Significant Correlation between Fractional Removal Rate (K_2) of Fat Emulsion and Serum Triglyceride or Apolipoprotein A-I in Subjects with ApoE Phenotype 3/3

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Summary We evaluated the relationship between the removal rate (K_2) of fat emulsion and serum lipids or apolipoproteins of apoA-I, A-II, and B to assess fat emulsion metabolism in subjects who were apoE3/3 homozygotes of wild-type apoE3. K_2 of fat emulsion, Intralipid®, was determined by the intravenous fat emulsion tolerance test (FETT) in 184 subjects with apoE phenotype 3/3, including normolipidemic subjects and those with primary hyperlipidemia. Other subjects ($n=47$) with normolipidemia or primary hyperlipidemia were also evaluated for comparison; they consisted of individuals with apoE phenotype 3/2 and 4/3. In subjects with apoE phenotype 3/3, when a single correlation coefficient was calculated, there were significant correlations between K_2 and the serum level of triglyceride (TG) ($r=-0.48$), total cholesterol ($r=-0.15$), apoB ($r=-0.27$), HDL cholesterol ($r=0.42$), and apoA-I ($r=0.26$). Multiple regression analysis for elimination of internal correlations revealed significant correlations between K_2 and the serum level of TG ($r=-0.46$, $p<0.001$) or apoA-I ($r=0.23$, $p<0.01$: multiple correlation coefficient=0.51, $p<0.001$). No significant correlations between K_2 and serum lipids or apolipoproteins were observed in subjects with apoE phenotypes other than 3/3. We suggest that K_2 is strongly related to serum TG and apoA-I in subjects with apoE phenotype 3/3 and that this relationship is unique and specific to subjects with this phenotype.

Key Words: fat emulsion tolerance test, hypertriglyceridemia, apoE phenotype 3/3

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Hypertriglyceridemia is a risk factor that is associated with ischemic heart disease [1, 2]. The presence of a serum triglyceride (TG)-processing disorder, an accelerated synthesis of TG, or their combination may be involved in the development of hypertriglyceridemia. A simple fat emulsion tolerance test (FETT), which uses Intralipid® (Vitrum AB, Stockholm) is used to determine whether a patient with hypertriglyceridemia has a disorder of serum TG processing [3–5]. In this test, the serum TG-processing capability can be deduced from the removal rate of Intralipid® (K_2 %/min).

Intralipid®, an artificial fat emulsion, is administered intravenously to patients as a supply of energy and essential fatty acids. The TG content, particle size, and metabolic pattern of Intralipid® resemble those of chylomicrons [6]. Once Intralipid® has entered the blood, it exchanges with lipids in high-density lipoprotein (HDL), accepts apolipoproteins (apo) E, C-II, and C-III from HDL, and is then metabolized via first-order kinetics [6–8].

Apolipoprotein E (apoE) is distributed throughout the chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and HDL. ApoE consists of 299 amino acids, and has three isoprotein types: E2, E3, and E4 [9, 10]. E3 is the wild type; E2 is a variant type in which Cys replaces Arg at the 158th amino acid residue (Arg₁₅₈→Cys); and E4 is a variant type in which Arg replaces Cys at the 112th amino acid residue (Cys₁₁₂→Arg) [9, 11].

It is reported that apoE is related to lipoprotein metabolism and that the influence of apoE3 on lipoprotein metabolism differs from that of apoE2 or apoE4 [9, 10, 12–14].

In the present study, we determined the correlation between K_2 obtained by FETT and serum lipids or apoA-I, A-II, and B in subjects who were apoE3/3 homozygotes of wild-type apoE3, so as to cast light on fat emulsion metabolism. To further allow assessment of the influence of the type of apoE on fat emulsion metabolism, other groups consisting of individuals with apoE phenotype 3/2 or 4/3 were evaluated to obtain comparative data.

METHODS

We evaluated 45 Japanese normolipidemic subjects [33 men and 12 women, 42.0 ± 1.5 (mean $\pm$ SD) years of age (age range: 25–76 years)], and 139 Japanese individuals with primary hyperlipidemia, 50 with Type IIa, 43 with Type IIb, and 46 with Type IV [68 men and 71 women, 52.2 ± 15.4 (mean $\pm$ SD) years of age (age range: 25–74 years)], all with apoE phenotype 3/3. While the principal subjects of study were those in the apoE phenotype 3/3 group, two other phenotypes were also evaluated for comparison: 26 individuals of apoE phenotype 3/2 and 21 of apoE phenotype 4/3. Included in these 47 subjects were 12 with normal blood lipid levels, 8 with primary hyperlipidemia Type IIa, 13 with Type IIb, and 14 with Type IV [25 men and 22 women, 53.2 ± 13.8 (mean $\pm$ SD) years of age (age range: 23–68 years)]. They presented at the outpatient clinic of The Third Department of

Internal Medicine, Nagoya City University Hospital, Nagoya, Japan, for their regular physical examinations. Informed consent for participation in the study was obtained from all subjects.

