Chronic Hyperinsulinemia Does Not Increase the Production Rate of High-Density Lipoprotein Apolipoprotein AI
Evidence From a Kinetic Study in Patients With Insulinoma
Laurence Duvillard, Emmanuel Florentin, Frédéric Pont, Jean-Michel Petit, Sabine Baiillot-Rudoni, Alfred Penfornis, Bruno Vergès

Objective—In vitro studies showed that insulin stimulates the production of apolipoprotein AI (apoAI). Thus, we hypothesized that chronic hyperinsulinemia could contribute to the increase in the production of high-density lipoprotein apoAI that is observed in metabolic syndrome.

Approach and Results—We performed an in vivo kinetic study with stable isotope in 7 patients with insulinoma who showed hyperinsulinemia but no insulin resistance, 8 patients with insulin resistance, and 16 controls. Insulinemia was 3.1× (P<0.01) higher in patients with insulinoma or insulin resistance than in controls in the fasting state and, respectively, 3.5× and 2.6× (P<0.05) higher in the fed state. The high-density lipoprotein apoAI pool size was smaller in patients with insulin resistance than in controls (49.3±5.4 versus 59.6±7.7 mg·kg⁻¹·d⁻¹; P<0.01), whereas both the high-density lipoprotein apoAI fractional catabolic rate and the high-density lipoprotein apoAI production rate were higher (0.30±0.07 versus 0.20±0.04 pool·d⁻¹; P<0.0001 and 14.6±1.5 versus 11.5±1.9 mg·kg⁻¹·d⁻¹; P<0.01, respectively). In contrast, no significant difference was observed for these parameters between patients with insulinoma and controls. In patients with insulinoma, the apoAI pool size tended to be greater than in patients with insulin resistance (56.3±8.6 versus 49.3±5.4 mg·kg⁻¹; P=0.078), whereas both the apoAI fractional catabolic rate and the production rate were lower (0.20±0.06 versus 0.30±0.07 pool·d⁻¹; P<0.01 and 11.1±1.6 versus 14.6±1.5 mg·kg⁻¹·d⁻¹; P<0.01, respectively). The apoAI fractional catabolic rate was the only variable associated with the apoAI production rate in multivariate analysis and explained 80% of its variance.

Conclusions—Chronic endogenous hyperinsulinemia does not induce any increase in the apoAI production rate, which seems to be more dependent on the apoAI fractional catabolic rate.

Key Words: apolipoprotein AI □ hyperinsulinism □ insulinoma □ kinetics □ metabolic syndrome

Low high-density lipoprotein (HDL) cholesterol and apolipoprotein AI (apoAI) concentrations are a key feature of metabolic syndrome and are likely to play a central role in the early development of atherosclerotic lesions in patients presenting with this syndrome.¹⁻³ Numerous kinetic studies have shown that the decrease in apoAI stems from a faster catabolic rate, which is observed early in patients with metabolic syndrome when glycemia is still normal, and worsens in patients with type 2 diabetes mellitus, in whom it can reach >90%.⁴⁻¹¹

The increase in the HDL apoAI fractional catabolic rate (FCR) is mainly explained by the remodeling of HDL particles under the combined action of cholesteryl ester transfer protein and hepatic lipase, the activities of which are increased in metabolic syndrome. Moreover, a lower concentration of adiponectin could also contribute to faster apoAI catabolism because a significant negative correlation between HDL apoAI catabolism and plasma adiponectin, independently of abdominal obesity, insulin sensitivity, age, sex, and plasma lipids, has been reported.¹²

In addition to the increased HDL apoAI FCR, several studies also showed an increase in the HDL apoAI production rate (PR).⁴⁻⁸ This increase is usually smaller than the increase in the FCR, but it explains why the concentration of HDL apoAI is decreased to a lesser extent than we could expect in view of the FCR values. The mechanisms underlying the increase in the apoAI PR are not clear. In vitro studies have demonstrated that insulin stimulates the transcription of apoAI via the transcription factor Sp.¹³⁻¹⁵ It has been postulated that hyperinsulinemia observed in patients with metabolic syndrome could contribute to the increase in the apoAI PR associated with this
Age, y 31.6±10.3 40.1±18.7 47.5±2.7 0.16 0.009 0.29
BMI, kg m−2 22.9±1.9 24.9±4.6 36.9±5.2 0.26 <0.0001 <0.0001

Table 1.

