**Clinical and Population Studies**

**Effect of Niacin on High-Density Lipoprotein Apolipoprotein A-I Kinetics in Statin-Treated Patients With Type 2 Diabetes Mellitus**

Jing Pang, Dick C. Chan, Sandra J. Hamilton, Vijay S. Tenneti, Gerald F. Watts, P. Hugh. R. Barrett

**Objective**—To investigate the effect of extended-release (ER) niacin on the metabolism of high-density lipoprotein (HDL) apolipoprotein A-I (apoA-I) in men with type 2 diabetes mellitus on a background of optimal statin therapy.

**Approach and Results**—Twelve men with type 2 diabetes mellitus were recruited for a randomized, crossover design trial. Patients were randomized to rosvastatin or rosvastatin plus ER niacin for 12 weeks and then crossed over to the alternate therapy after a 3-week washout period. Metabolic studies were performed at the end of each treatment period. HDL apoA-I kinetics were measured after a standardized liquid mixed meal and a bolus injection of d3-leucine for 96 hours. Compartmental analysis was used to model the data. ER niacin significantly decreased plasma triglyceride, plasma cholesterol, non-HDL cholesterol, low-density lipoprotein cholesterol, and apoB (all \( P < 0.05 \)) and significantly increased HDL cholesterol and apoA-I concentrations (\( P < 0.005 \) and \( P < 0.05 \), respectively). ER niacin also significantly increased HDL apoA-I pool size (6088±292 versus 5675±305 mg; \( P < 0.001 \)), and this was attributed to a lower HDL apoA-I fractional catabolic rate (0.33±0.01 versus 0.37±0.02 pools/d; \( P < 0.005 \)), with no significant changes in HDL apoA-I production (20.93±0.63 versus 21.72±0.85 mg/kg per day; \( P = 0.28 \)).

**Conclusions**—ER niacin increases HDL apoA-I concentration in statin-treated subjects with type 2 diabetes mellitus by lowering apoA-I fractional catabolic rate. The effect on HDL metabolism was independent of the reduction in plasma triglyceride with ER niacin treatment. Whether this finding applies to other dyslipidemic populations remains to be investigated. (Arterioscler Thromb Vasc Biol. 2014;34:427-432.)

**Key Words:** diabetes mellitus, type 2 ■ high-density lipoproteins ■ triglycerides

Type 2 diabetes mellitus is an important risk factor for cardiovascular disease (CVD). Diabetic dyslipidemia plays a major role in the progression of atherosclerosis. Reduced levels of circulating high-density lipoprotein (HDL) cholesterol and its major apolipoprotein, apolipoprotein A-I (apoA-I), are particularly common in this population. In type 2 diabetes mellitus, reduced levels of circulating apoA-I are likely to be a result of an increased HDL fractional catabolic rate (FCR).

Statin therapy is the cornerstone of treatment of dyslipidemia in diabetes mellitus. However, despite reaching the low-density lipoprotein (LDL) cholesterol target, only modest effects are exerted on triglyceride (TG) and HDL cholesterol, and patients often have residual CVD risk. This residual risk suggests that additional therapeutic interventions may be required to reduce CVD risk further.

Nicotinic acid, or niacin, is an essential B-complex vitamin (vitamin B3). In pharmacological doses, it is a potent agent for raising HDL cholesterol (29%--35%) and lowering plasma TG with moderate effects on LDL cholesterol. Niacin has been shown to regress coronary atherosclerosis and reduce the rate of coronary mortality. Niacin has complex mechanisms of actions that are yet to be fully elucidated.

To address residual risk in the dyslipidemic diabetic population, the combination of statin–niacin therapy may offer potent effects in lowering plasma TG and LDL cholesterol and increasing HDL cholesterol. Data from several trials have supported the use of niacin and statins to treat dyslipidemia. The combination of statin and niacin has been shown to delay the progression of atherosclerosis in individuals with known coronary heart disease.

