Effect of Niacin on Triglyceride-Rich Lipoprotein Apolipoprotein B-48 Kinetics in Statin-Treated Patients with Type 2 Diabetes

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ABSTRACT

Aim: To investigate the effects of extended-release niacin (ERN) on apolipoprotein B-48 (apoB-48) kinetics in statin-treated men with type 2 diabetes.

Methods: Twelve men with type 2 diabetes were randomized to rosuvastatin (R) or R plus ERN for 12 weeks and then crossed to the alternate therapy. Postprandial metabolic studies were performed at the end of each treatment period. D3-leucine tracer was administered as subjects consumed a high-fat liquid meal. ApoB-48 kinetics were determined using stable isotope tracer kinetics with fractional catabolic rates (FCR) and secretion rates (SR) derived using a non-steady state compartmental model. Area-under-the-curve (AUC) and incremental AUC for plasma triglyceride and apoB-48 were also calculated over the 10-hour period after ingestion of the fat meal.

Results: In statin-treated patients with type 2 diabetes, apoB-48 concentration was lower with ERN (8.24 ± 1.98 vs 5.48 ± 1.14 mg/L, $p=0.03$) compared with statin alone. Postprandial triglyceride and apoB-48 AUC were also significantly lower on ERN treatment (-15% and 26%, respectively; $p<0.05$) without any change to triglyceride and apoB-48 incremental AUC. ApoB-48 SR in the basal state (3.21 ± 0.34 vs 2.50 ± 0.31 mg/kg/day; $p=0.04$) and number of apoB-48 containing particles secreted in response to the fat load (1.35 ± 0.19 vs 0.84 ± 0.12 mg/kg; $p=0.02$) were lower on ERN. ApoB-48 FCR was not altered with ERN (8.78 ± 1.04 vs 9.17 ± 1.26 pools/day; $p=0.79$).

Conclusions: ERN reduces apoB-48 concentration by lowering fasting and postprandial apoB-48 SR. This effect may be beneficial for lowering atherogenic postprandial lipoproteins and may provide cardiovascular disease risk benefit in patients with type 2 diabetes.

Introduction

Patients with type 2 diabetes (T2DM) often have atherogenic dyslipidaemia, which is characterized by high plasma triglycerides and low high-density lipoprotein (HDL) cholesterol concentrations [1]. This dyslipidaemic state is further exacerbated by the secretion of triglyceride-rich chylomicrons (CM) following a meal. Although statins are front-line therapy to lower plasma cholesterol and reduce cardiovascular risk, a large residual risk remains owing to modest effects on plasma triglyceride and HDL cholesterol concentrations [2].

Insulin resistance is associated with increased CM and very-low density lipoprotein (VLDL) remnant particle concentrations, independent of hyperglycaemia and hyperinsulinaemia [3]. Insulin resistance can stimulate de novo lipogenesis, increasing microsomal triglyceride transfer protein, and enhancing intracellular CM stability in the intestine; increase free fatty acid (FFA) delivery to the enterocytes impairing insulin signalling, and increase intestinal lipid absorption during the postprandial period [4, 5]. Collectively, these could increase the enterocytic secretion of apolipoprotein B-48 (apoB-48) containing CM. Insulin resistance also stimulates triglyceride synthesis, thereby driving VLDL secretion. Such effects would result in increased competition between CM and VLDL remnants for hepatic receptors, further delaying the uptake of CM remnants by this pathway. Insulin resistance may also decrease lipoprotein lipase (LPL) production and down-regulate LDL receptor expression, limiting remnant lipolysis and removal [4, 5].

There is an accumulating body of evidence to suggest that apoB-48 particles play an important role in the development of atherosclerosis via effects on endothelial dysfunction, inflammation, oxidative stress, foam cell formation and, direct delivery of

cholesterol to the arteries [6]. Elevations of apoB-48 levels have been demonstrated in patients with T2DM, both in the fasted state and postprandial periods [7].

Nicotinic acid, or niacin, is an essential B-complex vitamin (vitamin B₃). In pharmacological doses, it is a potent agent for raising HDL cholesterol and lowering plasma triglyceride with moderate effects on LDL cholesterol [8-10]. Niacin has previously been shown to regress coronary atherosclerosis [11] and reduce the rate of coronary mortality [12, 13]. Data from several earlier trials have supported the use of niacin and statin to treat dyslipidaemia [14-16], although recent trials have not produced evidence of additional clinical benefit [17, 18]. Niacin has complex mechanisms of actions that are yet to be fully elucidated.

