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ORIGINAL ARTICLE

Exploring the value of apoB48 as a marker for atherosclerosis in clinical practice

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ABSTRACT

Background Postprandial accumulation of atherogenic remnants has been described in patients with type 2 diabetes mellitus (T2DM), familial combined hyperlipidaemia (FCH), familial hypercholesterolaemia (FH) and coronary artery disease (CAD). Scarce data are available on fasting plasma apolipoprotein (apo) B48 levels in relation to these conditions and atherosclerosis.

Design Treated patients with FCH (18), FH (20), T2DM (26), CAD (65), T2DM with CAD (T2DM/CAD) (28) and 33 healthy controls were included. Intima-media thickness (IMT) measurements were carried out to investigate subclinical atherosclerosis.

Results LDL-C and total apoB were lowest in patients with T2DM/CAD owing to the more frequent use of lipid-lowering medication. Fasting plasma apoB48 was elevated in patients with FCH (11·38 ± 1·50 mg/L) and T2DM/CAD (9·65 ± 1·14 mg/L) compared with the other groups (ANOVA, $P < 0·01$). CAD patients (8·09 ± 0·57 mg/L) had higher apoB48 levels than controls (5·74 ± 0·55 mg/L) and FH patients (5·40 ± 0·51 mg/L) ($P = 0·02$). IMT was highest in subjects with T2DM/CAD (0·77 ± 0·03 mm) ($P < 0·01$). The lowest IMT was measured in controls (0·56 ± 0·02 mm) and FCH patients (0·60 ± 0·03 mm). In the total group, the best association for apoB48 was found with fasting triglyceride (Pearson's $r = 0·72$, $P < 0·001$). In the subjects not using statins ($n = 74$), the best correlation was found with IMT ($r = 0·52$; $P < 0·001$), whereas total apoB was not associated with IMT ($r = 0·20$, $P = 0·12$).

Conclusions ApoB48 concentrations are highest in patients with FCH and in atherosclerotic subjects with T2DM. In patients not using statins, the surrogate atherosclerosis marker IMT correlates best with apoB48, suggesting that fasting apoB48 may help to detect subjects at risk.

Keywords Apolipoprotein B48, coronary artery disease, familial lipid disorders, intima-media thickness, type 2 diabetes mellitus.

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Introduction

Several investigators have suggested that postprandial remnants may be atherogenic [1–4]. Postprandial hyperlipidaemia has been associated with many disorders such as abdominal obesity [5], type 2 diabetes mellitus (T2DM) [5,6] and familial combined hyperlipidaemia (FCH) [7]. It has been shown that patients with significant atherosclerosis also have postprandial hyperlipidaemia [3,8,9]. Finally, in the literature, contradictory data have been published on postprandial hyperlipidaemia and familial hypercholesterolaemia (FH) showing normal [10,11] or disturbed postprandial chylomicron remnant clearance [12,13].

Postprandial lipaemia is characterized by the accumulation of both hepatic and intestinal lipoproteins [14]. The atherogenic particles in these lipoproteins contain apolipoprotein (apo) B48 and apoB100, of which by far the majority consists of apoB100 particles. We and others have shown that postprandial chylomicrons, containing apoB48, are able to induce leucocyte activation and may therefore cause atherosclerosis [15,16]. Single

measurements of apoB48 have been shown to predict postprandial apoB48 and triglyceride (TG) response [17].

Intima-media thickness (IMT) has been shown to be a good surrogate marker of subclinical atherosclerosis, and its close relationship with cardiovascular risk has subsequently been confirmed in several studies [18].

In this study, we aimed to investigate whether fasting plasma levels of apoB48 can help to differentiate subjects with different conditions with remnant accumulation [e.g. FCH, T2DM and coronary artery disease (CAD)] from subjects without remnant accumulation. The relationship between apoB48 and IMT was also investigated.

Materials and methods

Participants

Subjects who visited the outpatient clinic of the Department of Vascular Medicine of the Sint Franciscus Gasthuis and met the diagnostic criteria for FH, FCH and T2DM were asked to participate. Also (untreated) subjects who were referred for cardiovascular risk management were included if they met the criteria described below. Healthy volunteers were recruited by means of advertisement. FH was defined as by the diagnostic criteria as outlined by the World Health Organization [19]. FCH was defined as following: familial hyperlipidaemia with a dominant inheritance pattern, elevated plasma apoB concentrations (> 1·2 g/L) and elevated TG levels (> 1·7 mmol/L) at the time of diagnosis [7,14]. Patients with T2DM diagnosed according to the guidelines published by the International Expert Committee in 1998 [20].

