Factors influencing elevated serum apolipoprotein B48 in diabetic and control participants

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ABSTRACT

Factors influencing the concentration of apolipoprotein B48 (apo B48) at fasting and post-prandial time frames are still being elucidated. This study assesses some possible contributing factors including the presence of type 2 diabetes and gender using an established enzyme-linked immunosorbent assay (ELISA) method. Apo B48 and triglyceride (TG) levels were measured before and for two, four and six hours post-prandially in 49 poorly controlled participants with type 2 diabetes and in 60 apparently healthy participants (controls). Apo B48 levels in the control participants increased post-prandially, peaking at four hours (14.81±7.72 μg/mL) with similar responses demonstrated in TG concentrations. Post-prandial apo B48 levels were significantly higher in male control participants as demonstrated by apo B48 area under the curve (AUC); similar responses were also confirmed in triglyceride AUC. Post-prandial apo B48 concentrations in control participants correlated with HOMA-IR ($P<0.05$). Apo B48 continued to increase throughout the six hours in participants with type 2 diabetes (17.73±13.46 μg/mL), when levels were significantly greater than in the control participants (13.04±7.67 μg/mL) ($P<0.05$) despite a decrease in accompanying TG levels in participants with type 2 diabetes. Using an ELISA method, this study demonstrated that gender, insulin resistance (as evidenced by HOMA-IR) and diabetes status influence serum apo B48 levels. These effects were only apparent post-prandially.

KEY WORDS: Apolipoprotein B-48.
Diabetes.
Post-prandial.

Introduction

Plasma triglyceride (TG) levels measured in the post-prandial state more closely predict cardiovascular end-points compared to levels measured under fasting conditions.[1–4] Post-prandial hypertriglyceridaemia occurs in patients with type 2 diabetes[5,6] and potentially contributes to the marked increase in cardiovascular disease associated with this condition.

Triglycerides circulate as TG-rich lipoproteins (TRL), which include both very low-density lipoproteins (VLDL), produced by the liver, and chylomicrons produced in the intestine in response to an intake of dietary fat. Intestinally derived lipoproteins are characterised by the presence of apolipoprotein B48 (apo B48), which in humans is exclusively produced by the intestine.[7,8] While diabetic hypertriglyceridaemia is at least partially due to increased production and reduced clearance of hepatically derived lipoproteins, there is also some evidence of an increase in intestinally derived lipoproteins.[9,10] However, the extent of this and whether or not it is altered by improved glycaemic control is not known.

In previous studies where intestinally derived lipoprotein metabolism was studied, the quantification of apo B48 in human serum has proved difficult. This was mainly due to low concentrations, structural similarity to B100 and lack of appropriate apo B48 standards.[11–17] Development of an enzyme-linked immunosorbent assay (ELISA) method has provided a rapid and simple means to determine the concentration of apo B48 in plasma. This technique does not require prior separation of TRL using ultracentrifugation and does not cross-react with apo B100.[18–23]

The aim of this study is to determine the apo B48 response to a mixed meal in apparently healthy participants and in participants with poorly controlled type 2 diabetes. Statin treatment is now a fundamental component of management of patients with diabetes, and participants with diabetes included similar numbers of patients receiving or not receiving statins.

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Materials and methods

Participants

Forty-nine participants with poorly controlled type 2 diabetes were recruited from the Diabetes Day Care Centre at the Adelaide and Meath incorporating the National Children's Hospital, Dublin. All participants were either newly diagnosed or poorly controlled patients with type 2 diabetes mellitus. Patients were excluded if there was hyperlipidaemia secondary to renal disease, liver disease and thyroid dysfunction, or a known primary (genetic) hyperlipidaemia.

All participants were aged 21 years and over. Six participants were smokers, 28 participants reported no alcohol units per week and 41 participants followed a low fat diet at initiation. Thirty-three were established on oral antihyperglycaemic agents at commencement of the study, while 21 were receiving statins (Table 1). Statin treatment is now a fundamental component of management of patients

with diabetes. The cohort with diabetes included similar numbers of participants receiving or not receiving statins based on assessment of presenting lipid profile.