In the present study, we applied a new stable isotope protocol to demonstrate that, despite statin treatment, apoB-48 metabolism is dysregulated in diabetes, and secondly to test the hypothesis that extended-release niacin (ERN) would lower apoB-48 secretion in this high-risk population.

Materials and Methods

Subjects and Study Design

Twelve men with type 2 diabetes aged between 18-75 years (age 63.0 ± 5.9 years, mean $\pm$ SD) with a body mass index (BMI) of less than 40 kg/m² and fourteen normolipidaemic lean men (age 61.6 ± 7.9 years, waist circumference < 90 cm and plasma triglyceride < 1.2 mmol/L) were recruited for this study. T2DM was defined at a fasting plasma glucose concentration of ≥ 7.0 mmol/L. This study was a randomized, cross-over design trial and approved by the Ethics Committee of the Royal Perth Hospital, and informed consent was obtained in all subjects. Details of subject characteristics, study design and clinical protocol had been previously reported [19] and are available in the online Supplement.

Postprandial kinetic studies

Metabolic studies were performed at the end of each treatment period in the cross-over trial. Metabolic studies were performed once only in the fourteen control subjects [19]. Briefly, all subjects were admitted to the metabolic ward in the morning after a 12 hour fast. After taking fasting blood samples, subjects consumed a liquid test meal over a 5 minute period. Immediately following the test meal, a single bolus of D₃-leucine (5 mg/kg) was administered intravenously within a 2 minute period into an antecubital vein via a Teflon cannula. Blood samples were taken at baseline and during this 10-hour period. TRL-apoB-48 fraction was isolated from plasma by ultracentrifugation and gel electrophoresis. The apoB-48 band was excised from the membrane, hydrolyzed and derivatized with isotopic enrichment determined using gas chromatography-mass spectrometry (GCMS) as described previously [20]

Kinetic analyses

A non-steady compartment model was developed using the SAAM II program (The Epsilon Group, VA) to account for changes in plasma apoB-48 concentration following consumption of the fat meal (Figure 1) [21]. Briefly, the leucine compartment model consists of a four-compartment subsystem (compartments 1-4) that describes plasma leucine kinetics. This subsystem is connected to an intrahepatic delay compartment (compartment 5) that accounts for the time required for leucine tracer to be incorporated into apoB-48 and subsequently secreted into plasma. The apoB-48 concentration compartment model consists of a delay compartment (compartment 7) that represents four compartments in series and an additional compartment represents plasma apoB-48 particles (compartment 8). The model could be used to estimate apoB-48 secretion in the fasted state, in the postprandial state, apoB-48 fractional catabolic rate (FCR) and number of apoB-48 secreted in response to the fat meal.

Biochemical analyses

The laboratory methods for measurements of biochemical analytes were previously described (19). Plasma apoB-48 levels were measured by enzyme immunoassay kit (Fujirebio, Japan). Plasma apoC-III was determined using a Hydragel LP CIII electroimmunodiffusion kit (Sebia, Moulineaux, France). Postprandial metabolism was quantified by calculating the area- and incremental under-the-curve (AUC) and iAUC, respectively, for plasma triglyceride and apoB-48 (0-10hr) using the trapezium rule. The iAUC was estimated as the difference between the area defined below the baseline concentration and the area under the plasma curve between 0 and 10 hour.

Statistics

Data are reported as mean $\pm$ SEM. Significance was defined at the 5% level using a two-tailed test. Groups were compared using independent t-tests. Paired t-tests were used between the diabetic men. All data were analysed using the SPSS 17.0 (SPSS, Chicago, IL) software. Carryover effect of the cross-over design was estimated using SAS 9.2 (SAS Institute, Cary, North Carolina, USA). P values were not adjusted for multiple comparisons.

Results

Age (63.0 ± 5.9 vs 61.6 ± 7.9 years, $p=0.61$) and blood pressure (systolic: 136.1 ± 3.4 vs 129.2 ± 4.1 mmHg, $p=0.21$; diastolic: 77.9 ± 2.2 vs 74.7 ± 1.2 mmHg, $p=0.19$) were not significantly different between the patients with diabetes and the controls. The diabetic men had a significantly higher BMI (31.9 ± 4.6 vs 23.2 ± 2.2 kg/m², $p<0.001$) and waist circumference (109.9 ± 3.7 vs 84.7 ± 1.0 cm, $p<0.001$) compared with the normolipidaemic men. On average, the patients with diabetes were middle-aged, centrally obese and normotensive.