Patients with CAD were selected from male and female subjects who visited the outpatient clinic of the Department of Cardiology and were scheduled to undergo diagnostic coronary angiography. CAD was defined as angiographically established coronary atherosclerosis, ranging from wall irregularities to multivessel disease.

Exclusion criteria were the presence of inflammatory disorders, e.g. rheumatoid arthritis, systemic lupus erythematosus and infections, MDRD-GFR ≥ 40, liver and thyroid function.

The Independent Ethics Committee of the Institutional Review Board of the St. Franciscus Gasthuis in Rotterdam and the regional independent medical ethical committee at the Maasstad Hospital in Rotterdam approved the study. The participants gave written informed consent.

Study design

During the first visit, the cardiovascular history, anthropometric measures and the use of medication were recorded.

For blood sampling, all participants visited the hospital after an overnight fast of at least 12 h, without drinking alcohol on the day before and without taking their regular medication.

Analytical methods

All clinical chemistry measurements were taken on the same day as the diagnostic coronary angiography. Basic parameters for renal and liver function as well as glucose, total cholesterol, HDL-C and TG were determined using a Synchron LX analyzer (Beckman Coulter, Brea, CA, USA) according to standard procedures in our laboratory for clinical chemistry. LDL-C values were calculated using the Friedewald formula. ApoAI and apoB were determined by rate nephelometry using IMMAGE with kits provided by Beckman (Beckman Coulter).

ApoB48 serum levels were quantified as previously reported [21], using a commercially available ELISA (Shibayagi Co. Ltd., Shibukawa, Japan), consisting of a sandwich-type ELISA with absorbance dichromatic reading at 450 nm/620 nm (reference for plate correction) wavelength [22]. All samples were assayed in three runs. As no commercial quality controls are available for apoB48, a local internal quality control was pooled according to WHO recommendations, stored at −80 °C and formerly assayed by duplicate on each plate in parallel to samples. Calculated variation coefficients were 5·7% (intra-assay), 11·0% (interassay) and 12·4% (total).

Intima-media thickness measurements of the carotid arteries

Measurements were taken according to the consensus guidelines for carotid ultrasound for CVD risk assessment [23]. They were carried out using the ART-LAB (Esaote, Genoa, Italy) by a single trained and experienced sonographer, who was unaware of the patient's medical history. Ultrasound scans were taken with the patients lying in a supine position with the head resting comfortably and the neck slightly hyperextended and rotated in the opposite direction of the probe. The ultrasound images were obtained of the distal 1 cm of the far wall of each common carotid artery (CCA) using B-mode ultrasound producing two echogenic lines. These lines represent the combined thickness of the intimal and medial layers of the arterial wall. Each CCA was imaged in three different projections: CCA right side 90–120–150 and CCA left side 210–240–270°. The segments were measured semi-automated in triplicate.

Statistics

Data are given as mean ± SEM in the text, Tables and Figures. Differences were tested by analysis of variance (ANOVA) with LSD test as *post hoc* test and Bonferroni correction for multiple comparisons. Fisher's exact test was used to evaluate dichotomous variables. Correlation analysis was carried out using Pearson correlation statistics. Data were analysed in SPSS 17.0. Probability values <0·05 (2-tailed) were considered statistically significant.

Results

Baseline characteristics

A total of 190 subjects were included consisting of 33 controls, and 20 FH, 18 FCH, 26 T2DM, 65 CAD and 28 T2DM/CAD patients.

Table 1 shows the baseline characteristics. Patients with CAD and T2DM/CAD were older and had higher systolic blood pressure than subjects in the other groups. Patients with T2DM and T2DM/CAD had higher fasting glucose concentration than the subjects in the other groups. Furthermore, their BMI and waist circumference was the highest, whereas controls and FH patients had the lowest BMI and waist circumference. Total cholesterol and LDL-C were highest in FH and FCH patients and lowest in patients with CAD and T2DM/CAD. FH patients had higher HDL-C and apoAI when compared to the other groups. TG was highest in FCH patients and lowest in controls and FH patients. Patients with FH, FCH and T2DM showed the highest levels of total apoB.

