients) or one risk factor (26.2%, 135 patients) for MetS, including hypertension (or a high BP status), hypertriglyceridemia, low HDL-cholesterolemia and a high FPG level.

Apo B-48 Concentrations and their Distribution in Several Classifications

We examined the fasting apo B-48 concentrations after classifying the patients into various groups. First, a sex difference was observed, namely, the mean apo B-48 concentration in men (284 patients) was higher than that observed in women (232 patients)

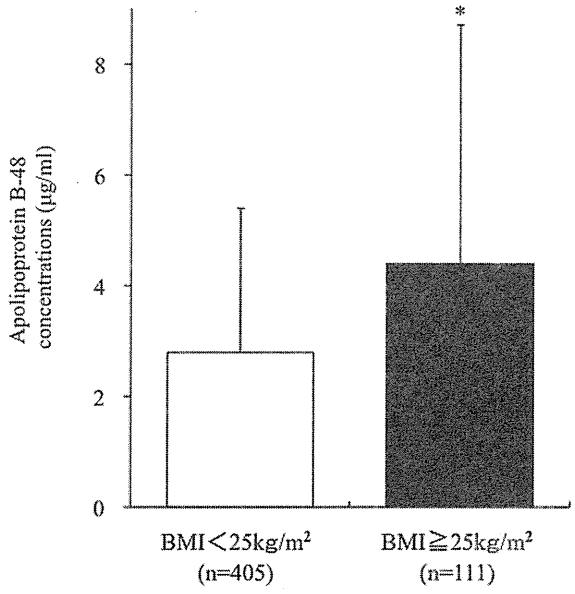

Fig. 1B. Comparison of the apolipoprotein B-48 concentrations in the subjects with a BMI of <25 kg/m^2 and those with a BMI of ≥25 kg/m^2.

The values indicate the mean ± standard deviation, as follows: BMI <25 kg/m^2 = 2.8 ± 2.4 μg/mL (n=405), BMI ≥25 kg/m^2 = 4.4 ± 3.7 μg/mL (n=111). The number of subjects is shown in brackets. Statistical significance was assessed using the Mann-Whitney *U* test. $^{*}p<0.001$

(3.8 ± 3.3 μg/mL vs 2.4 ± 1.9 μg/mL, $p<0.001$, Mann-Whitney *U* test) (**Fig. 1A**). A significant difference was also observed between the pre- and postmenopausal