Clinical and Biochemical Parameters of Subjects Participating in the Study

<table>
<thead>
<tr>
<th></th>
<th>Controls (C; n=16)</th>
<th>Patients With Insulinoma (Ins; n=7)</th>
<th>Patients With Insulin Resistance (IR; n=8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31.6±10.3</td>
<td>40.1±18.7</td>
<td>47.5±2.7</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI, kg ⋅ m−2</td>
<td>22.9±1.9</td>
<td>24.9±4.6</td>
<td>36.9±5.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Fasting serum values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol ⋅ L−1</td>
<td>4.76±0.39</td>
<td>3.37±0.61</td>
<td>5.37±0.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, mU ⋅ L−1</td>
<td>4.8±1.6</td>
<td>14.8±8.3</td>
<td>14.7±8.0</td>
<td>0.003</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.02±0.28</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>SSPG, mmol ⋅ L−1</td>
<td>...</td>
<td>4.42±2.58</td>
<td>13.28±4.87</td>
<td>...</td>
</tr>
<tr>
<td>Triglycerides, mmol ⋅ L−1</td>
<td>0.75±0.33</td>
<td>0.80±0.28</td>
<td>1.50±0.66</td>
<td>0.81</td>
</tr>
<tr>
<td>Total cholesterol, mmol ⋅ L−1</td>
<td>4.56±0.81</td>
<td>4.85±1.34</td>
<td>4.95±0.63</td>
<td>0.48</td>
</tr>
<tr>
<td>LDL cholesterol, mmol ⋅ L−1</td>
<td>2.71±0.78</td>
<td>2.98±1.16</td>
<td>3.16±0.75</td>
<td>0.48</td>
</tr>
<tr>
<td>HDL cholesterol, mmol ⋅ L−1</td>
<td>1.51±0.26</td>
<td>1.43±0.34</td>
<td>1.11±0.28</td>
<td>0.56</td>
</tr>
<tr>
<td>Mean serum values during the kinetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol ⋅ L−1</td>
<td>4.78±0.83</td>
<td>3.19±1.21</td>
<td>5.94±1.37</td>
<td>0.012</td>
</tr>
<tr>
<td>Insulin, mU ⋅ L−1</td>
<td>8.5±5.7</td>
<td>30.1±15.5</td>
<td>22.1±3.7</td>
<td>0.024</td>
</tr>
<tr>
<td>Triglycerides, mmol ⋅ L−1</td>
<td>0.96±0.36</td>
<td>1.09±0.48</td>
<td>2.22±0.73</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Data are means±SD. BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and SSPG, steady-state plasma glucose.
In contrast, HDL from patients with insulin resistance was 17% less rich in cholesteryl esters than was that from controls \((P=0.009)\) and patients with insulinoma \((P=0.033)\). The percentage of proteins, free cholesterol, and phospholipids was similar in the 3 groups.

**ApoAI Kinetic Parameters**

Kinetic curves are shown in Figure 1 for the 3 groups of subjects.