Few studies have investigated the effect of niacin on lipoprotein kinetics. The results of these studies have been undertaken in small groups of subjects with inconclusive results. Despite studies on the effect of niacin on plasma lipid and lipoprotein concentrations in patients with type 2 diabetes mellitus, no studies have investigated the effects of niacin, in combination with statin, on the metabolism of lipoproteins. In the present study, we hypothesized that niacin, on a background of optimal statin therapy, would increase HDL apoA-I concentrations by reducing catabolism in patients with type 2 diabetes mellitus.
Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Table 1 shows the clinical and biochemical characteristics of the 12 subjects with type 2 diabetes mellitus at recruitment. Table 2 shows the plasma lipid, lipoprotein, apolipoprotein, glucose, and insulin concentrations at the end of each treatment phase. Compared with rosuvastatin, the rosuvastatin plus extended-release (ER) niacin combination significantly decreased total plasma cholesterol, TG, LDL cholesterol, non-HDL cholesterol, and apolipoprotein B (all P<0.05) and significantly increased HDL cholesterol and apoA-I concentrations (P<0.001 and P<0.05, respectively). HDL cholesterol:apoA-I ratio increased significantly with ER niacin (0.77±0.02 versus 0.83±0.02; P=0.005) compared with rosuvastatin alone. TG area under the curve was significantly lower with ER niacin (39.1±4.1 versus 33.0±3.9 mmol/L·h; P<0.001). There was a significant increase in glucose on rosuvastatin plus ER niacin treatment compared with rosuvastatin alone. There was no significant alteration in body weight between treatments. Carryover effects were tested and found to be nonsignificant for total cholesterol, TG, LDL cholesterol, HDL cholesterol, apoB, apoA-I, insulin, and glucose. We performed tracer metabolic studies in the nonsteady state. Patients consumed a fatty mixed meal that resulted in postprandial changes in plasma TG, HDL cholesterol, and HDL apoA-I concentrations during the first 10 hours of the metabolic study (Figure 1). The dip and subsequent increase in postprandial HDL cholesterol curves mirrors the postprandial rise in TG, whereas the HDL apoA-I concentration remained, in 21 of 24 metabolic studies, constant over time. Carryover effects were tested and found to be nonsignificant for total cholesterol, TG, LDL cholesterol, HDL cholesterol, apoB, apoA-I, insulin, and glucose. We performed tracer metabolic studies in the nonsteady state. Patients consumed a fatty mixed meal that resulted in postprandial changes in plasma TG, HDL cholesterol, and HDL apoA-I concentrations during the first 10 hours of the metabolic study (Figure 1). The dip and subsequent increase in postprandial HDL cholesterol curves mirrors the postprandial rise in TG, whereas the HDL apoA-I concentration remained, in 21 of 24 metabolic studies, constant over time. Carryover effects were tested and found to be nonsignificant for total cholesterol, TG, LDL cholesterol, HDL cholesterol, apoB, apoA-I, insulin, and glucose.

Table 2 shows isotopic tracer curves and the model fits for HDL apoA-I after the administration of d3-leucine in a representative subject on each treatment arm. Plasma leucine tracer curves did not differ significantly between treatments. The flatter decay of the HDL apoA-I tracer curve on rosuvastatin plus niacin treatment suggests a slower catabolic rate compared with rosuvastatin alone.

Table 3 shows the HDL apoA-I kinetic parameters after treatment with rosuvastatin and rosuvastatin plus ER niacin. HDL apoA-I FCR was significantly lower (P<0.005) and pool size was significantly higher (P<0.001) on rosuvastatin plus ER niacin treatment compared with rosuvastatin alone. HDL apoA-I clearance is more delayed with addition of niacin. Table 4 shows that total plasma tocopherol, HDL-cholesterol, and apoA-I increased significantly (P<0.05) compared with rosuvastatin alone. HDL apoA-I clearance is more delayed with addition of niacin. Table 4 shows that total plasma tocopherol, HDL-cholesterol, and apoA-I increased significantly (P<0.05) compared with rosuvastatin alone.