Gender distribution, smoking behaviour and the use of relevant drugs are listed in Table 2. There were significantly more male subjects in the groups consisting of patients with CAD

Table 1 Baseline characteristics of the total group consisting of controls, and patients with FH, FCH, T2DM, CAD and T2DM/CAD

	Controls (n = 33)	FH (n = 20)	FCH (n = 18)	T2DM (n = 26)	CAD (n = 65)	T2DM/CAD (n = 28)	P-value
Age (years)	55·1 (1·7)	55·1 (1·4)	54·1 (1·6)	56·7 (1·5)	66·0 (1·4)*	67·9 (1·9)*	< 0·005
BMI (kg/m^2)	25·8 (0·8)	25·6 (0·9)	27·9 (0·6)‡	30·0 (1·0)†	27·0 (0·5)‡	28·7 (1·0)†	< 0·005
Waist circumference (m)	0·94 (0·03)	0·92 (0·02)	1·01 (0·02)‡	1·11 (0·04)†	1·04 (0·02)‡	1·10 (0·04)†	< 0·005
Systolic BP (mmHg)	126·3 (3·0)	128·7 (2·7)	126·9 (3·6)	133·8 (2·9)	143·3 (3·0)*	140·4 (4·1)*	< 0·005
Diastolic BP (mmHg)	78·6 (2·1)	76·7 (1·5)	78·5 (1·6)	78·1 (1·9)	78·7 (1·1)	75·5 (1·8)	NS
Glucose (mM)	5·28 (0·13)	5·09 (0·14)	5·61 (0·11)	10·13 (0·60)†	6·23 (0·13)	8·53 (0·32)†	< 0·005
Total cholesterol (mM)	5·13 (0·13)	6·11 (0·33)§	5·82 (0·35)§	5·08 (0·32)	4·68 (0·14)*	4·45 (0·20)*	< 0·005
LDL-C (mM)	3·23 (0·10)	3·80 (0·32)§	3·37 (0·37)§	2·99 (0·25)	2·67 (0·13)*	2·29 (0·17)*	< 0·005
HDL-C (mM)	1·43 (0·08)	1·79 (0·12)$^{¥}$	1·12 (0·06)	1·20 (0·06)	1·21 (0·04)	1·29 (0·09)	< 0·005
TG (mM)	1·07 (0·07)	1·16 (0·10)	2·62 (0·47)$^{$}$	1·73 (0·18)$^{#}$	1·74 (0·11)$^{#}$	1·89 (0·25)$^{#}$	< 0·005
ApoAI (g/L)	1·52 (0·06)	1·76 (0·08)$^{¥}$	1·34 (0·05)	1·46 (0·05)	1·34 (0·04)	1·40 (0·07)	< 0·005
ApoB (g/L)	0·95 (0·04)	1·15 (0·07)**	1·25 (0·09)**	1·12 (0·09)**	0·92 (0·03)	0·88 (0·05)	< 0·005

Data are mean (±SEM). FH: familial hypercholesterolaemia. FCH: familial combined hyperlipidaemia. T2DM: type 2 diabetes mellitus. CAD: coronary artery disease. BP: blood pressure. *P < 0·005 vs. controls and patients with FH, FCH and T2DM. $^{†}P$ < 0·005 vs. controls and patients with FH, FCH and CAD. $^{‡}P$ < 0·005 vs. controls and patients with FH, T2DM and T2DM/CAD. $^{§}P$ < 0·005 vs. controls and patients with T2DM, CAD and T2DM/CAD. $^{¥}P$ < 0·005 vs. controls and patients with FCH, T2DM, CAD and T2DM/CAD. $^{$}P$ < 0·005 vs. controls and patients with FH, T2DM, CAD and T2DM/CAD. $^{#}P$ < 0·005 vs. controls and patients with FH and FCH. **P < 0·005 vs. controls, CAD patients and T2DM/CAD. TG, triglyceride.

Table 2 Gender distribution, smoking behaviour and the use of lipid-lowering drugs in controls, and patients with FH, FCH, T2DM, CAD and T2DM/CAD

	Controls (n = 33)	FH (n = 20)	FCH (n = 18)	T2DM (n = 26)	CAD (n = 65)	T2DM/CAD (n = 28)	P-value
Gender (% male)	36·4†	35·0†	55·6	42·3†	66·2*	64·3*	0·02
Smoking (% users)	9·1	10·0	11·1	19·2	12·3	10·7	0·89
Statins (% users)	0	50·0	61·2	69·2	76·9	85·7‡	< 0·005
Ezetimibe (% users)	0§	25·0	38·9	7·7§	18·5	17·9	0·007
Fibrate (% users)	0	0	11·1$^{¥}$	0	1·5	7·1$^{¥}$	0·09