Table 1 shows the plasma lipid, lipoprotein, apolipoprotein, glucose and insulin concentrations of the normolipidaemic controls and the patients with T2DM at the end of the R and R plus ERN treatment phases. As anticipated, the R-treated diabetic men had significantly higher fasting glucose, insulin and HOMA-IR score compared with the control group ($p<0.01$). The statin-treated men with diabetes had significantly higher fasting plasma triglyceride, but lower total cholesterol, LDL cholesterol, non-HDL cholesterol and apoA-I (all $p<0.01$) compared with the normolipidaemic controls. There were no statistically significant differences in plasma apoB, apoB-48 and apoC-III concentrations in the fasted state. TRL-apoC-III, however, was significantly lower on ERN (10.4 ± 2.7 vs 18.6 ± 3.3 mg/L; $p<0.001$). Proprotein convertase subtilisin/kexin type 9 (PCSK9) concentrations were significantly higher in the patients with diabetes. Statin-treated diabetic men had significantly lower lathosterol and higher campesterol indices compared with controls. These were not altered with niacin treatment.

No significant differences were observed in urea, creatinine and ALT concentrations between treatment groups (data not shown), however, uric acid was significantly higher on niacin treatment (0.36 ± 0.2 vs 0.32 ± 0.01 mmol/L, $p=0.02$).

Compared with R alone, the R plus ERN combination significantly decreased total plasma cholesterol, triglycerides, LDL cholesterol, non-HDL cholesterol and apolipoprotein B (all $p<0.05$), and significantly increased HDL cholesterol and apoA-I concentrations (both $p<0.05$). In addition, glucose increased significantly with niacin treatment ($p=0.03$). HOMA-IR, insulin and HbA1c levels were not significantly altered with ERN, nor were PCSK9, lathosterol and campesterol concentrations.

Body weight (94.4 ± 4.8 vs. 94.7 ± 4.8 kg, $p=0.34$), systolic blood pressure (130.6 ± 3.0 vs. 131.4 ± 3.3 mmHg, $p=0.74$), diastolic blood pressure (74.6 ± 2.2 vs. 75.8 ± 2.2 mmHg, $p=0.30$) and dietary intake ($7,306\pm523$ vs. $7,659\pm508$ kJ/day, $p=0.42$) were not different between R and R plus ERN treatments. Carry-over effects were tested and found not significant for total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, apoB, apoA-I, insulin and glucose.

Figure 2A and 2B shows the postprandial excursion of triglycerides and apoB-48 over 10 hours following consumption of a liquid mixed meal. Concentration curves display a monophasic rise and then fall across the postprandial period. The statin-treated men with diabetes exhibited a postprandial concentration profile that peaked at 5hr. In contrast, the triglyceride and apoB-48 concentrations peaked between 2 and 3hr in the lean controls. Compared with normolipidaemic controls, postprandial triglyceride and apoB-48 AUC were significantly higher in the statin-treated men with diabetes (both $p<0.01$, Table 2). Moreover, the incremental AUC for plasma triglyceride and apoB-48 were also significantly higher in the patients compared with the normolipidaemic men.

In the statin-treated patients with T2DM, triglyceride and apoB-48 AUCs were significantly lower on niacin treatment (-15% and 26%, respectively; $p<0.05$).

Triglyceride and apoB-48 incremental AUCs were not different with niacin (Table 2). As we previously reported [19], HDL cholesterol concentration changed during the postprandial period with the dip and subsequent return to pre-meal concentrations mirroring the postprandial rise in triglycerides, whereas HDL apoA-I concentration did not alter significantly during the postprandial state.

Figure 3 shows the isotopic enrichment for apoB-48 with D₃-leucine in control subjects and patients with T2DM on rosuvastatin and rosuvastatin plus ERN therapy. Although the FCR of apoB-48 was significantly lower in the statin-treated diabetic men compared with the non-obese controls ($p < 0.05$), basal apoB-48 PR was not different between the two groups (Table 2). The significant difference in apoB-48 FCR between the two groups remained after adjustment for BMI ($p = 0.022$), waist circumference ($p = 0.022$) and fat free mass ($p = 0.028$).

Compared with R alone, R plus ERN treatment lowered apoB-48 secretion in the fasted state ($p = 0.04$, Table 2). Furthermore, in the postprandial state, 38% less apoB-48 particles were secreted ($p = 0.02$, Table 2) on ERN therapy. The apoB-48 FCR was not altered with ERN, suggesting that the reduction in apoB-48 concentration was due to lower rates of apoB-48 secretion. These findings remained after adjustment for change in fasting glucose concentration (basal apoB-48 PR $p = 0.034$; apoB-48 secreted $p = 0.022$; apoB-48 FCR $p = 0.679$).