The serum HDL apoAI pool size in patients with insulinoma was similar to that in controls \((56.3±8.6 \text{ versus } 59.6±7.7 \text{ mg·kg}^{-1}; P=0.34; \text{Figure 2})\). In contrast, it was significantly lower in patients with insulin resistance \((49.3±5.4 \text{ versus } 59.6±7.7 \text{ mg·kg}^{-1}; P=0.003)\) and tended to be lower than in patients with insulinoma \((49.3±5.4 \text{ versus } 56.3±8.6 \text{ mg·kg}^{-1}; P=0.078)\). The HDL apoAI FCR in patients with insulinoma was similar to that in controls \((0.20±0.06 \text{ versus } 0.20±0.04 \text{ pool·d}^{-1}; P=0.88)\). It was 1.5× higher in patients with insulin resistance than in controls \((0.30±0.07 \text{ versus } 0.20±0.04 \text{ pool·d}^{-1}; P<0.0001)\) and in patients with insulinoma \((0.30±0.07 \text{ versus } 0.20±0.06 \text{ pool·d}^{-1}; P=0.0011)\). The HDL apoAI PR in patients with insulinoma was very similar to that in controls \((11.1±1.6 \text{ versus } 11.5±1.9 \text{ mg·kg}^{-1}·d^{-1}; P=0.72)\), whereas it was 27% and 232% higher in patients with insulin resistance than in controls \((14.6±1.5 \text{ versus } 11.5±1.9 \text{ mg·kg}^{-1}·d^{-1}; P=0.005)\) and in patients with insulinoma \((14.6±1.5 \text{ versus } 11.1±1.6 \text{ mg·kg}^{-1}·d^{-1}; P=0.005)\), respectively.

**Correlations**

In univariate analysis, the HDL apoAI PR correlated with the HDL apoAI FCR in the control group \((r=0.69; P=0.007)\), in patients with insulinoma \((r=0.79; P=0.050)\), and in patients with insulin resistance \((r=0.81; P=0.032; \text{Table 3})\). In multivariate analysis, with the apoAI PR as a dependent variable and the apoAI FCR, HDL triglyceride content, the presence of insulin resistance (yes/no), the pathology (insulinoma, insulin resistance, none), age, and sex as independent variables, only the apoAI FCR was significantly correlated with the apoAI PR and explained 80% of the variance of the apoAI PR \((β=6.56; P<0.0001; \text{Figure 3})\).

**Discussion**

This stable isotope kinetic study of HDL apoAI conducted in patients with insulinoma, a very rare pathology, showed that, in contrast to what could be suggested by in vitro studies, endogenous chronic hyperinsulinemia is not associated

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**Table 2.** HDL Composition in Subjects Participating in the Study

<table>
<thead>
<tr>
<th></th>
<th>Controls (C; n=16)</th>
<th>Patients With Insulinoma (Ins; n=7)</th>
<th>Patients With Insulin Resistance (IR; n=8)</th>
<th>P Value</th>
<th>P Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteins, %</td>
<td>43.3±4.7</td>
<td>45.4±4.0</td>
<td>0.359</td>
<td>0.073</td>
<td>0.562</td>
</tr>
<tr>
<td></td>
<td>Phospholipids, %</td>
<td>28.5±2.5</td>
<td>26.2±3.3</td>
<td>0.100</td>
<td>0.118</td>
<td>0.765</td>
</tr>
<tr>
<td></td>
<td>Free cholesterol, %</td>
<td>4.2±1.02</td>
<td>3.9±0.8</td>
<td>0.614</td>
<td>0.058</td>
<td>0.298</td>
</tr>
<tr>
<td></td>
<td>Cholesteryl esters, %</td>
<td>21.4±2.3</td>
<td>21.5±3.1</td>
<td>0.940</td>
<td>0.009</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Triglycerides, %</td>
<td>2.7±1.9</td>
<td>2.9±0.8</td>
<td>0.761</td>
<td>0.002</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Data are mean±SD. The values represent the percentage of the total mass of HDL. HDL indicates high-density lipoprotein.
with an increase in the apoAI PR and is thus very unlikely to explain the overproduction of apoAI that is frequently observed in patients with metabolic syndrome.