*P < 0·05 vs. patients in the other groups. $^{†}P$ < 0·05 vs. the other groups. $^{‡}P$ < 0·005 vs. controls and FH patients. $^{§}P$ < 0·05 vs. patients with FH, FCH CAD and T2DM/CAD. $^{¥}P$ = 0·09 vs. controls, and patients with FH, T2DM and CAD.
CAD, coronary artery disease; FCH, familial combined hyperlipidaemia; FH, familial hypercholesterolaemia; T2DM, type 2 diabetes mellitus.

and T2DM/CAD. More female subjects were found in controls and patients with FH and T2DM. No differences were found for smoking behaviour between the groups. Most statins were used in patients with T2DM/CAD, but it reached only statistical significance when compared to controls and FH patients. Patients with FH, FCH, CAD and T2DM/CAD used more ezetimibe than the other groups. There was a trend for a more frequent use of fibrates in patients with FCH and T2DM/CAD.

Differences in apoB48 and IMT

ApoB48 levels were highest in patients with FCH (11·38 ± 1·50 mg/L) and T2DM/CAD (9·65 ± 1·14 mg/L), when compared to controls (5·74 ± 0·55 mg/L) and patients with FH (5·40 ± 0·51 mg/L), T2DM (7·07 ± 0·83 mg/L) and CAD (8·09 ± 0·57 mg/L) ($P < 0·01$) (Fig 1). CAD patients had also higher apoB48 levels than controls and patients with FH ($P < 0·01$; Fig. 1).

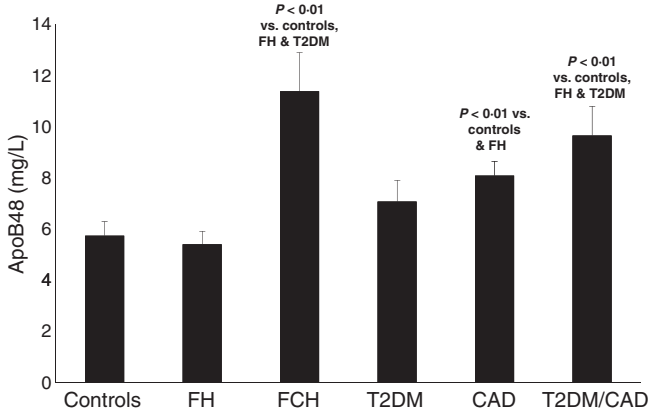

Figure 1 Mean ± SEM fasting apoB48 (mg/L) levels in controls, and patients with familial hypercholesterolaemia (FH), familial combined hyperlipidaemia (FCH), type 2 diabetes mellitus (T2DM), coronary artery disease (CAD) and T2DM/CAD.

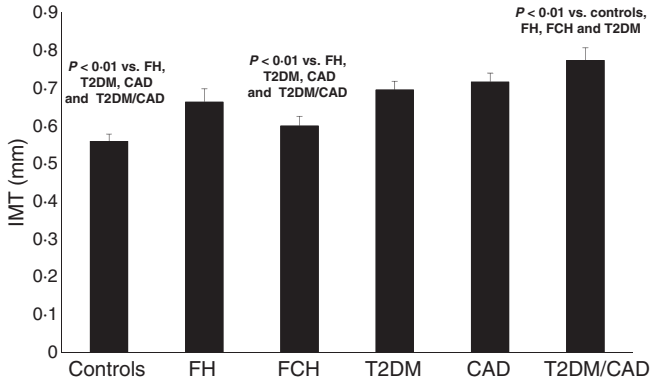

Figure 2 Mean ± SEM IMT (mm) measures in controls, and patients with familial hypercholesterolaemia (FH), familial combined hyperlipidaemia (FCH), type 2 diabetes mellitus (T2DM), coronary artery disease (CAD) and T2DM/CAD. IMT, intima-media thickness.

Of 190 subjects, 113 gave consent to undergo the IMT measurements. The distribution was as follows: 19 controls, 16 FH patients, 15 FCH patients, 17 T2DM patients, 31 CAD patients and finally 15 patients with T2DM/CAD. In general, the highest IMT was measured in patients with T2DM/CAD (0·77 ± 0·03 mm; $P < 0·01$) (Fig. 2). Controls (0·56 ± 0·02 mm) and patients with FCH (0·60 ± 0·03 mm) showed the lowest IMT measurements ($P < 0·01$). Patients with FH (0·66 ± 0·03 mm), T2DM (0·69 ± 0·02 mm) and CAD (0·72 ± 0·02 mm) showed comparable IMT's.