Table 1. Clinical and Biochemical Characteristics of the 12 Subjects With Type 2 Diabetes Mellitus at Randomization

<table>
<thead>
<tr>
<th>Subjects With Type 2 Diabetes Mellitus</th>
<th>n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63.0±5.7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>94.1±15.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.7±4.4</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>110.0±12.4</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>127.4±9.7</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>73.8±7.0</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.8±1.3</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>12.8±6.0</td>
</tr>
<tr>
<td>HOMA-IR score</td>
<td>3.9±1.9</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.5±0.5</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.4±0.4</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.5±0.6</td>
</tr>
<tr>
<td>On lipid-lowering medication, n</td>
<td>12</td>
</tr>
<tr>
<td>On antidiabetic medication, n</td>
<td>11</td>
</tr>
<tr>
<td>On antihypertensive medication, n</td>
<td>8</td>
</tr>
</tbody>
</table>

Data are mean±SD. BMI indicates body mass index; BP, blood pressure; HOMA-IR, Homeostasis Model of Assessment - Insulin Resistance; and LDL, low-density lipoprotein.

Table 2. Plasma Lipid, Lipoprotein, Apolipoprotein, Glucose, and Insulin Concentrations on Rosuvastatin and Rosuvastatin Plus Niacin Treatments

<table>
<thead>
<tr>
<th></th>
<th>Rosuvastatin</th>
<th>Rosuvastatin Plus Niacin</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.66±0.23</td>
<td>1.28±0.20</td>
<td>0.013</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.59±0.14</td>
<td>3.73±0.14</td>
<td>0.034</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.32±0.12</td>
<td>1.99±0.10</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mmol/L</td>
<td>2.59±0.13</td>
<td>2.20±0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.00±0.04</td>
<td>1.17±0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein A-I, g/L</td>
<td>1.31±0.05</td>
<td>1.41±0.06</td>
<td>0.020</td>
</tr>
<tr>
<td>Apolipoprotein B, g/L</td>
<td>0.71±0.04</td>
<td>0.60±0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>7.59±0.69</td>
<td>8.26±0.67</td>
<td>0.034</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>13.43±2.53</td>
<td>16.59±3.69</td>
<td>0.095</td>
</tr>
<tr>
<td>HOMA-IR score</td>
<td>4.49±0.80</td>
<td>5.75±1.00</td>
<td>0.065</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.20±0.30</td>
<td>7.53±0.26</td>
<td>0.119</td>
</tr>
<tr>
<td>CETP, mg/L</td>
<td>15.72±1.51</td>
<td>14.44±1.00</td>
<td>0.306</td>
</tr>
<tr>
<td>Hepatic lipase, mg/L</td>
<td>51.20±3.73</td>
<td>50.32±2.45</td>
<td>0.812</td>
</tr>
<tr>
<td>HDL cholesterol:apoA-I</td>
<td>0.77±0.02</td>
<td>0.83±0.02</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Data are mean±SEM. apoA-I indicates apolipoprotein A-I; CETP, cholesteryl ester transfer protein; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model of Assessment - Insulin Resistance; and LDL, low-density lipoprotein.

rosuvastatin plus ER niacin treatment. However, insulin and the Homeostasis Model of Assessment - Insulin Resistance score did not alter significantly with niacin. Cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) concentrations were not significantly different between treatments. There were no significant alterations in body weight (94.4±4.8 versus 94.7±4.8 kg; P=0.34), systolic blood pressure (130.6±3.0 versus 131.4±3.3 mm Hg; P=0.74), diastolic blood pressure (74.6±2.2 versus 75.8±2.2 mm Hg; P=0.30), and dietary intake (7306±523 versus 7659±508 kJ/d; P=0.42) between treatments. Carryover effects were tested and found to be nonsignificant for total cholesterol, TG, LDL cholesterol, HDL cholesterol, apoB, apoA-I, insulin, and glucose.
ApoA-I production rate was not altered with the addition of ER niacin (*P*=0.28).

No significant correlations were found between changes in HDL apoA-I kinetic parameters and changes in lipid concentrations. Surprisingly, change in HDL apoA-I FCR was not associated with change in plasma TG concentration (*r*=0.082; *P*=0.80), TG area under the curve (*r*=0.370; *P*=0.24), or TG incremental area under the curve (*r*=0.475; *P*=0.12).