The kinetic data of HDL apoAI we report here for subjects with insulin resistance but not diabetes mellitus are consistent with previous reports by our and other research groups. Indeed, the FCR in patients with insulin resistance was 1.5× higher than that in lean control subjects with normal insulin sensitivity, which is characteristic of metabolic syndrome. An increase in FCR has been reported in various pathological conditions, including metabolic syndrome and familial hypercholesterolemia or familial defective apoB100. In vitro studies on HepG2 cells demonstrated the positive regulation of apoAI by insulin at the transcriptional level, which led us to hypothesize that hyperinsulinemia could explain the overproduction of apoAI in subjects with metabolic syndrome. If this hypothesis were true, we would have observed an overproduction of apoAI in patients with insulinoma, and we did not. The strength of our study is that the serum insulin level in our insulin-resistant subjects was comparable with that in patients with insulinoma, and that in both groups of patients, hyperinsulinemia resulted from endogenous secretion with delivery in the portal vein. The reasons for the discrepancies between the results obtained on HepG2 cells and those of our in vivo kinetic study in patients with hyperinsulinemia are not clear. In vitro studies showed the induction of apoAI transcription with concentrations of insulin, such as 10 mU L−1, and greater transcription with insulin at 100 mU L−1. ApoAI is mainly secreted by the liver, meaning that apoAI production strongly depends on insulinemia in the portal vein. As it is difficult to reach the portal vein, we were not able to measure portal insulinemia. However, we know that it is higher than peripheral insulinemia because of liver extraction. Thus, given the insulinemia in the portal vein of our patients with insulinoma was very probable in the range of concentrations that should have induced apoAI overproduction. In vitro studies have shown that the insulin-induced increase in apoAI production strongly depends on insulinemia in the portal vein. Therefore, we hypothesized that hyperinsulinemia could explain the overproduction of apoAI in subjects with metabolic syndrome.

### Table 3. Correlation Coefficients Between the HDL apoAI PR and Clinical or Metabolic Parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=16)</th>
<th>Patients With Insulinoma (n=7)</th>
<th>Patients With Insulin Resistance (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>−0.46 (P=0.072)</td>
<td>−0.11 (P=0.79)</td>
<td>0.05 (P=0.90)</td>
</tr>
<tr>
<td>Fasting triglyceridemia, mmol·L⁻¹</td>
<td>−0.28 (P=0.27)</td>
<td>0.39 (P=0.34)</td>
<td>−0.67 (P=0.077)</td>
</tr>
<tr>
<td>Triglyceridemia during kinetics, mmol·L⁻¹</td>
<td>−0.04 (P=0.87)</td>
<td>0.04 (P=0.93)</td>
<td>−0.62 (P=0.10)</td>
</tr>
<tr>
<td>HDL triglycerides (% of HDL mass)</td>
<td>0.16 (P=0.55)</td>
<td>0.60 (P=0.23)</td>
<td>0.17 (P=0.66)</td>
</tr>
<tr>
<td>HDL apoAI FCR, pool·d⁻¹</td>
<td>0.69 (P=0.007)</td>
<td>0.79 (P=0.050)</td>
<td>0.81 (P=0.032)</td>
</tr>
<tr>
<td>HDL apoAI pool, mg·kg⁻¹</td>
<td>0.23 (P=0.38)</td>
<td>−0.51 (P=0.20)</td>
<td>0.02 (P=0.97)</td>
</tr>
<tr>
<td>BMI, kg·m⁻²</td>
<td>−0.09 (P=0.74)</td>
<td>0.029 (P=0.94)</td>
<td>−0.17 (P=0.66)</td>
</tr>
</tbody>
</table>

Data are mean±SD. apoAI indicates apolipoprotein AI; BMI, body mass index; FCR, fractional catabolic rate; HDL, high-density lipoprotein; and PR, production rate.