Discussion

We provide new knowledge of postprandial apoB-48 metabolism in statin-treated men with T2DM. Despite being optimally treated with statin, the diabetic men had high plasma concentrations of triglycerides and low HDL in the fasted state compared with healthy, non-obese controls. Furthermore, during the postprandial state, apoB-48 concentrations were significantly higher than in controls. This reflects an abnormality in apoB-48 metabolism, which is likely a consequence of increased intestinal secretion together with impaired catabolism of apoB-48. We showed that treatment with niacin significantly lowered fasting plasma triglyceride and apoB-48 concentrations and, increased HDL cholesterol, as previously reported [19]. Niacin treatment lowered apoB-48 secretion in the basal and postprandial states without altering apoB-48 catabolism.

Several studies have previously examined the metabolism of apoB-48 in insulin resistant subjects and patients with T2DM during the postprandial state [22-24]. They demonstrated that triglyceride and apoB-48 concentrations were elevated in the postprandial states. We have recently reported that in insulin resistant obese men, the accumulation of apoB-48 in the postprandial state was a consequence of both increased intestinal secretion and decreased catabolism of apoB-48 in both the fasted and postprandial periods [21], consistent with the study by Hogue et al in patients with T2DM [25]. None of these studies have, however, examined apoB-48 metabolism in statin-treated patients with T2DM. Our new data extends previous studies by investigating apoB-48 metabolism following an oral fat load in statin-treated men with T2DM.

Despite ingesting the same fat load, the postprandial patterns exhibited for triglyceride and apoB-48 were markedly different in the statin-treated diabetic men compared with normolipidaemic controls (Figure 2A and 2B). For apoB-48, our kinetic data support that the postprandial elevation is attributable to a combination of overproduction and impaired catabolism of apoB-48 particles, consistent with the mechanisms described by others [25, 26]. However, we did observe that the fasting plasma concentration and secretion rate of apoB-48 were not significantly different between control subjects and statin-treated patients with diabetes. This observation may relate to the effect of statin on apoB-48 secretion in diabetes. In support of this, Hogue et al reported that atorvastatin lowers the intestinal secretion of apoB-48 in diabetic men [27]. Rosuvastatin may have a similar effect on CM secretion; it is known to significantly lower fasting apoB-48 concentrations in mixed hyperlipidaemia [28]. Statins inhibit cholesterol synthesis, but also increase intestinal cholesterol absorption [29], consistent with lower lathosterol and higher campesterol indices in the statin-treated patients with diabetes compared with the controls.

Niacin has previously been shown to reduce apoB-48 concentrations by up to 28% [30]. The present study shows that niacin, on a background of optimal rosuvastatin therapy, lowered apoB-48 concentrations by 33%. In the fasted state, ERN lowered apoB-48 concentration by decreasing the secretion of apoB-48 without altering its catabolism. Niacin inhibits diglyceride acyltransferase 2 (DGAT2), the terminal and rate-limiting enzyme in triglyceride synthesis, leading to lower rates of triglyceride secretion [31], which may decrease the lipidation of CM particles and thus lower the secretion of apoB-48.

In the postprandial state, ERN significantly lowered apoB-48 AUC and the number of apoB-48 particles secreted during the postprandial study, without any change to apoB-48 FCR. Although plasma apoC-III concentration was not different on ERN, TRL apoC-III concentration was significantly lowered by 45%. That the apoB-48 FCR was not altered on ERN may suggest the fall in TRL apoC-III was insufficient or that the fall was largely in apoC-III associated with the apoB-100-containing TRL particles.

The absence of significant change in apoB-48 incremental AUC with ERN suggests an inconsistency between the methods of numerical analysis. While AUC, and incremental AUC, provide a measure of the effect of ERN treatment, they do not provide insight into the mechanisms, secretion and catabolic rates, that account for the change in plasma concentration over time. In this regard, the AUC (and incremental AUC) results are supportive, but the definitive mechanistic information arises from the compartment model. As noted previously [21], the apoB-48 compartmental model used in this study is based upon assumptions that are difficult to test in the postprandial state. Despite this, the model describes the plasma apoB-48 concentration and tracer enrichment data. That the apoB-48 concentration rises before the appearance of tracer in apoB-48 is consistent with the initial secretion of pre-formed apoB-48 particles from the enterocyte, followed by an up-regulated secretion of apoB-48 to transport the ingested lipid load. This up-regulation accounts for the rise in plasma apoB-48 concentration post meal.