**Discussion**

This is the first study to examine the effect of ER niacin, added to background statin therapy, on HDL apoA-I metabolism in patients with type 2 diabetes mellitus. We demonstrated that ER niacin significantly increases HDL cholesterol concentration and apoA-I concentration by reducing the FCR of apoA-I without significant changes to the production rate. These changes were achieved without alteration to body weight, blood pressure, and dietary intake. The effects of niacin in lowering plasma TG concentrations and elevating HDL cholesterol concentrations are consistent with the literature.

A previous study on the effects of niacin monotherapy (3 g/d) showed a reduction in the catabolism of HDL apoA-I in 2 normolipidemic subjects. We confirm this in a population of patients with type 2 diabetes mellitus. Patients with type 2 diabetes mellitus have elevated plasma TGs and hypercatabolism of apoA-I. The results of the present study may not extrapolate to other dyslipidemic populations. A previous study in type IV hyperlipoproteinemic subjects treated with niacin (3 g/d) also showed an increase in apoA-I concentration owing to a reduction in FCR. In contrast, no differences in kinetic parameters were found in patients with type II hyperlipoproteinemia. Shepherd et al observed an increase in HDL apoA-I concentration in the absence of change in apoA-I FCR and production rate in 5 normolipidemic subjects. More recently, Lamon-Fava et al demonstrated an increased production rate of apoA-I with 2 g/d ER niacin alone and also with a lovastatin combination but no change in HDL apoA-I catabolism in subjects with combined hyperlipidemia. These findings have been summarized in the online-only Data Supplement (Table I in the online-only Data Supplement).

These previously published studies are limited by a small sample size (n=2–6) and studied different patient populations compared with the present study. Discrepancies between the findings may be because of differences in experimental models, study design, subject characteristics, niacin dosage, and schedule and background of concomitant medication. Furthermore, differences in the methods of kinetic analysis, including use of apoA-II FCR as a measure of apoA-I catabolism, add to the heterogeneity of findings.

No significant associations were found between the changes in HDLapoA-I kinetic parameters and the changes in TG concentrations. Because TG is a strong determinant of apoA-I remodeling and is associated with HDL apoA-I FCR, our results imply that the effect of ER niacin on apoA-I catabolism may be independent of change in TG. However, the remodeling effect of TG on HDL cannot be completely excluded on the basis of a lack of a significant correlation. Previous studies have also failed to find associations between plasma TG and HDL apoA-I FCR. Furthermore, the slower
rate of HDL apoA-I catabolism with ER niacin is consistent with increased HDL particle size, as suggested by the higher HDL cholesterol:apoA-I ratio (Table 2). 20

Mechanistically, niacin can downregulate β-ATP synthase and prevent HDL holoparticle endocytosis. 22 The niacin receptor, GPR109A, is not involved in the effects of niacin on the hepatic catabolism of HDL apoA-I because GPR109A is not expressed in the liver. 23 Furthermore, niacin-treated Hep G2 cells exhibit a reduced uptake of HDL protein but not of HDL cholesteryl esters; 24 thus, niacin does not act via the scavenger receptor class B type 1 pathway because this is selective to HDL cholesteryl esters. 25 The exact mechanism of niacin on HDL apoA-I catabolic pathways is yet to be completely elucidated. In terms of HDL apoA-I synthesis, niacin did not alter secretion rate, consistent with recent findings in HepG2 cells in which apoA-I gene expression was constant with niacin treatment. 26

A recent animal study demonstrated that reduced CETP-mediated transfer of cholesteryl from HDL to very-LDL leads to a reduced apoA-I uptake by the kidneys. 27 The study also demonstrated that HL activity was inhibited by niacin, thereby contributing to the preservation of larger HDL particles that are cleared slowly from the circulation. However, neither CETP nor HL concentrations changed significantly in the present study, a finding that is consistent with other studies. 37 No significant correlations between changes in apoA-I FCR and changes in CETP and HL were found, suggesting that changes in FCR are not CETP or HL mediated. Importantly, HL was measured as HL concentration and not as postheparin HL activity. The possible effects of niacin on the activities of these enzymes cannot be excluded. Endothelial lipase is another HDL-modifying enzyme potentially influenced by niacin.