In vitro studies on HepG2 cells demonstrated the positive regulation of apoAI by insulin at the transcriptional level, which led us to hypothesize that hyperinsulinemia could explain the overproduction of apoAI in subjects with metabolic syndrome. If this hypothesis were true, we would have observed an overproduction of apoAI in patients with insulinoma, and we did not. The strength of our study is that the serum insulin level in our insulin-resistant subjects was comparable with that in patients with insulinoma, and that in both groups of patients, hyperinsulinemia resulted from endogenous secretion with delivery in the portal vein. As it is difficult to reach the portal vein, we were not able to measure portal insulinemia. However, we know that it is higher than peripheral insulinemia because of liver extraction. Thus, given the results obtained in vitro, insulinemia in the portal vein of our patients with insulinoma was very probable in the range of concentrations that should have induced apoAI overproduction. In vitro studies have shown that the insulin-induced increase in apoAI production strongly depends on insulinemia in the portal vein. Therefore, we hypothesized that hyperinsulinemia could explain the overproduction of apoAI in subjects with metabolic syndrome.

### Figure 3. Correlation between high-density lipoprotein (HDL) apolipoprotein AI (apoAI) production rate (PR) and HDL apoAI fractional catabolic rate (FCR).

**A.** Control subjects. **B.** Patients with insulinoma. **C.** Patients with insulin resistance.
Moreover, in multivariate analysis, the apoAI FCR was the only parameter that correlated significantly with the apoAI PR and explained 80% of the variance. Chan et al also reported a very strong correlation between these 2 parameters in both a subgroup of obese subjects and a subgroup of nonobese subjects. The molecular mechanisms underlying the correlation between the apoAI FCR and PR remain to be determined. In univariate analysis, the apoAI PR also correlated with HDL triglycerides and insulin resistance, but these correlations were no longer significant in multivariate analysis. This observation can be explained by the fact that HDL triglycerides (and triglyceridemia) and insulin resistance are major determinants of the HDL apoAI FCR, as clearly demonstrated by previous works. Indeed, hypertriglyceridemia stimulates the transfer of cholesteryl esters from HDL toward apoB-containing lipoproteins in exchange for triglycerides that are secondly hydrolyzed by hepatic lipase. This remodeling of HDL promotes the release of apoAI and the catabolism of both the released apoAI and the HDL remnants.

Alternatively, one might speculate that the increase in apoAI PR is the primary abnormality in metabolic syndrome and is responsible for the increase in apoAI FCR. However, to our knowledge, no data in the literature sustain such an hypothesis. Indeed, beside metabolic syndrome, in some conditions HDL apoAI PR is increased and HDL particles are of smaller size, with no change in apoAI FCR. Such data have been observed, for example, in women on estrogen plus progestin as oral contraceptive. An issue of our work is how hypoglycemia or the increased food intake might interfere with apoAI PR and our conclusion. On cultured cells glucose represses apoAI production. Thus, hypoglycemia is unlikely to mask an increase in apoAI production induced by insulin. However, insulin plus glucose increases apoAI production, as does insulin alone, which suggests that food intake associated with hyperinsulinemia did not mask an increase in apoAI production.

Our control subjects were younger than our patients with insulin resistance. However, age correlated negatively with the PR in the control group with a borderline significance (P=0.07) and no correlation was found in the group of patients with insulin resistance. Thus, the difference in age cannot explain the higher apoAI PR in patients with insulin resistance than in controls. However, the apoAI PR was also higher in patients with insulin resistance than in patients with insulinoma, although the age in these 2 groups was similar. We, therefore, think that endogenous hyperinsulinemia is not responsible for the increased apoAI PR, which is not affected by differences in age.

In conclusion, in patients with insulinoma, showing chronic hyperinsulinemia without insulin resistance, there is no overproduction of HDL apoAI. These results indicate that hyperinsulinemia per se is not responsible for the increased PR of HDL apoAI observed in patients with metabolic syndrome.

Acknowledgments

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Disclosures

None.