In the present study, the effect of ERN in lowering apoB-48 AUC was consistent with the decreased secretion of apoB-48 particles, as determined by compartmental modelling by Wong *et al* [21]. This result is also consistent with the mechanism of action of niacin whereby DGAT2 is inhibited, resulting in less triglyceride available for

the lipidation of chylomicrons and hence lowers rates of secretion. That the incremental AUCs are not different on ERN suggests that the lipid load, derived from the meal, overcomes the effect of niacin by providing sufficient substrate to lipidate chylomicrons and thus maintain “normal” rates of apoB-48 particle secretion. Recent evidence supports that the intestine is an insulin-sensitive tissue and that deregulation by oxidative stress and inflammation is associated with a pronounced increase in TRLs and apoB-48 secretion [32]. Whether this is also related to the effect of niacin on inflammation requires further investigation.

To date, only one other study has investigated the effect of niacin on apoB-48 metabolism. A study by Lamon-Fava *et al* employed a primed constant tracer infusion and constant feeding protocol in combined hyperlipidaemic subjects [n=5]. Their study showed that niacin, relative to placebo, lowered apoB-48 concentrations by increasing apoB-48 FCR [30]. Their study did not test the addition of niacin to patients already on statin therapy. Interestingly, the addition of lovastatin (40mg/d) to niacin did not result in an increased FCR [30]. The increase in FCR with niacin differs with the current findings. Discrepancies between the results may be attributable to differences in subject characteristics study design (postabsorptive vs postprandial states). A further explanation for the difference may relate to the current study being done on a background of rosuvastatin, whilst the study by Lamon-Fava *et al* employed a design in which the combination of a weak statin (lovastatin) with ERN was compared with ERN alone and placebo. In the same study, the ERN-lovastatin combination had significantly higher sitosterol and lower lathosterol relative to ERN alone [30]. However, this difference does not reflect the effect of ERN. In the present study, ERN had no significant effect on plasma sterols.

It is well known that HMG Co-A reductase inhibitors lower apoB-100-containing lipoproteins by upregulating LDL receptor activity and thus catabolism. Given that our patients were already on rosuvastatin, LDL receptor activity may have already been maximally upregulated. The potential for ERN to further raise particle FCR may therefore be limited.

There is evidence that statins upregulate PCSK9 expression, which may blunt the LDL cholesterol lowering effects of the statin [33]. Confirming this, a significantly higher PCSK9 concentration was observed in the statin-treated men with diabetes compared with lean, control subjects. That ERN did not alter fasting PCSK9 levels suggests that it does not mediate the effect of niacin on lowering LDL cholesterol, although PCSK9 may already be maximally regulated by statin.

In this study, we employed a compartmental model to describe the non-steady-state kinetics of apoB-48 based on that developed previously (21) using concepts from Le et al (34). The model enabled the integration of apo48 concentration and tracer data into a single model that could estimate rates of secretion in the basal and postprandial period in addition to apoB-48 FCR. Initially, the model assumed a constant rate of apoB-48 secretion; however, it failed to describe the apoB-48 tracer and concentration data. In contrast, a model where FCR was time invariant and apoB-48 secretion changed, as a consequence of the fat load, simultaneously fit the apoB-48 concentration and tracer data. This model permitted the precise estimation of apoB-48 FCR and secretion. To further validate the model in the postprandial setting, the iv administration of labeled apoB-48 concurrently with the fat load is warranted. This approach would demonstrate whether or not the FCR of apoB-48 particles changes during the postprandial period.

Our study has limitations. The sample size is small, although the current study investigated more patients than other studies studying the effect of niacin on apoB-48 kinetics. The study was restricted to men and, therefore, may not be reflective of females with diabetes. Further studies with larger sample size, in women and, in other ethnic groups with type 2 diabetes are required to confirm our results. Differences in body weight between the patients with diabetes and the non-obese controls may confound interpretation of the case-control results. Although a better comparator would have been weight-matched subjects, our findings persisted after adjusting for differences in BMI, waist circumference and fat-free mass. We did not study patients with T2DM off statins. Nevertheless, the inclusion of the control subjects highlighted the degree of apoB-48 metabolic dysregulation in patients with diabetes. We did not measure hepatic lipase and LPL activities or the kinetics of TRL triglycerides. This information may have helped elucidate the metabolic regulation of apoB-48 catabolism and triglyceride hydrolysis.