Concern has been raised on the effect of niacin on glycemic control in patients with type 2 diabetes mellitus. Two prospective, randomized, double-blinded trials reported that the effect of niacin on glycemia was minimal in patients with stable diabetes mellitus, particularly at lower doses of niacin. 28, 29 In the current study, a significant 8% increase in glucose concentration was observed with ER niacin. This warrants the careful monitoring of glycemia to ensure that control is maintained because glycation of HDL during hyperglycemia can impair HDL functionality 30 and reduce its antiatherogenic effects, 3 in addition to other adverse effects of hyperglycemia.

Owing to the ingestion of a high-fat liquid mixed meal, a new compartmental model was developed to describe HDL apoA-I kinetics. Although a postprandial study, the plasma concentration of HDL apoA-I did not change significantly, despite changes in plasma TG and HDL cholesterol concentration. Previous HDL models were developed using data derived in the fasting state, in which a single plasma compartment and an extravascular HDL pool were sufficient to describe the tracer data. 31 In the nonfasting state, apoA-I levels are, on average, elevated compared with fasting values, 32 owing possibly to the intestinal biosynthesis of apoA-I. 33 In consideration of this, 2 biosynthesis pathways were included in the model to potentially account for the hepatic and intestinal secretion of apoA-I (Figure 3), analogous to the modeling concepts proposed by Fisher et al. 34

This study has limitations. Only male subjects were recruited, and given that there are differences in HDL metabolism between men and women, the results of this study may not apply to women. The sample size is small, although the current study investigated more patients than other studies investigating the effect of niacin on HDL kinetics. Further work is also required to confirm the results in other ethnic groups. Additional measurements such as CETP activity, HL activity, endothelial lipase activity, and HDL size would also be of interest.

Elevated TG and low HDL cholesterol levels are significant risk factors for coronary heart disease. 35, 36 Correcting for these residual risk factors with ER niacin therapy may provide clinical benefit, as suggested in a subgroup analysis of the Atherothrombosis Intervention in Metabolic Syndrome with low HDL/high Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) trial. 37 Our study provides a kinetic

![Figure 3. Compartment model describing high-density lipoprotein (HDL) apolipoprotein A-I (apoA-I) tracer kinetics. Compartments 1 to 4 describe leucine kinetics. Leucine tracer is injected into plasma, compartment 2. Compartments 5 and 6 are delay compartments (one = 0.4 hours, the other 2.5 hours), accounting for the synthesis and secretion of apoA-I into the plasma HDL pool, compartment 7.](image-url)

### Table 3. Kinetic Estimates of the Metabolism of High-Density Lipoprotein Apolipoprotein A-I After Treatment With Rosuvastatin and Rosuvastatin Plus Niacin

<table>
<thead>
<tr>
<th></th>
<th>Rosuvastatin</th>
<th>Rosuvastatin Plus Niacin</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool size, mg</td>
<td>5675±305</td>
<td>6088±292</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fractional catabolic rate, pools/d</td>
<td>0.37±0.02</td>
<td>0.33±0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Production rate, mg/kg per day</td>
<td>21.72±0.85</td>
<td>20.93±0.63</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
explanation for the changes in lipoproteins with the addition of ER niacin to current frontline statin therapy. The results of our study indicate that in subjects with type 2 diabetes mellitus with at-target LDL cholesterol, adjunctive therapy with ER niacin increases HDL by lowering the catabolism of apoA-I. This effect was independent of changes in plasma TG with niacin treatment. Despite this, ER niacin therapy may provide CVD risk benefit to patients with type 2 diabetes mellitus by increasing HDL concentrations. Studies that investigate HDL functionality would also be important to complement this knowledge. Given that statin has a limited effect on apoA-I metabolism, combination therapy may be important in the prevention of CVD in diabetic dyslipidemia that is characterized by apoA-I hypercatabolism.

Since the early termination of the AIM-HIGH\(^\text{38}\) and Heart Protection Study 2 Treatment of HDL to Reduce the Incidence of Vascular Events (HP52-THRIVE)\(^\text{39}\) trials, the future use of niacin remains unclear. Careful consideration for the prevention of coronary heart disease. \(JAMA.\) 1975;231:360–381.