The control participants comprised 60 non-diabetic, apparently healthy volunteers on no medications, recruited from the general population. The exclusion criteria for control participants included the following conditions: myocardial infarction, angina or heart disease, liver disease, thyroid disease, hypertension, renal failure and other debilitating illness. The use of lipid-lowering drugs was also grounds for exclusion. Eight participants smoked, six participants reported no alcohol units per week and eight participants followed a low-fat diet. All control participants underwent a standard 75 g oral glucose tolerance test.[24]

The study was approved by the SJH/AMNCH Research Ethics Committee, Dublin, and each research participant gave written and informed consent before commencement.

Study design

The study was a cross-sectional comparison of post-prandial lipoproteins in 49 participants with poorly controlled type 2 diabetes and 60 apparently healthy volunteers.

Test meal

Participants were advised to avoid alcohol and rigorous physical activity for two days prior to the study. Each participant was required to fast overnight (12–14 hours) and to attend the Diabetes Day Care Centre the following morning. A 20-G venous cannula was inserted at initiation of the visit and fasting blood was drawn into serum tubes. Each participant was given a 1250 kcal fat challenge meal consisting of approximately 67.3 g carbohydrates, 47.2 g protein and 82 g fat (24 g saturates, 32 g monounsaturated, 26 g polyunsaturated and 285 mg cholesterol). Subsequent blood samples were taken at two, four and six hours after consuming the meal. Participants were required to refrain from exercise during the visit and were permitted only water to drink after the meal. All participants remained in the hospital until study completion six hours after the test meal.

Table 1. Medication used in the treatment of participants with type 2 diabetes.

Medication	All participants (baseline)	Intensification (Pre-)	(Post-)
	n=49	*n*=41	*n*=41
Metformin only	30	28	24
Gliclazide only	3	2	3
Metformin + gliclazide	2	2	10
Metformin + rosiglitazone	1	1	1
No oral antihyperglycaemic agent	13	8	3
Statin	21	15	24
No statin	28	26	17

Table 2. Baseline data and metabolic variables in control participants (control) and participants with type 2 diabetes (cohort) subdivided into those receiving and not receiving statin treatment.

	Control	Cohort	Cohort on statin	Cohort – no statin
	(*n*=60)	(*n*=49)	(*n*=21)	(*n*=28)
Age (years)	49.9±5.3	56.3±8.5*	55.1±9.8*	57.3±7.4*
Gender (m/f)	20/40	22/27	11/10	11/17
BMI (kg/m^2)	28.6±6.6	32.2±6.31*	33.5±7.5*	31.3±5.2
HOMA-IR	1.71±1.40	4.89±2.97*	4.81±2.34*	4.95±3.42*
HbA1c (%)	5.5±0.5	8.6±1.4†	8.3±1.1*	8.9±1.5*
ApoB (g/dL)	1.01±0.27	0.93±0.24	0.84±0.23*	0.99±0.23‡
Total cholesterol (mmol/L)	5.1±1.0	4.6±1.1*	4.2±1.0*	4.85±1.12‡
HDL-C (mmol/L)	1.40±0.42	1.25±0.33	1.23±0.40	1.27±0.28
LDL-C (mmol/L)	3.35±1.01	2.88±0.86*	2.66±0.85*	3.05±0.85
TG fasting (mmol/L)	1.46±0.64	2.26±2.01*	1.95±1.13*	2.49±2.47*
TG 2 h (mmol/L)	†2.03±0.84	†2.82±2.20*	†2.52±1.31	†3.04±2.69*
TG 4 h (mmol/L)	†2.19±1.08	†3.05±2.34*	†2.65±1.45	†3.34±2.82*
TG 6 h (mmol/L)	†1.77±0.88	†2.66±1.58*	†2.34±1.53	†3.13±2.70*
TG AUC (mmol/h/L)	11.97±6.00	16.77±13.17*	14.64±7.82	18.37±16.03*
TGi AUC (mmol/h/L)	2.92±1.96	3.37±2.50	3.12±2.18	3.56±2.74
Apo B48 fasting (μg/mL)	7.41±4.07	9.59±9.64	10.85±13.29	8.66±5.66
Apo B48 2 h (μg/mL)	†13.86±7.09	†14.23±9.18	†14.35±10.78	†14.31±7.98
Apo B48 4 h (μg/mL)	†14.81±7.72	†16.44±10.42	†15.11±10.95	†17.43±10.08*
Apo B48 6 h (μg/mL)	†13.04±7.67	†17.73±13.46*	†17.84±16.22	†17.65±11.28
Apo B48 AUC (μg/h/mL)	77.78±36.02	88.66±52.53	87.62±62.07	89.43±45.28
Apo B48i AUC (μg/h/mL)	33.49±20.15	35.45±26.94	31.20±21.99	38.64±30.12