The use of niacin in T2DM has been questioned owing to its effect on glycaemia and insulin resistance. Two prospective randomized double-blinded trials have addressed this concern and demonstrated that niacin's effect on glycaemic control was minimal in patients with stable diabetes, particularly at lower doses of niacin [35, 36]. In the currently study, a significant 8% increase in glucose concentration was observed with ER niacin. Whether the impact of niacin on glycemic control would have diminished its favourable effect on apoB-48 FCR and PR remains to be investigated. Monitoring glucose to ensure control is maintained is essential for niacin use in this population.

Evidence for the use of niacin has been challenged by two large clinical trials, AIM-HIGH and HPS2-THRIVE [17, 18] that failed to demonstrate positive effects of niacin

on CVD endpoints. However, a sub-analysis in patients with both high triglyceride and low HDL cholesterol from the AIM-HIGH study showed a trend towards CVD benefit with ERN [37]. Hence, correcting for these residual risk factors with niacin therapy may provide clinical benefit. Our study provides a kinetic explanation for the changes in atherogenic TRLs with the addition of ER niacin to current frontline statin therapy.

In conclusion, in statin-treated patients with T2DM, CM metabolism remains significantly impaired owing to over secretion and reduced catabolism of apoB-48-containing lipoproteins in the postprandial state. We demonstrated that ERN lowers apoB-48 secretion in this patient group on optimal rosuvastatin therapy. Given that statins have a limited impact on TRL metabolism, combination therapy may be important in the prevention of CVD in diabetic dyslipidaemia. Our results lay the foundation for the kinetic effects of new agents, including niacin-analogues that affect lipid metabolism by similar mechanisms to ERN. Future studies should examine the additive effects of fibrates, n-3 fatty acids and ezetimibe to statin therapy and their effects on apoB-48 kinetics.

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Contribution by the authors

JP researched data and wrote the manuscript. DCC, GFW and PHBR researched data and reviewed/edited the manuscript. SJH coordinated the clinical trial, reviewed/edited the manuscript. VST helped with the clinical trial.

Disclosure statement

Abbott Australasia provided the ER niacin for the study. Gerald F Watts serves on an Abbott Australasia Pty Ltd advisory board and has received honoraria.

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Tables

Table 1. Plasma lipid, lipoprotein, apolipoprotein, glucose, insulin and PCSK9 concentrations in the normolipidaemic controls and patients with type 2 diabetes at the end of each treatment period.

	Control subjects (C)	Patients with Type 2 diabetes (R)	Patients with Type 2 diabetes (R+ERN)	p-value (C vs R)	p-value (R vs R+ERN)
Total Cholesterol (mmol/L)	4.80 ± 0.14	3.59 ± 0.14	3.37 ± 0.14	<0.001	0.03
Triglycerides (mmol/L)	0.78 ± 0.06	1.66 ± 0.23	1.28 ± 0.20	<0.001	0.01
LDL cholesterol (mmol/L)	3.15 ± 0.13	2.32 ± 0.12	1.99 ± 0.10	<0.001	<0.01
Non-HDL cholesterol (mmol/L)	3.27 ± 0.51	2.59 ± 0.13	2.20 ± 0.12	0.001	<0.01
HDL cholesterol (mmol/L)	1.53 ± 0.07	1.00 ± 0.04	1.17 ± 0.06	<0.001	<0.01
Total apolipoprotein B (g/L)	0.81 ± 0.03	0.71 ± 0.04	0.60 ± 0.04	0.07	<0.01
Apolipoprotein B-48 (mg/L)	6.36 ± 1.04	8.24 ± 1.98	5.48 ± 1.14	0.39	0.03
Apolipoprotein A-I (g/l)	1.60 ± 0.06	1.31 ± 0.05	1.41 ± 0.06	0.001	0.02
Apolipoprotein C-III (mg/L)	28.67 ± 3.84	35.48 ± 4.62	31.73 ± 4.00	0.26	0.10
Glucose (mmol/L)	5.23 ± 0.09	7.59 ± 0.69	8.26 ± 0.67	<0.005	0.03
Insulin (mU/L)	3.04 ± 0.30	13.43 ± 2.53	16.59 ± 3.69	<0.001	0.10
HOMA-IR score	0.70 ± 0.07	4.49 ± 0.80	5.75 ± 1.00	<0.001	0.07
HbA1c (%)	<6	7.20 ± 0.30	7.53 ± 0.26	-	0.12
PCSK9 (µg/L)	182.5 ± 12.6	252.7 ± 15.3	233.7 ± 13.8	0.002	0.21
Lathosterol (mmol/mol of cholesterol)	0.91 ± 0.08	0.44 ± 0.05	0.40 ± 0.04	<0.001	0.45
Campesterol (mmol/mol of cholesterol)	1.14 ± 0.14	2.04 ± 0.40	1.91 ± 0.33	0.04	0.63

Data are presented as mean ± SEM.