12. McKenney JM, Jones PH, Bays HE, Knopp RH, Kashyap ML, Ruoff GE, McGovern ME. Comparative effects on lipid levels of combination therapy with a statin and extended-release niacin or ezetimibe versus a statin alone (the COMPEL study). \(Atherosclerosis.\) 2007;192:432–437.


lipoprotein levels and glycemic control in patients with diabetes and peripheral arterial disease the admit study: a randomized trial. JAMA. 2000;284:1263–1270.


**Significance**

Usual care for diabetic dyslipidemia is statin treatment, but a significant proportion of patients have residual dyslipidemia, with elevated plasma triglyceride (TG) and low high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I concentrations. We conducted a tracer metabolic study in 12 men with diabetes mellitus on optimal statin treatment (low-density lipoprotein concentrations <95 mg/dL [2.5 mmol/L]). Despite this, plasma TG and HDL cholesterol concentrations remained abnormal. Niacin has widely been used to lower plasma TG and raise HDL, but the mechanism by which it increases HDL concentrations is unknown. Patients were studied in random sequence with niacin in addition to statin therapy or statin therapy alone. Compared with statin alone, niacin significantly lowered plasma TG concentrations and increased HDL cholesterol and apolipoprotein A-I concentrations. The increase in HDL apolipoprotein A-I concentration was a function of reduced apolipoprotein A-I catabolism. Niacin may provide cardiovascular disease risk benefit to patients with high-risk type 2 diabetes mellitus, those with elevated TG and low HDL, by increasing HDL concentrations.
Effect of Niacin on High-Density Lipoprotein Apolipoprotein A-I Kinetics in Statin-Treated Patients With Type 2 Diabetes Mellitus
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ONLINE SUPPLEMENTAL MATERIAL

Supplemental Table I. Summary of clinical studies investigating the effects of niacin on apolipoprotein A-I kinetics.

<table>
<thead>
<tr>
<th>n</th>
<th>Males (%)</th>
<th>Population</th>
<th>Niacin Intervention</th>
<th>ApoA-I Concentration (g/L)</th>
<th>ApoA-I PR (pools/day)</th>
<th>ApoA-I FCR (mg/kd/day)</th>
<th>Principal apoA-I kinetic result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No Niacin</td>
<td>After Niacin</td>
<td>p-value</td>
<td>No Niacin</td>
</tr>
<tr>
<td>Blum (1977)&lt;sup&gt;16&lt;/sup&gt;</td>
<td>2</td>
<td>1 (50%)</td>
<td>Normolipidemic</td>
<td>1g thrice/day</td>
<td>1.13</td>
<td>1.16</td>
<td>12.40</td>
</tr>
<tr>
<td>Blum (1977)&lt;sup&gt;16&lt;/sup&gt;</td>
<td>Mean</td>
<td>1.06</td>
<td>1.12</td>
<td>0.31</td>
<td>11.03</td>
<td>9.84</td>
<td>0.17</td>
</tr>
<tr>
<td>Shepherd* (1979)&lt;sup&gt;17&lt;/sup&gt;</td>
<td>5</td>
<td>5 (60%)</td>
<td>Normolipidemic</td>
<td>1g thrice/day</td>
<td>1.22</td>
<td>1.34</td>
<td>14.60</td>
</tr>
<tr>
<td>Shepherd* (1979)&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Mean</td>
<td>1.35</td>
<td>1.45</td>
<td>0.03</td>
<td>12.60</td>
<td>12.48</td>
<td>0.63</td>
</tr>
<tr>
<td>Packard (1980)&lt;sup&gt;18&lt;/sup&gt;</td>
<td>6</td>
<td>NA</td>
<td>Type II lipoproteinemic</td>
<td>3g/day</td>
<td>1.26</td>
<td>1.35</td>
<td>8.90</td>
</tr>
<tr>
<td>Packard (1980)&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Mean</td>
<td>1.13</td>
<td>1.17</td>
<td>0.29</td>
<td>10.18</td>
<td>9.88</td>
<td>0.61</td>
</tr>
<tr>
<td>Lamon-Fava (2008)&lt;sup&gt;19&lt;/sup&gt;</td>
<td>4</td>
<td>NA</td>
<td>Type IV lipoproteinemic</td>
<td>3g/day</td>
<td>1.28</td>
<td>1.46</td>
<td>13.80</td>
</tr>
<tr>
<td>Lamon-Fava (2008)&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Mean</td>
<td>1.08</td>
<td>1.25</td>
<td>0.004</td>
<td>11.23</td>
<td>10.13</td>
<td>0.16</td>
</tr>
<tr>
<td>Lamon-Fava (2008)&lt;sup&gt;19&lt;/sup&gt;</td>
<td>5</td>
<td>5 (100%)</td>
<td>Combined hyperlipidemic</td>
<td>2 g/day (extended-release niacin)</td>
<td>0.90</td>
<td>1.03</td>
<td>10.30</td>
</tr>
<tr>
<td>Lamon-Fava (2008)&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Mean</td>
<td>1.03</td>
<td>1.19</td>
<td>0.001</td>
<td>9.52</td>
<td>11.50</td>
<td>0.04</td>
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</table>
Shepherd et al. assumed that the FCR of apoA-II and apoA-I were equivalent.
MATERIALS AND METHODS