Correlation studies

Total group. In the total group, the best correlation for apoB48 was found with fasting plasma TG ($R = 0·72$, $P < 0·001$). Moreover, apoB48 correlated positively with total apoB ($r = 0·26$, $P = 0·001$), total cholesterol ($R = 0·22$, $P = 0·003$) and waist circumference ($R = 0·21$, $P = 0·02$). Negative correlations were found for HDL-C ($R = -0·32$, $P < 0·001$) and apoAI ($R = -0·18$, $P = 0·02$).

In this group of subjects, IMT correlated positively with age ($R = 0·57$, $P < 0·001$), systolic blood pressure ($R = 0·34$, $P < 0·001$), fasting glucose ($R = 0·30$, $P = 0·002$) and waist circumference ($R = 0·29$, $P = 0·007$).

Subjects not using statins, ezetimibe and fibrates. In the group of subjects not using statins, ezetimibe and fibrates ($n = 74$), the best correlation for apoB48 was found with fasting plasma TG ($R = 0·69$, $P < 0·001$) and IMT ($R = 0·52$, $P < 0·001$, Fig. 3). ApoB48 also correlated positively with waist

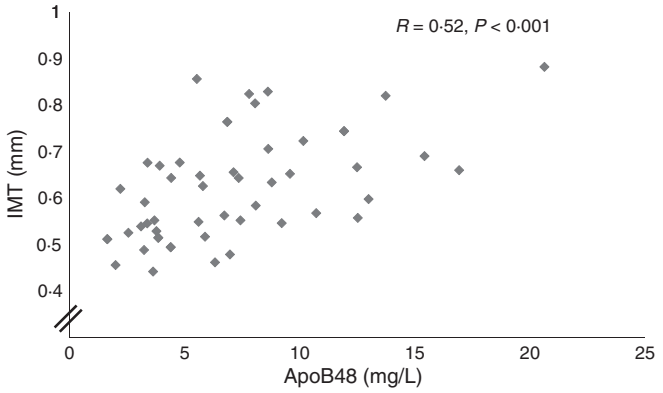

Figure 3 Correlation plot between apoB48 levels (mg/L) and IMT (mm) measures in subjects not using lipid-lowering drugs. IMT, intima-media thickness.

circumference ($R = 0{\cdot}38$, $P = 0{\cdot}009$) and fasting glucose ($R = 0{\cdot}21$, $P = 0{\cdot}04$). No correlation was found between apoB48 and apoB ($R = 0{\cdot}20$, $P = 0{\cdot}12$).

After exclusion of controls, still a strong correlation persisted between apoB48 and IMT ($n = 27$, $R = 0{\cdot}47$, $P = 0{\cdot}01$), and no correlation was found between IMT and total apoB ($R = -0{\cdot}02$, $P = 0{\cdot}91$).

In the group of subjects not using lipid-lowering drugs, besides correlating with apoB48, IMT also correlated positively with age ($R = 0{\cdot}57$, $P < 0{\cdot}001$), fasting TG ($R = 0{\cdot}55$, $P < 0{\cdot}001$), fasting glucose ($R = 0{\cdot}43$, $P = 0{\cdot}003$) and waist circumference ($R = 0{\cdot}38$, $P = 0{\cdot}02$). Negative correlations were found with HDL-C ($R = -0{\cdot}29$, $P < 0{\cdot}05$). IMT did not correlate with total apoB ($R = 0{\cdot}20$, $P = 0{\cdot}12$).

Discussion

In this study, we show for the first time that there is a strong positive correlation between *fasting* plasma apoB48 concentrations and the surrogate marker for subclinical atherosclerosis IMT in subjects not using lipid-lowering drugs. Another interesting finding is that a single measurement of fasting apoB48 differentiates (treated) patients with FCH and T2DM/CAD from the other groups of patients. CAD patients also showed higher fasting apoB48 levels than controls, and FH and T2DM patients. These data are in line with previous studies showing that patients with FCH, CAD and T2DM have a delayed clearance of chylomicron remnants. Although we did not carry out postprandial studies, these data are also in line with the study by Smith *et al.* [17], showing that one single apoB48 measurement correlates with postprandial remnant clearance. As patients with CAD and T2DM/CAD reached overall LDL and total apoB targets, our data showing elevated apoB48 levels may have therapeutic implications in the future. Indeed, these patients may still be at a higher risk for future cardiovascular disease. To establish the risk associated with elevated apoB48 levels, prospective studies including this measurement will be necessary. In addition, new strategies to reduce apoB48 levels and postprandial hyperlipidaemia need to be developed.