Table 2. Postprandial metabolic and kinetic parameters in the normolipidaemic control subjects and in patients with type 2 diabetes at the end of each treatment period.

	Control subjects (C)	Patients with Type 2 diabetes (R)	Patients with Type 2 diabetes (R+ERN)	p-value (C vs R)	p-value (R vs R+ERN)
Triglyceride AUC (mmol/L.h)	13.3 ± 1.1	39.1 ± 4.1	33.0 ± 3.9	<0.001	0.05
Triglyceride incremental AUC (mmol/L.h)	6.4 ± 0.7	22.9 ± 2.2	20.5 ± 2.2	<0.001	0.33
Apolipoprotein B-48 AUC (mg/L.h)	91.5 ± 10.8	163.4 ± 22.0	121.4 ± 16.2	0.005	0.02
Apolipoprotein B-48 iAUC (mg/L.h)	37.6 ± 5.0	75.9 ± 8.2	67.2 ± 11.0	<0.001	0.51
Basal apoB-48 PR (mg/kg/day)	3.72 ± 0.53	3.21 ± 0.34	2.50 ± 0.31	0.44	0.04
ApoB-48 secreted (mg/kg)*	0.69 ± 0.07	1.36 ± 0.19	0.84 ± 0.12	0.002	0.02
ApoB-48 FCR (pools/day)	15.59 ± 2.31	8.78 ± 1.04	9.17 ± 1.26	0.02	0.80

Data are presented as mean ± SEM.

*ApoB-48 secreted in response to the fat meal on the top of basal apoB-48 PR

Figure legends

Figure 1. Compartmental model to describe apolipoprotein B-48 (apoB-48) tracer kinetics. Compartments 1 to 4 describe leucine kinetics, connected to an intrahepatic delay compartment (5); incorporation of leucine tracer into apoB-48 and then secretion into plasma (6). Compartment 7 represents a delay compartment (intestinal motility and the absorption processes) for apoB-48 concentration and compartment 8, the plasma apoB-48 particles.

Figure 2. Postprandial plasma concentrations of (A) triglycerides and (B) apolipoprotein B-48 from 0 to 10 hours in normolipidaemic control subjects (●), rosuvastatin treated patients with type 2 diabetes (■) and rosuvastatin plus niacin (▲). Mean and SEM is shown.

Figure 3. Isotopic enrichment for apoB-48 with D₃-leucine in normolipidaemic control subjects (●), rosuvastatin treated patients with type 2 diabetes (■) and rosuvastatin plus niacin (▲). Mean and SEM is shown.

A **B**

Appendix

Subjects

Twelve men with T2DM aged between 18-75 years (age 63.0 ± 5.9 years, mean $\pm$ SD) with a body mass index (BMI) of less than 40 kg/m^2 and fourteen normolipidaemic lean men (age 61.6 ± 7.9 years, waist circumference $< 90 \text{ cm}$ and plasma triglyceride $< 1.2 \text{ mmol/L}$) were recruited for this study. T2DM was defined at a fasting plasma glucose concentration of $\geq 7.0 \text{ mmol/L}$.

Subjects with fasting cholesterol $> 6.0 \text{ mmol/L}$ or triglyceride $> 4.5 \text{ mmol/L}$, genetic hyperlipidaemia, proteinuria, hypothyroidism, cholelithiasis, alcohol consumption $> 30 \text{ g alcohol/day}$, HbA1c $> 8.5\%$, daytime insulin treatment, uncontrolled hypertension ($> 150/90 \text{ mmHg}$), creatinemia ($> 150 \text{ } \mu\text{mol/L}$), hepatic dysfunction (AST or ALT $> 3 \times \text{ULN}$), abnormal thyroid function, muscle disorders or creatinine kinase $> 3 \times \text{ULN}$, major systemic illness or used steroids or other agents that may influence lipid metabolism, including fish oils, cardiovascular event within the last 6 months, lactose intolerance or intolerance to cream and eggs were excluded. All subjects were non-smokers and were advised to continue their habitual isocaloric diet and to keep physical activity constant. Dietary intake was assessed using a 3-day food record and FoodWorks 2007 Version 5 (Xyris Software, Brisbane, Australia).