*P<0.05 *vs.* normal; †P<0.05 *vs.* fasting, ‡P<0.05 *vs.* cohort on statin.

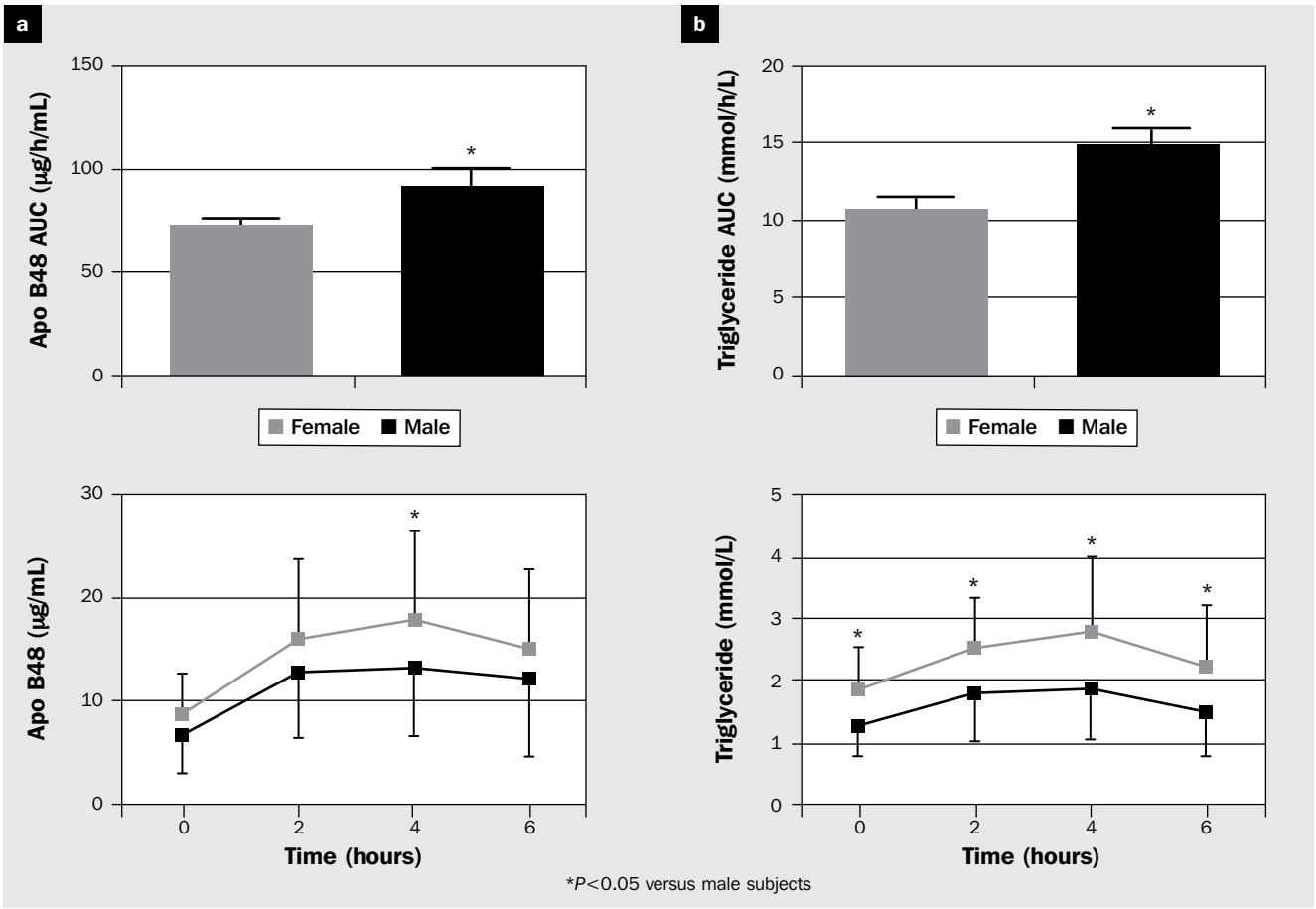

Fig. 1. a) Apo B48 concentration in the male and female control populations; and **b)** Triglyceride concentration in the male and female control populations.

Analytical methods

All samples if not for immediate analysis were separated and stored at –20˚C or –70˚C as appropriate (for no longer than 3–12 months as appropriate).

Lipid and glucose analyses were performed on a Roche Hitachi MODULAR P autoanalyser. All reagents and calibrators were provided by Roche Diagnostics. Insulin and C-peptide analysis was performed at St. James's Hospital, Dublin, on the Autodelphia automatic immunoassay system. Direct LDL and HDL methodology was provided by Randox Laboratories.

Fasting glucose and insulin levels were used to calculate the HOMA-IR value (Homeostasis Model Assessment of Insulin Resistance).[25] Apolipoprotein B measurement was performed on the Dade Behring Nephelometer Analyser II. High-performance liquid chromatography using the A1c 2.2 Plus glycohaemoglobin analyser was used to determine HbA1c concentrations. The ELISA for apo B48 was obtained from Shibayagi (Gunma, Japan; www.shibayagi.co.jp). The assay characteristics have been described previously.[20,22,26] There was no cross-reactivity with apo B100 as previously described.[22]

Statistical analysis

Variables are expressed as mean±standard deviation (SD). Statistical evaluation was performed using SPSS 16.0.1 software provided by SPSS. Significance was set at $P<0.05$. The area under the time concentration curve (AUC) was calculated using the trapezoidal method, with GraphPad Prism 5.01 software (GraphPad Software). The incremental area under the curve (iAUC) was calculated from serum concentrations after the subtraction of baseline values. Analytical variables did not demonstrate normal Gaussian distribution; therefore, non-parametric statistics were