In our study, the strong correlation between fasting apoB48 and IMT persisted after excluding controls. This finding suggests that apoB48 measurements may help to detect subjects at risk for postprandial hyperlipidaemia and higher cardiovascular disease. Interestingly, in both the total group and the group of subjects not using lipid-lowering drugs, total apoB did not correlate with IMT. This finding is in contrast to data published in the literature in the same patient groups as in our study [24–26]. One of the explanations may be the limited number of subjects included, in contrast to the other publications. However, the strong correlations with apoB48 suggest that this may be a stronger marker than total apoB. Chylomicron remnants have been shown to be atherogenic [27,28]. Remnant lipoproteins and postprandial TG concentrations have also been associated with IMT [29,30]. Our study not only confirms those data but also shows that fasting levels of apoB48, reflecting the presence of chylomicron remnants, are also associated with IMT. These data suggest that IMT may be more associated with intestinal lipoproteins than with hepatic lipoproteins. Another explanation for the lack of association between total apoB and IMT comes from studies on atherosclerotic plaques. Previously, it has been shown that apoB48 (intestinal) lipoproteins were found in relatively higher concentrations in the plaques than apoB100-containing lipoproteins [31–33]. The final explanation comes from the large cohort studies on predicting the role of lipids in cardiovascular disease. *Total* apoB has been shown to predict fatal myocardial infarction in both genders [24,34]. However, measurements of *apoB100* have been suggested to have no additional value for the prediction of CVD when compared to non-HDL-C and the ratio of total cholesterol to HDL-C [35,36]. Despite the fact that these studies were only carried out in women, the overall evidence points at a significant role for apoB48 in the association between apoB and atherosclerosis.

In the total population, including subjects on lipid-lowering drugs, no relationship was found between apoB48 and IMT measurements. The impact of statins on apoB48 levels is not conclusive. It has been shown that both atorvastatin and rosuvastatin caused significant decreases in TG and apoB48 [37,38] compared with combination therapy. Monotherapy with either ezetimibe or simvastatin was associated with smaller, nonsignificant reductions in apoB48 particle size and production rate [39]. The authors linked this lack of significance to measurement variances and the relatively small sample size. Moreover, differences on the impact of statins on apoB48 levels within clinical disease have been described before. For example, reduction in both TG and apoB48 in the chylomicron fraction during atorvastatin treatment, compared with placebo, was shown in subjects with hypertriglyceridaemia [40] and type III hyperlipoproteinaemia [41], but not in subjects with FCH [14], most likely due to the small number of subjects included in the latter study. Despite these inconsistencies on the role of statins on apoB48 levels, the majority of the data point towards a reduction of apoB48 levels by statins. Therefore, in our opinion, the lack of relationship between apoB48 and IMT could be explained by the use of statins.

Preferential accumulation of fat in the abdominal region (visceral abdominal fat) is expressed as waist circumference. It has been shown that waist circumference is associated with fasting and postprandial TG [5] and postprandial apoB48 concentrations [42]. As there is a strong relationship between fasting and postprandial TG and apoB48 levels [17,42], it is not a surprise that this correlation was found.

We did not find elevated apoB48 levels in FH patients. As mentioned before, conflicting data on FH and postprandial lipaemia have been published [10–13]. Already in 1987, Weintraub *et al.* [10] showed that subjects with type IIa hyperlipoproteinaemia, who have similar lipoprotein phenotypes as FH patients, exhibit 'abnormally low chylomicron fractions'. Later, in 1990, Rubinsztein *et al.* [11] described that homozygote FH patients have normal postprandial clearance of chylomicron remnants. In contrast to these data, others showed that patients with FH do have a delayed postprandial remnant clearance [12,13]. This finding was confirmed by Dane-Steward *et al.* [43]. Tremblay *et al.* [44] demonstrated elevated levels of intestinally derived triglyceride-rich lipoproteins because of an increased production rate without abnormalities in the plasma apoB48 catabolism in patients with heterozygous FH. So, mechanistic postprandial studies on apoB48 levels in FH patients in comparison with healthy controls are needed to explain these conflicting data.

In conclusion, in this clinical study, fasting apoB48 concentrations are highest in patients with FCH and in patients with both CAD and T2DM. In patients not using statins, the surrogate atherosclerosis marker IMT correlates best with apoB48, suggesting that fasting apoB48 may help to detect subjects at risk for cardiovascular disease.

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Disclosures

None declared.

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