References


**Significance**

Apolipoprotein AI (apoAI) production rate is increased in patients with metabolic syndrome. In vitro experiments, performed on rat genome, showed that insulin induces apoAI secretion. As hyperinsulinemia is a key feature of metabolic syndrome, we hypothesized that hyperinsulinemia could be responsible for the increase in apoAI production observed in this syndrome. We performed a stable isotope kinetic study of apoAI in controls with neither insulin resistance nor hyperinsulinemia, obese patients with both insulin resistance and hyperinsulinemia, and patients with insulinoma showing hyperinsulinemia but not insulin resistance. The production rate of high-density lipoprotein apoAI was similar in patients with insulinoma and controls, suggesting that isolated chronic hyperinsulinemia does not induce any apoAI oversecretion in human. However, apoAI secretion rate and apoAI fractional catabolic rate were significantly correlated, both in the whole group and in each subgroup separately, suggesting that apoAI oversecretion is a consequence of increased apoAI catabolic rate, another abnormality characteristic of metabolic syndrome.
Chronic Hyperinsulinemia Does Not Increase the Production Rate of High-Density Lipoprotein Apolipoprotein A1: Evidence From a Kinetic Study in Patients With Insulinoma

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Materials and Methods

Subjects

For this study, we enrolled seven patients (five males, two females) with insulinoma that induced hyperinsulinemia without insulin resistance, eight obese patients (four males, four females) with both insulin resistance and hyperinsulinemia and 16 control subjects (eight males, eight females) without insulin resistance. The distribution of males and females was not statistically different between groups (chi2-test). The recruited subjects were non-smokers and did not drink alcohol, had no other disease and were not taking any medication known to affect lipid metabolism. Control subjects showed no clinical or biological signs of insulin resistance (BMI < 25 kg.m⁻², fasting serum triglyceride level < 1.7 mmol.l⁻¹, HDL cholesterol > 1.04 mmol.l⁻¹ for men and 1.30 mmol.l⁻¹ for women, fasting plasma glucose level < 6.10 mmol.l⁻¹).¹ The HOMA method showed a value below 1.3 in each of them, indicating they were not insulin resistant.² All of the patients with insulinoma had been presenting severe symptomatic hypoglycemia (plasma glucose level < 2.5 mmol.l⁻¹) for 6 to 48 months. They had undergone surgery after the kinetic study, and diagnosis was confirmed by the histology of the removed lesion. In patients with insulinoma, insulin resistance was estimated during Reaven's insulin suppressive test by measuring steady state plasma glucose (SSPG) concentrations achieved during the last 60 min of a 180-min continuous infusion of somatostatin, glucose and insulin.³ Somatostatin was used to suppress endogenous insulin infusion. Insulin and glucose were infused at a dose of 0.8 mU.kg⁻¹.min⁻¹ and 6 mg. kg⁻¹.min⁻¹, respectively. Normal subjects have an SSPG below 6.66 mmol.l⁻¹. Every obese patient had a BMI > 30 kg.m⁻² and was insulin resistance, based on an SSPG concentration above 6.66 mmol.l⁻¹. None of the obese patients had any history of cardiovascular disease. Three of them were treated for high blood pressure by an inhibitor of angiotensin-converting enzyme.

The protocol was approved by the Dijon University Hospital Ethics Committee and written informed consent was obtained from each subject before the study.
**Experimental protocol**

The kinetic study was performed in the fed state. Food intake, consisting of a leucine-poor diet (1700 kcal.day\(^{-1}\), 55% carbohydrates, 38% fats (34% saturated fatty acids, 45% monounsaturated fatty acids, 21% polyunsaturated fatty acids) and 7% proteins), was divided into small equal portions which were provided every 2 hours starting 6 hours before the tracer infusion and continuing to the end of the study in order to avoid large variations in apolipoprotein secretion, as previously performed by our group.\(^4,5\) The endogenous labelling of apolipoproteins was achieved by the infusion of L-[\(^{1-13}\)C] leucine (99 atom %; Eurisotop, Saint Aubin, France), dissolved in 0.9% NaCl solution. At 08.00 hours, each subject received an intravenous priming infusion of 0.7 mg.kg\(^{-1}\) of tracer immediately followed by a 16-hour constant infusion of 0.7 mg.kg\(^{-1}\).h\(^{-1}\). Blood samples were drawn in tubes without anticoagulant but with a gel separator (Becton Dickinson, Meylan, France) at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15 and 16 hours after the priming infusion. Serum was separated by centrifugation for 10 min at 4°C and 3000 g.