employed. Variables were compared between groups using a Mann-Whitney U test. Variables for which there was a significant gender effect were compared following adjustment for gender. A Friedman test was used to determine an overall time effect in variables measured post-prandially. Where differences were identified, post-hoc analysis was carried out using Wilcoxon Signed-Rank tests.

Results

Triglyceride and ApoB48 responses in control participants

Data from control participants are shown in Table 2. The female to male ratio in the control group was 2:1 (40 females, 20 males; mean age: 49.9±5.3; mean BMI: 28.6 kg/m^2). Control participants had higher fasting cholesterol and LDL-C levels ($P<0.05$) when compared to participants with type 2 diabetes. There was an 86% increase in apo B48 level at the two-hour time-point compared to baseline, and a further increase at the four-hour time-point. There was a decrease between the four-hour and the six-hour time-point. Similar changes were seen in plasma triglyceride levels.

Table 3 and Figures 1a and 1b demonstrate the effect of gender. Male control participants were more insulin-

resistant and had lower HDL-C ($P<0.05$). Fasting TG, TG AUC, TG iAUC, and TG measured at all time-points were greater in male participants ($P<0.05$). Fasting apo B48 did not differ between gender but apo B48 AUC and apo B48 measured at the four-hour time-point were greater in men ($P<0.05$). These effects remained significant following adjustment for HOMA-IR.

Table 4 demonstrates univariate relationships between apo B48 levels and other relevant variables. There was no effect of age or BMI on apo B48 levels. HOMA-IR correlated with apo B48 measured at the four- and six-hour time-points (P<0.05). Fasting apo B48 levels correlated strongly with apo B48 measured at all time-points ($P<0.05$). Apo B48 levels at all time-points also correlated strongly with fasting triglyceride and triglyceride AUC ($P<0.05$).