After blood had been collected early in the morning from overnight fasting subjects for measurement of serum lipids and apoproteins, the FETT [3-5] was conducted by administering Intralipid® intravenously, 0.25 ml/kg body weight over 90 s. Blood samples were collected at 3, 5, 7, 9, 11, 14, 17, and 20 min thereafter. Serum samples were then diluted 100-fold with physiologic saline solution. The light-scattering index (LSI) for three specimens was determined at each time point with a nephelometer (model DN-2110, Kyoto Daiichi Kagaku, Kyoto, Japan) to obtain the mean value. Using the logarithmic value of the mean LSI of samples at each time point minus the mean LSI of samples obtained before fat emulsion loading, we calculated by the least-squares method the fractional removal rate (K_2) of fat emulsion (10% Intralipid®) as the regression coefficient of a successively declining curve of LSI.

VLDL ($d < 1.006$ g/ml) was isolated by ultracentrifugation in NaBr solution [15], and the apoE phenotype was identified by isoelectric focusing of apoE from VLDL separated on polyacrylamide gels, according to the method of Warnick *et al.* [16].

Serum TG and total cholesterol (TC) levels were measured by enzymatic methods [17, 18], HDL cholesterol (HDL-C) was determined by the heparin-Ca precipitation method [19], and apolipoproteins were measured by immunoturbidimetry [20]. Normolipidemic subjects were classified on the basis of a TG level under 150 mg/dl and a TC level under 220 mg/dl. The type of hyperlipidemia was determined according to the WHO classification, based on serum lipid levels and agarose gel electrophoresis results.

The correlations between K_2 and serum lipids or apoproteins were determined by single correlation coefficients and multiple regression coefficients [21]. A level of $p < 0.05$ was considered to be statistically significant.

RESULTS

The apoE phenotype 3/3 group demonstrated significant negative correlations between K_2 and the TG level ($r = -0.48$, $p < 0.001$; Fig. 1), the TC level ($r = -0.15$, $p < 0.05$), and the apoB level ($r = -0.27$, $p < 0.01$); in addition, there were significant positive correlations between K_2 and the HDL-C level ($r = 0.42$, $p < 0.001$), and the apoA-I level ($r = 0.26$, $p < 0.001$) (Table 1).

In the apoE phenotype 3/3 group, the multiple regression model revealed significant correlations between K_2 and TG ($r = -0.46$, $p < 0.001$) or apoA-I ($r = 0.23$, $p < 0.01$; multiple correlation coefficient = 0.51, $p < 0.001$; Table 2). K_2 was most strongly related to the TG level and apoA-I level, with the rate of contribution being 25%.

In the apoE phenotypes other than 3/3, i.e., in the group that consisted of

Fig. 1. Relationship between K_2 and serum TG in apoE3/3 phenotype subjects. A significant correlation between K_2 and serum TG level is evident ($r = -0.48$, $p < 0.001$).

Table 1. Correlations between K_2 and serum lipids and apolipoproteins in apoE3/3 phenotype subjects.

Parameter	Mean $\pm$ SD	Correlation with K_2
n	184	
K_2 (%/min)	9.3 ± 3.9	
Triglyceride (mg/dl)	192 ± 140	-0.48^{***}
Cholesterol (mg/dl)	230 ± 60	-0.15^*
HDL cholesterol (mg/dl)	44 ± 14	0.42^{***}
apoA-I (mg/dl)	122 ± 25	0.26^{***}
apoA-II (mg/dl)	32 ± 8	0.05
apoB (mg/dl)	124 ± 30	-0.27^{**}

$***p < 0.001$, $**p < 0.01$, $*p < 0.05$.

Table 2. Significant correlations between K_2 and serum lipids and apolipoproteins in apoE3/3 phenotype subjects (multiple regression analysis: backward elimination).

Parameter	Regression coefficient	Partial correlation coefficient	t -value
TG	-0.010	-0.46	6.37^{**}
apoA-I	0.025	0.23	2.96^*

Multiple correlation coefficient = 0.51^{**} . $F = 27.13$. $**p < 0.001$, $*p < 0.01$.

individuals with apoE phenotype 3/2 or 4/3, no significant correlations were observed between K_2 and serum TG level (Fig. 2), HDL-C level, or apoprotein levels. Similarly, we observed no significant correlations between K_2 and serum TG level, HDL-C level, or apoprotein levels in either the apoE phenotype 3/2 group or the apoE phenotype 4/3 group.

Fig. 2. Relationship between K_2 and serum TG in subjects with apoE4/3, apoE3/2 phenotypes. ●, apoE4/3; ○, apoE3/2. No significant correlations are evident between K_2 and serum TG level.

DISCUSSION

In the apoE phenotype 3/3 group selected for study, there were significant negative correlations between K_2 and TG level, TC level, or apoB level, and significant positive correlations between K_2 and HDL-C level, or apoA-I level. However, when internal correlations were eliminated by a multiple regression analysis, we observed a significant inverse correlation between K_2 and TG level, with a rate of contribution of 17%, and a significant positive correlation between K_2 and apoA-I level, with a rate of contribution of 7%. After log transformation of data of K_2 and TG, the calculated data did not show improvement over the data mentioned above. These results suggest that the serum TG level is involved in the fat emulsion-processing capability, K_2 . Because apoA-I is the primary apoprotein in HDL [22] and the apoA-I level reflects the number of HDL particles, the results also suggest that HDL is involved in K_2 .