Of the patients with diabetes, three were already on rosuvastatin at recruitment, the other nine subjects were on atorvastatin ($n=6$), pravastatin ($n=2$) and simvastatin ($n=1$). None of the subjects were on fibrates or niacin at recruitment. Eleven subjects were on anti-diabetic medication (biguanide [$n=5$], on both biguanide and sulfonylurea [$n=6$]) and eight subjects were on anti-hypertensive medication (calcium channel blocker [$n=3$], angiotensin-converting-enzyme inhibitor [$n=5$], angiotensin receptor

blocker [n=3], beta blocker [n=1]; three subjects were on multiple antihypertensive medication). Duration of diabetes was 5.67 ± 2.77 years (mean $\pm$ SD).

Participants provided informed written consent and the study was approved by the Ethics Committee of Royal Perth Hospital (EC2009/014 and EC2009/018) and registered on the Australian New Zealand Clinical Trials Registry (ACTRN12609000382279 and ACTRN12609000448246).

Study Design and Clinical Protocol

In the randomized, cross-over design trial, the patients with T2DM were treated with rosuvastatin (Crestor, Astrazeneca) at a stable dose for a ≥ 6 week run-in period and attained a target LDL cholesterol of <2.5 mmol/L, fasting triglyceride <4.5 mmol/L and HDL cholesterol ≤ 1.0 mmol/L to be eligible for the study. At the end of the run-in period, subjects were randomized to either rosuvastatin (R) or R plus extended-release niacin (ERN) (Niaspan, Abbott Laboratories, Australasia) (10 subjects were titrated to 2g, 1 subject to 1.5g and 1 subject to 1g) for 12 weeks, then crossed over to the alternate therapy with a 3 week washout period. Titration was based on patient response. During the washout period, only niacin was discontinued, background rosuvastatin therapy and other concomitant medication did not cease and was not altered. Subjects were asked to take 100mg of aspirin once daily in the evening, prior to taking niacin to help reduce flushing. In order to match both treatment periods, subjects were asked to take aspirin for the duration of the study. Compliance with study medication was checked by tablet count at the end of each treatment period.

Postprandial Studies

Metabolic studies were performed at the end of each treatment period in the cross-over trial. Metabolic studies were performed once only in the fourteen control subjects. All subjects were admitted to the metabolic ward in the morning after a 12 hour fast. Body weight and height were measured and arterial blood pressure recorded using a Dinamap1846 SX/P monitor (Critikon, Tampa, USA). Fasting venous bloods were collected for biochemical measurements. Plasma volume was determined by multiplying body weight by 0.045.

After taking fasting blood samples, subjects consumed a liquid test meal over a 5 minute period. The test meal consisted of 100mL milk (3.4% fat), 150ml cream (35% fat), 70mL corn oil, 90g egg, 10g sugar, and 3.5g flavouring, totalling 1305kcal with an energy distribution of 87% fat, 7% carbohydrates and 6% protein.

Immediately following the test meal, a single bolus of D3-leucine (5 mg/kg) was administered intravenously within a 2 minute period into an antecubital vein via a Teflon cannula. Blood samples were taken at baseline and at 5, 10, 20, 30, and 40 minutes, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 hours after isotope injection. During this 10-hour period, subjects were asked to rest quietly in a semi-recumbent posture and were allowed to drink water only.

Isolation and measurement of isotopic enrichment of apoB-48

TRL fraction was isolated from 3.5ml plasma by ultracentrifugation (Optima XL-100K, Beckman Coulter, Australia) at density of 1.006 (40,000 rpm, 16 h, 4°C) and subsequent extraction using diethyl ether. The TRL samples were then prepared for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Tris-

glycine buffer system and a 5% acrylamide gel. The gel was then electroblotted onto polyvinylidene fluoride (PVDF) membrane using a 1 x Tris-glycine (10% methanol) buffer. The PVDF membrane was stained with amido black protein stain and destained with 100% methanol. Two bands, apoB-100 (550kDa) and apoB-48 (240kDa), were clearly visible after destaining. The apoB-48 band was excised from the membrane, hydrolyzed with 200 μ L 6M HCl at 110°C for 16 hours. Derivatization of leucine to the oxazolinone derivative was described previously [Dwyer et al J Lipid Res 2002]. Isotopic enrichment was determined using gas chromatography-mass spectrometry (GCMS) with selected ion monitoring of samples at a mass to charge ratio (m/z) of 212 and 209 and negative ion chemical ionization.

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