Triglyceride and ApoB48 responses in participants with type 2 diabetes

Baseline data from participants with diabetes are compared to the control participants in Table 2. Mean age and BMI were greater in participants with type 2 diabetes. The ratio of female to male participants was greater in control participants and therefore statistical comparisons are reported following adjustment for gender. Fasting TG, TG AUC and TG measured at all time-points were greater in participants with type 2 diabetes ($P<0.05$). In contrast to control participants, apo B48 levels rose throughout the measurement period in participants with diabetes, with levels at each time point significantly greater than control participants ($P<0.05$).

Twenty-one participants with type 2 diabetes were receiving statin treatment in the baseline studies, while 28 were not. Participants receiving statin treatment demonstrated lower apo B and LDL-C levels when compared with those not receiving treatment; no significant changes in post-prandial lipaemia were noted between the groups. When results from those receiving and not receiving statin treatment were compared separately with control participants, differences only emerged in those not receiving statins. Triglyceride and apo B48 levels in participants with type 2 diabetes receiving statin treatment did not differ from control participants, while among participants with diabetes not receiving statin treatment the fasting TG, TG AUC and TG measured at all time-points and apo B48 measured at the four-hour time-point were greater in participants with diabetes ($P<0.05$).

The participants with type 2 diabetes were treated with a range of medications outlined in Table 1. The reported impact of such medications on post-prandial lipaemia is incongruous; a number of studies have attempted to assess impact of metformin therapy on post-prandial lipaemia with conflicting results.[27–30] Sulfonylureas have led to an increase in post-prandial insulin concentrations,[29,31,32] glipizide resulted in a decrease in VLDL TG concentrations[29] but not chylomicron triglyceride concentrations. Thiazolinediones have demonstrated varying effects on post-prandial lipaemia; some studies have reported no effects,[33,34] as opposed to decreases in post-prandial triglyceride levels[35,36] with decreases in chylomicron concentrations.[37]

Table 3. Characteristics of control participants (male and female).

	Female ($n=40$)	Male ($n=20$)
Age (years)	50.4±5.5	49.0±4.8
Weight (kg)	78.4±20.2	92.1±15.9*
BMI (kg/m^2)	28.5±7.7	28.7±3.6
HOMA-IR	1.56±1.45	2.01±1.26*
HbA1c (%)	5.4±0.5	5.6±0.5
Glucose (mmol/L)	5.1±0.5	5.2±0.6
Apo B (g/dL)	0.97±0.30	1.10±0.19*
Cholesterol (mmol/L)	5.1±1.1	5.2±0.8
HDL-C (mmol/L)	1.55±0.41	1.10±0.25*
LDL-C (mmol/L)	3.26±1.04	3.53±0.96

*$P<0.05$ *vs.* female.

Discussion

This study has characterised the response of apo B48 and TG to a standard meal in participants with and without type 2 diabetes. The results show that apo B48 levels increased following a mixed meal, peaking at four hours in control participants, but continued to increase in participants with type 2 diabetes six hours post-prandially when a small but significant difference in levels between groups was observed. This differed from the TG response which demonstrated decreasing levels at six hours post-prandially with participants from both cohorts. Apo B48 levels were significantly greater in normal male participants compared to female participants and this difference persisted following adjustment for HOMA-IR, which was an independent correlate of apo B48. This was accompanied by significantly greater TG levels at all measurement points.

Table 4. Univariate relationships (Spearman's rho correlation) in control participants.

	Apo B48 (fasting)	Apo B48 (2 h)	Apo B48 (4 h)	Apo B48 (6 h)	Apo B48 (AUC)
Age	0.182	0.11	0.039	0.209	0.133
BMI	0.067	–0.01	–0.01	0.238	0.123
HOMA-IR	0.081	0.028	0.282*	0.297*	0.189
Apo B48 (fasting)	–	0.628†	0.643†	0.656†	0.766†
Triglyceride (fasting)	0.568†	0.474†	0.452†	0.506†	0.562†
Triglyceride (AUC)	0.557†	0.567†	0.54†	0.541†	0.615†

*$P<0.05$; †$P<0.001$.