There is reportedly a good correlation between the K_2 of Intralipid® and the fractional catabolic rate (FCR) of VLDL-apoB [23]. A good correlation has also been reported between HDL-C and FCR of VLDL-apoB [24]. The good correlation between K_2 and HDL-C level may be attributable to the fact that K_2 reflects the metabolism of VLDL-apoB.

As in the case with apoA-I, apoA-II [25] is an apolipoprotein that exists primarily in the HDL fraction. However, the fact that there was no significant correlation between K_2 and apoA-II level suggests that apoA-II does not play an active role in the metabolism of Intralipid®.

Even though there was difference in age between normolipidemic subjects and hyperlipidemic subjects in this study, there was no significant correlation between K_2 and age.

It is known from *in vitro* studies that apoE is related to the hydrolysis of VLDL- ($d < 1.006$ g/ml) TG by lipoprotein lipase (LPL), as well as its conversion into LDL ($1.019 < d < 1.063$ g/ml) [12, 26, 27]. It appears that apoE is involved in the hydrolysis of Intralipid® by LPL as well.

The activity of LPL with respect to the hydrolysis of VLDL-TG is reported to be positively correlated with HDL₂ and inversely correlated with HDL₃ [28]. It is known that individuals with hypertriglyceridemia have low blood levels of HDL [29] and that the HDL of individuals with hypertriglyceridemia consists primarily of HDL₃, with little HDL₂ [30]. It is also known that apoE and apoC groups are distributed primarily in HDL₂ within the HDL subfraction and that only extremely small quantities of apoE and apoC groups are present in HDL₃ [31]. These findings suggest that, because the HDL fraction of individuals with hypertriglyceridemia has a low content of apoE and apoC-II, Intralipid® accepts smaller quantities of apoE and apoC-II from HDL, thus delaying its metabolism by LPL, resulting in lower K_2 values in individuals with hypertriglyceridemia than in those with normal blood lipid levels.

Meanwhile, we reported a significant positive correlation between K_2 and post heparin lipoprotein lipase activity in a group ($n=42$) consisting of normolipidemic subjects and hyperlipidemic subjects ($r=0.76$, $p<0.01$) [32].

It may also be inferred that these mechanisms are involved in the inverse correlation observed between the serum TG level and K_2 when the apoE phenotype 3/3 group was considered as a whole, including subjects with normal serum levels of lipid and patients with hypertriglyceridemia.

There are many reports on apoE with respect to lipoprotein metabolism. We previously observed that, in patients with Type III hyperlipidemia with LDL-receptor abnormality, serum LDL levels were low; whereas β -VLDL had increased [33]. In other words, it appears that E2 inhibits the conversion of VLDL into LDL *in vivo* as well.

It is believed that when chylomicrons and VLDL-TG undergo hydrolysis by LPL and become remnants, and are then incorporated into the liver through remnant receptors or apoB/E (LDL) receptors, apoE plays an essential role as a ligand [13]. E3 and E4 show stronger binding capability to LDL receptors than E2 [34]. In oral fat load tests, the removal of chylomicrons and chylomicron remnants in humans with apoE phenotype 2/2 was shown to be delayed [35].

In recent study of oral fat loading test, a delayed chylomicron remnant clearance was observed only in apoE2 homozygotes; and this delay was neither correlated with fasting lipids nor with peak lipoprotein concentration [36]. In non-apoE2/2 individuals, in contrast, the chylomicron remnant response was highly correlated with the magnitude of chylomicron and VLDL responses, and with fasting triglycerides. In apoE2/3 heterozygotes, the defective isoform E2 appears to be compensated for by the normal apoE isoform E3.

It has also been reported that the metabolism of TG-rich lipoproteins in humans with apoE phenotypes 4/3 and 4/4 is faster than in homozygotes for the

wild-type apoE3/3 [14]. *In vitro* studies have shown that E4 binds easily to VLDL and is distributed in smaller quantities in HDL [37] and that E-A-II complexes are not easily formed, such that E4 binds more readily with apoB/E receptors [38] and particles that contain E4 are incorporated more readily into the liver and are catabolized more quickly than are those that contain E3.

Although apoE2, E3, and E4 have differing functions in the metabolism of lipoproteins that are produced *in vivo*, their role in metabolism of artificial fat emulsions is not well understood. Since the present results indicate that the significant correlations between K_2 and serum TG and apoA-I seen in human subjects with apoE phenotype 3/3 were not seen in those with apoE phenotype 3/2 or 4/3, it appears that apoE2 and apoE4 have a different effect than apoE3 on the metabolism of Intralipid®.

In conclusion, the present results indicate that K_2 is strongly related to serum TG and apoA-I in subjects with apoE phenotype 3/3 and that this relationship is unique and specific to subjects having this phenotype.

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