Subjects

Twelve type 2 diabetic men aged between 18-75 years with a body mass index (BMI) of less than 40kg/m² (mean±SD, age 63.0±5.7 years, BMI 31.7±4.4 kg/m²) were recruited for this study (Table 1). Type 2 diabetes was defined at a fasting plasma glucose concentration of ≥6.1mmol/L. Subjects with fasting cholesterol >6.0mmol/L or fasting TG >4.5mmol/L, genetic hyperlipidaemia, proteinuria, hypothyroidism, cholelithiasis, alcohol consumption >30 g alcohol/day, HbA1c >8.5%, daytime insulin treatment, uncontrolled hypertension (>150/90mmHg), creatinaemia (>150µmol/L), hepatic dysfunction (AST or ALT>3x ULN), abnormal thyroid function, muscle disorders or creatinine kinase >3xULN, 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 (ingredients in the test meal) were excluded. All subjects were non-smokers. Three subjects 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 anti-hypertensive medication).

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

Study Design and Clinical Protocol

This was a randomized, crossover design trial. Subjects were treated with rosuvastatin (Crestor, Astrazeneca) at a stable dose for a ≥6 week run-in period and attained a target LDL cholesterol of <2.5mmol/L, fasting TG <4.5mmol/L and HDL cholesterol ≤1.0mmol/L to be eligible for the study. Subjects were advised to continue their habitual isocaloric diet and to keep physical activity constant throughout the study. Dietary intake was assessed using four 3-day food record and FoodWorks 2007 Version 5 (Xyris Software, Brisbane, Australia). At the end of the run-in period, subjects were randomised to either rosuvastatin or rosuvastatin plus extended-release (ER) niacin (Niaspan, Abbott Laboratories) (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.
Metabolic studies were performed at the end of each treatment period. 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\(^1\).

After taking fasting blood samples, subjects consumed a liquid test meal and two 50,000U vitamin A capsules 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. This fat load yielded 1305kcal with an energy distribution of 87% fat, 7% carbohydrates and 6% protein.

Immediately following the test meal, a single bolus of D\(_3\)-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. Additional fasting blood samples were collected in the morning on the following 4 days (24, 48, 72 and 96 hours).

*Isolation and measurement of isotopic enrichment of apoA-I*

The apoB-containing lipoproteins were removed from 3.5ml of fresh plasma by ultracentrifugation (Optima XL-100K, Beckman Coulter, Fullerton, Australia). 200µl of the remaining supernatant was density adjusted with CsCl to 1.21g/ml and HDL was isolated by ultracentrifugation. ApoA-I was isolated using SDS-PAGE and blotted onto polyvinylidene difluoride membrane. The apoA-I bands were excised from the membrane, hydrolysed with 6M HCl at 110°C overnight and dried for derivatisation using the oxazolinone method\(^2\). Plasma-free leucine was isolated by cation-exchange chromatography using AG 50 W-X8 resin (Biorad, Richmond, CA) following removal of plasma protein with 60% perchloric acid. Isotopic enrichment was determined using gas chromatography–mass spectrometry (GCMS) with selected ion monitoring at a mass-to-charge ratio (m/z) of 212 and 209 and negative ion chemical ionization. The leucine and apoA-I enrichment at each time point was calculated as tracer-to tracee ratios.