Improved glycaemic control did not influence apo B48 levels at any time-point but differences were observed at the end of the study between participants with diabetes receiving statin treatment compared to those who were not. Gender differences, the effect of insulin resistance, diabetes status and statin use were not observed under fasting conditions and were most clearly demonstrable at the later time-points of the study. The ELISA method used provides a relatively quick and simple procedure for the quantification of apo B48 in serum, eliminating the need for ultracentrifugation.

The mean fasting concentration of apo B48 in control participants was similar to those reported in two previous studies of healthy controls, also using the ELISA method.[18,20] Apo B48 levels in control participants peaked at the four-hour time-point post-prandially and had returned to baseline at completion of the study protocol in seven control participants. These observations are broadly similar to other studies in control participants.[17,18,22,38]

Significant correlations were noted in control participants between HOMA-IR and apo B48 measured at the four- and six-hour time-points post-prandially. A mechanistic explanation for this is provided by recent studies in human participants, which have demonstrated association of hyperinsulinaemia with increased apo B48 production rate.[39] The observation that there were no differences in apo B48 AUC or iAUC in poorly controlled participants with diabetes compared to control participants contrasts with differences in apo B48 levels that were observed in some previous studies,[10] and also with the significant differences that were observed in post-prandial TG levels between participant groups in the current study.

There are mechanistic reasons to expect apo B48 levels to be increased in participants with diabetes relating to both increased production and reduced clearance of intestinally derived lipoprotein particles.[10,41–44] It is possible that the relatively minor difference observed in the current study may be reflective of the use of statin therapy. Some studies have reported a decrease in post-prandial lipaemia with the use of various statin medication[45–48] thought to be directly linked to the lowering of LDL-C concentration.[49] Additionally, apo B48 levels were still increasing in participants with diabetes by the six-hour time-point, unlike control participants, indicating a continuing dyslipidaemia as the next meal approached.

Conclusions

In summary, post-prandial apo B48 levels measured in the post-prandial setting are influenced by gender, HOMA-IR, diabetes status and statin use. None of these effects were observed under fasting conditions and in general only emerged four or six hours following the meal challenge. The authors conclude that apo B48 measured by ELISA is a useful addition to techniques used to study post-prandial lipoprotein metabolism. □

References

1 Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation* 1979; **60** (3): 473–85.

2 Nordestgaard BF, Benn M, Schnor P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007; **298** (3): 299–308.

3 Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and the risk of cardiovascular events in women. *JAMA* 2007; **298** (3): 309–16.

4 Patel A, Barzi F, jamrozik K *et al*.; Asia Pacific Cohort Studies Collaboration. Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. *Circulation* 2004; **110** (17): 2678–86.

5 Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. *J Intern Med* 1999; **246** (4): 341–55.

6 Ginsberg HN, Illingworth DR. Postprandial dyslipidemia: an atherogenic disorder common in patients with diabetes mellitus. *Am J Cardiol* 2001; **88** (6A): 9H–15H.

7 Young SG. Recent progress in understanding apolipoprotein B. *Circulation* 1990; **82** (5): 1574–94.

8 Tushuizen ME, Diamant M, Heine RJ. Postprandial dysmetabolism and cardiovascular disease in type 2 diabetes. *Posgrad Med J* 2005; **81** (951): 1–6.

9 Reyes-Soffer G, Holleran S, Karmally W *et al*. Measures of postprandial lipoproteins are not associated with the presence of coronary artery disease in patients with type 2 diabetes mellitus. *J Lipid Res* 2009; **50** (9): 1901–9.

10 Hogue JC, Lamarche B, Tremblay AJ, Bergeron J, Gagné C, Couture P. Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes. *J Lipid Res* 2007; **48** (6): 1336–42.

11 Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *J Lipid Res* 1988; **29** (7): 925–36.

12 Cohn JS, Johnson EJ, Millar JS *et al*. Contribution of apoB-48 and apoB-100 (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *J Lipid Res* 1993; **34** (12): 2033–40.

13 Castro Cabezas M, de Bruin TWA, Kock LAW, de Bruin TW. Postprandial apolipoprotein B100 and B48 in familial combined hyperlipidemia before and after reduction of fasting plasma triglycerides. *Eur J Clin Invest* 1994; **24** (10): 669–78.