**Determination of leucine\(^{13}\)C enriched apolipoprotein**

ApoB100 of VLDL and apoAI of HDL were isolated and hydrolyzed as previously described in reference 6.

Amino acids were converted to N-acetyl O-propyl esters and were analyzed on a Finnigan Mat Delta Plus Advantager isotope ratio mass spectrometer (Finnigan Mat, Bremen, Germany). \(^{13}\)C leucine enrichment was initially expressed in delta %\(,\) and converted into the tracer/tracee ratio prior to modelling.\(^6\)

**Modelling**

ApoAI data were analyzed with the Simulation Analysis And Modelling (SAAM) II program (SAAM Institute, Inc., Seattle, WA) using the same multicompartmental model as that previously published in reference 7.\(,\) others. It was assumed that the majority of apoAI was synthesized by the liver.\(^8\) In this case, the enrichment of the leucine precursor pool for apoAI is the same as that for
VLDL apoB and was assumed to correspond to the tracer-to-tracee ratio at the plateau of the VLDL apoB curve. Two dummy points with enrichment equal to that of the plateau were added at late times (650 and 680 hours) in order to facilitate the compartmental modeling, as previously published. Without doing that, the model could not determine the plateau and could not calculate FSR. As our study was performed in the steady state, the fractional catabolic rate (FCR) was equal to the fractional synthetic rate.

The ApoAI PR was calculated as the product of its FCR and its pool size, and was divided by body weight. Pool size was estimated by the product of HDL apoAI concentration and plasma volume established from body weight, age and sex. The pool size of HDL apoAI was calculated by averaging apoAI plasma measurements at four different times (0, 4, 8 and 12 hours after the beginning of the tracer infusion). HDL apoAI was estimated from plasma measurements since chylomicron apoAI concentrations can be neglected, and by so doing the approximation is far less important than measuring apoAI in the HDL fractions after ultracentrifugation, for which recovery is not 100% because of apoAI loss into the infranatant.

**Laboratory measurements**

Total serum and HDL cholesterol, triglyceride and apoAI concentrations were measured on a Dimension analyzer with dedicated reagents (Siemens, Newark, DE, US). Since each subject had a fasting triglyceride level below 3.74 mmol.l⁻¹. LDL cholesterol was calculated using the Friedewald formula. ApoAI was measured by immunoturbidimetry. The coefficient variation for this parameter was less than 3%. Glycemia was measured on a Vitros 950 analyzer using a glucokinase method (OrthoClinical Diagnostics, Rochester, NY). Insulin was quantified by a chemiluminescent method on an Immulite analyzer (Siemens) with dedicated reagents.

Fasting parameters were measured at 8 am the day before the kinetic study. Triglyceridemia, glycemia and insulinemia during the kinetic study were the mean of four values obtained throughout the study (0, 4, 8 and 12 hours after the beginning of tracer infusion).
**Statistical analysis**

Data are reported as means ± SD. Statistical calculations were performed using the Statview software package (SAS Institute Inc, Berkeley, CA, US). Data comparison between the different groups of subjects was performed using ANOVA and the Fisher's test. Correlation coefficients were calculated using the Pearson test for groups with n>30 and the Spearman test for smaller groups. A multivariate regression analysis was performed to analyze the influence of different factors on the apoAI FCR. A 2-tailed value of 0.05 was considered statistically significant.