*Biochemical analyses*

Plasma total cholesterol, TG, HDL cholesterol, insulin and glucose concentrations were determined by standard enzymatic methods (Architect c16000 Analyser, Abbott Diagnostics, USA) and LDL cholesterol by Friedewald calculation\(^3\). Homeostasis model assessment: insulin resistance (HOMA-IR) score was calculated according to Matthews et al.\(^4\) Plasma total apoB and apoA-I concentrations were determined by immunonephelometry (BNII, Siemens Healthcare Diagnostics Inc., Delaware, USA). HbA1c was measured by a turbidometric inhibition immunoassay (Tina-quant® Hemoglobin A1c Gen.2, Roche Diagnostics, Basel, Switzerland). Biochemical analyses of CETP and HL concentrations (in pre-heparin plasma) were assessed with ELISA (KA1152, Abnova Corporation, Taiwan and KA1241, Abnova Corporation, Taiwan).
Kinetic analyses

Analysis of the tracer data in this study commenced with the understanding that this was a postprandial study, and as such the concentration of lipids and apoproteins would be changing over the course of the study. While this was true for plasma TG and HDL cholesterol concentrations, in only 3 of the 24 kinetic studies did plasma apoA-I concentrations change significantly (using the Wald-Wolfowitz–Runs test) during the course of the kinetic study. As a consequence, we modeled the tracer data assuming constant apoA-I concentrations.

A compartment model was developed using the SAAM II software (The Epsilon Group, VA, USA) to fit the observed tracer data. A simple model with one synthesis pathway and a single plasma compartment was first developed. This model provided a reasonable fit but the tracer curve did not reach the peak apoA-I enrichment. The model was modified to include two synthesis pathways, potentially to mimic the hepatic and intestinal secretion of HDL apoA-I (Figure 3). A similar model was proposed by Fisher et al (1995) with two independent pools within the plasma, however, the present model differs by having two independent sites of synthesis. The inclusion of an extra-vascular HDL exchange compartment did not improve the fit of the model. The Akaike Information Criterion was used to assess goodness of fit of the three models. The model shown in Figure 3 provided significantly better fits to the tracer data compared with the other models. Compartments 1-4 describe leucine kinetics, compartment 2 is the plasma leucine compartment. Compartments 5 and 6 are delay compartments, which account for the synthesis and secretion of apoA-I into the plasma pool, compartment 7. The time of the delays did not change significantly with treatment (compartment 5: 0.39±0.05 vs. 0.47±0.04 h, p=0.42, compartment 6: 2.30±0.32 vs. 2.87±0.40 h, p=0.36).

In three kinetic studies we explored the postprandial nature of this study. We added TG concentration as a modulator of HDL apoA-I fractional catabolic rate (FCR), hypothesising that as TG concentrations increase, the FCR of apoA-I would also increase. To counter the fall in apoA-I concentration with increasing FCR, a transient increase in apoA-I production was required to restore plasma apoA-I concentrations. Although this model provided reasonable fits to the tracer and apoA-I concentration data, estimation of parameters was poor. Furthermore, as apoA-I concentration did not change significantly during the course of each metabolic study as investigated by the Wald-Wolfowitz test, such a model was not supported by the data. The model presented in Figure 3 was fit to the HDL apoA-I tracer data to estimate FCR. HDL apoA-I pool size (PS) was determined as the product of plasma volume and the mean apoA-I concentration. Production rate (PR) was determined as the product of FCR and apoA-I concentration, and expressed as mg/kg/day.

Statistics

SPSS 17 (SPSS Software, Chicago, Illinois, USA) was used for statistical analyses. Significance was defined at the 5% level using a two-tailed test. The paired t-test was used to compare the differences in parameters between treatment groups. The Wald-
Wolfowitz–Runs test was used to test for randomness across apoA-I concentration time courses. Carryover effect of the crossover design was estimated using SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

References