14 Lemieux S, Fontani R, Uffelman KD, Lewis GF, Steiner G. Apolipoprotein B-48 and retinyl palmitate are not equivalent markers of postprandial intestinal lipoproteins. *J Lipid Res* 1998; **39** (10): 1964–71.

15 Batista MC, Welty FK, Diffenderfer MR *et al*. Apolipoprotein A-I, B-100 and B-48 metabolism in subjects with chronic kidney disease, obesity, and the metabolic syndrome. *Metabolism* 2004; **53** (10): 1255–61.

16 Verseyden C, Meijssen S, Cabezas MC. Effects of atorvastatin on fasting plasma and marginated apolipoproteins B48 and B100 in large, triglyceride-rich lipoproteins in familial combined hyperlipidemia. *J Clin Endocrinol* Metab 2004; **89** (10): 5021–9.

17 Campos H, Khoo C, Sacks FM. Diurnal and acute patterns of postprandial apolipoprotein B-48 in VLDL, IDL and LDL from normolipidemic humans. *Atherosclerosis* 2005; **181** (2): 345–51.

18 Sakai N, Uchida Y, Ohashi K *et al*. Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. *J. Lipid Res* 2003; **44** (6): 1256–62.

19 Jackson KG, Williams CM. Apolipoprotein B-48: comparison of fasting concentrations measured in normolipidaemic individuals using SDS-PAGE, immunoblotting and ELISA. *Atherosclerosis* 2004; **176** (2): 207–17.

20 Kinoshita M, Kojima M, Matushima T, Teramoto T. Determination of apolipoprotein B-48 in serum by a sandwich ELISA. *Clin Chim Acta* 2005; **351** (1–2): 115–20.

21 Lamon-Fava S, Diffenderfer MR, Barrett HR *et al*. Extended-release niacin alters the metabolism of plasma apolipoprotein (Apo) A-I and ApoB-containing lipoproteins. *Arterioscler Thromb Vasc Biol* 2008; **28** (9): 1672–8.

22 Otokozawa S, Ai M, Diffenderfer MR *et al*. Fasting and postprandial apolipoprotein B-48 levels in healthy, obese, and hyperlipidemic subjects. *Metabolism* 2009; **58** (11): 1536–42.

23 Lorec AM, Juhel C, Pafumi Y *et al*. Determination of apolipoprotein B-48 in plasma by a competitive ELISA. *Clin Chem* 2000; **46** (10): 1638–42.

24 World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultantion. Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva: WHO, 1999.

25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Tracher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting glucose and insulin concentrations in man. *Diabetologia* 1985; **28** (7): 412–9.

26 Uchida Y, Kurano Y, Ito S. Establishment of monoclonal antibody against human apo B-48 and measurement of apo B-48 in serum by ELISA method. *J Clin Lab Anal* 1998; **12** (5): 289–92.

27 Wu MS, Johnston P, Sheu WH *et al*. Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. *Diabetes Care* 1990; **13** (1): 1–8.

28 Hollenbeck CB, Johnston P, Varasteh BB, Chen YD, Reaven GM. Effects of metformin on glucose, insulin and lipid metabolism patients with mild hypertriglyceridaemia and non-insulin dependent diabetes by glucose tolerance test criteria. *Diabete Metab* 1991; **17** (5): 483–9.

29 Jeppesen J, Zhou MY, Chen YD, Reaven GM. Effect of metformin on postprandial lipemia in patients with fairly to poorly controlled NIDDM. *Diabetes Care* 1994; **17** (10): 1093–9.

30 James AP, Watts GF, Mamo JC. The effect of metformin and rosiglitazone on postprandial lipid metabolism in obese insulin resistant subjects. *Diabetes Obes Metab* 2005; **7** (4): 381–9.

31 Skrapari I, Perrea D, Ioannidis I *et al*. Glibenclamide improves postprandial hypertriglyceridaemia in type 2 diabetic patients by reducing chylomicrons but not very low-density lipoprotein subfraction levels. *Diabet Med* 2001; **18** (10): 781–5.

32 Vakkilainen J, Mero N, Schweizer A, Foley JE, Taskinen MR. Effects of nateglinide and glibenclamide on postprandial lipid and glucose metabolism in type 2 diabetes. *Diabetes Metab Res Rev* 2002; **18** (6): 484–90.

33 Raskin P, Rappaport EB, Cole ST, Yan Y, Patwardhan R, Freed MI. Rosiglitazone short-term monotherapy lowers fasting and post-prandial glucose in patients with type II diabetes. *Diabetologia* 2000; **43** (3): 278–84.

34 Shimono D, Kuwamura N, Nakamura Y, Koshiyama H. Lack of effect of pioglitazone on postprandial triglyceride levels in type 2 diabetes. *Diabetes Care* 2001; **24** (5): 971.

35 Tan GD, Fielding BA, Currie JM *et al.* The effects of rosiglitazone on fatty acid and triglyceride metabolism in type 2 diabetes. *Diabetologia* 2005; **48** (1): 83–95.

36 Mori Y, Itoh Y, Obata T, Tajima N. Effects of pioglitazone *vs.* glibenclamide on postprandial increases in glucose and triglyceride levels and on oxidative stress in Japanese patients with type 2 diabetes. *Endocrine* 2006; **29** (1): 143–8.

37 van Wijk JPH, Castro-Cabezas MC, de Koning EJP, Rabelink TJ. Rosiglitazone improves postprandial triglyceride and free fatty acid metabolism in type 2 diabetes. *Diabetes Care* 2005; **28** (4): 844–9.

38 Nakano T, Tanaka A, Okazaki M, Tokita Y, Nagamine T, Nakajima K. Particle size of apoB-48 carrying lipoproteins in remnant lipoproteins isolated from postprandial plasma. *Ann Clin Biochem* 2011; **48** (Pt 1): 57–64.

39 Duez H, Lamarche B, Uffelman KD, Valero R, Cohn JS, Lewis GF. Hyperinsulinemia is associated with increased production rate of intestinal apolipoprotein B-48-containing lipoproteins in humans. *Arterioscl Thromb Vasc Biol* 2006; **26** (6): 1357–63.

40 Duez H, Lamarche B, Valero R *et al*. Both intestinal and hepatic lipoprotein production are stimulated by an acute elevation of plasma free fatty acids in humans. *Circulation* 2008; **117** (18): 2369–76.

41 Cianflone K, Paglialunga S, Roy C. Intestinally derived lipids: metabolic regulation and consequences. An overview. *Atheroscler Suppl* 2008; **9** (2): 63–8.

42 Leonard CA. A study of postprandial lipoprotein metabolism and glycaemic control in a population with type 2 diabetes mellitus. PhD Thesis, Trinity College, Dublin, 2010.

43 Philips C, Murugasu G, Owens D, Collins P, Johnson A, Tomkin GH. Improved metabolic control reduces the number of postprandial apolipoprotein B-48-containing particles in type 2 diabetes. *Atherosclerosis* 2000; **148** (2): 283–91.

44 Eriksson JW, Buren J, Svensson M, Olivecrona T, Olivecrona G. Postprandial regulation of blood lipids and adipose tissue lipoprotein lipase in type 2 diabetes patients and healthy control subjects. *Atherosclerosis* 2003; **166** (2): 359–67.

45 Battula SB, Fitzsimons O, Moreno S *et al*. Postprandial apolipoprotein B48- and B100-containing lipoproteins in type 2 diabetes: do statins have a specific effect on triglyceride metabolism? *Metabolism* 2000; **49** (8): 1049–54

46 Dane-Stewart CA, Watts GF, Pal S *et al*. Effect of atorvastatin an apolipoprotein B48 metabolism and low-density lipoprotein receptor activity in normolipidemic patients with coronary artery disease. *Metabolism* 2003; **52** (10): 1279–86.

47 Ceriello A, Assaloni R, Da Ros R *et al*. Effect of atorvastatin and irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. *Circulation* 2005; **111** (19): 2518–24.

48 Ceriello A, Taboga C, Tonutti L *et al*. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 2002; **106** (10): 1211–8.

49 Kolovou GD, Anagnostopoulou KK, Salpea KD, Daskalopoulou SS, Mikhailidis DP. The effects of statins on postprandial lipemia. *Curr Drug Targets* 2007; **8** (4): 551–60.