

PAPER

High-density lipoprotein apolipoprotein A-I kinetics in obese insulin resistant patients. An *in vivo* stable isotope study

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AIMS/HYPOTHESIS: Mechanisms responsible for the decreased high-density lipoprotein (HDL) cholesterol level associated with insulin resistance in obese patients are not clearly understood. To determine the influence of insulin resistance at an early stage on HDL metabolism, we performed a stable isotope kinetic study of apolipoprotein (apo) A-I, in five obese insulin resistant women with normal fasting triglycerides and without impaired glucose tolerance, and in five age-matched control women.

METHODS: Each subject received a 16 h constant infusion of L-[1-¹³C]leucine at 0.7 mg/kg/h following a primed bolus of 0.7 mg/kg.

RESULTS: ApoA-I fractional catabolic rate (FCR) was significantly increased in insulin-resistant women compared to controls (0.316 ± 0.056 vs 0.210 ± 0.040 per day, $P < 0.01$), indicating a significant 50% increase of apoA-I catabolism, leading to an important reduction of plasma apoA-I residence time (3.25 ± 0.59 vs 4.92 ± 1.11 , $P < 0.01$). ApoA-I production rate tended to be higher in insulin resistant women than in controls (364 ± 77 vs 258 ± 60 mg/l/day, $P = 0.13$), but the difference was not statistically significant. ApoA-I FCR was correlated with triglycerides during the fed state ($r = 0.69$; $P = 0.026$) and HDL triglycerides–esterified cholesterol ratio ($r = 0.73$; $P = 0.016$), suggesting that alteration of apoA-I metabolism in insulin resistance may be partly related to HDL enrichment in triglycerides.

CONCLUSIONS: Our kinetic study shows that patients, at an early stage of insulin resistance (without impaired glucose tolerance nor fasting hypertriglyceridaemia), already have a significant alteration of apoA-I metabolism (increased apoA-I catabolism), which is consistent with the increased risk of atherosclerosis in this population.

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Introduction

Insulin resistance is common in obese patients. It is a metabolic feature of the insulin resistance syndrome (IRS) including also hyperinsulinaemia, central obesity, dyslipidaemia, glucose intolerance and hypertension, which is associated with an increased risk of cardiovascular disease.¹ Insulin resistant patients often show elevated triglycerides and low high density lipoprotein (HDL) cholesterol levels. The abnormalities of HDL cholesterol metabolism are likely to

play a key role in the development of atherosclerosis, in insulin-resistant patients. Mechanisms involved in the modification of HDL metabolism in insulin-resistant patients are not yet fully understood. Kinetic studies of apolipoprotein A-I have been performed in diabetic patients.^{2,3} In these studies, the true influence of insulin resistance, hypertriglyceridaemia and hyperglycaemia on apolipoproteins kinetics cannot be precisely determined. So far, one kinetic study of apoA-I in 57 human subjects with a broad range of HDL cholesterol levels has been performed with radioisotopes by Brinton *et al*, suggesting that insulin sensitivity could influence apoA-I metabolism.⁴ However, the relationship between insulin resistance and the metabolism of apoA-I is still unclear. In order to get further insight into the influence of insulin resistance on apoA-I kinetics and to see whether

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abnormalities of apoA-I metabolism are observed early in the development of the insulin resistance syndrome, we performed a kinetic study of apoA-I, the major apolipoprotein of HDL particles using stable isotopes in insulin-resistant patients at an early stage of insulin resistance, before the onset of impaired glucose tolerance or diabetes mellitus and with fasting triglyceride levels in the normal range. So far, no kinetic study using stable isotopes has been performed in such patients. In addition to apolipoprotein kinetic study, an evaluation of insulin resistance was performed in each patient.

Research design and methods

Subjects

Five obese insulin-resistant women and five lean, normolipidaemic, age-matched control women were studied. All the women (obese and control) were pre-menopausal and not taking oral contraceptives. They were not consuming any alcohol. Their clinical and biochemical characteristics are shown in Tables 1 and 2.

Table 1 Clinical and biological characteristics of the study group

	Control women (n = 5)	Insulin-resistant women (n = 5)	P value
Age (y)	35.0 ± 11.5	39.0 ± 8.0	NS
BMI (kg/m ²)	23.2 ± 1.5	40.5 ± 3.9	0.009
Waist circumference (cm)	75 ± 6	125 ± 2	0.009
Waist-hip ratio	0.81 ± 0.07	1.04 ± 0.01	0.009
Fasting plasma glucose (mmol/l)	4.46 ± 0.52	4.60 ± 0.66	NS
Post load plasma glucose (mmol/l)	4.34 ± 0.44	6.27 ± 0.98	0.009
SSPG (mmol/l)	—	15.48 ± 5.38	—
Fasting insulin (mU/l)	4.5 ± 1.1	20.3 ± 13.5	0.009
Post load insulin (mU/l)	15.2 ± 4.2	108.7 ± 63.5	0.016
HOMA	0.9 ± 0.2	4.1 ± 2.6	0.009

Values are mean ± s.d.

NS, not significant.

Table 2 Lipid and apolipoprotein concentration of study subjects

Concentrations	Control women	Insulin-resistant women	P value
<i>Screening values (in fasting state)</i>			
Plasma cholesterol (mmol/l)	5.03 ± 0.98	4.92 ± 0.85	NS
Plasma TG (mmol/l)	0.75 ± 0.27	1.04 ± 0.30	NS
HDL cholesterol (mmol/l)	1.70 ± 0.28	1.03 ± 0.43	0.04
LDL cholesterol (mmol/l)	2.99 ± 0.75	3.43 ± 0.82	NS
<i>Experimental values (in fed state)</i>			
Plasma cholesterol (mmol/l)	4.72 ± 0.87	4.56 ± 1.23	NS
Plasma free cholesterol (mmol/l)	1.44 ± 0.23	1.41 ± 0.21	NS
Plasma TG (mmol/l)	1.03 ± 0.19	1.43 ± 0.45	0.02
HDL-TG-EC	0.12 ± 0.01	0.24 ± 0.12	0.016
apo B (mg/dl)	64 ± 9	104 ± 23	0.009
apo A-I (mg/dl)	122 ± 12	119 ± 34	NS

Values are mean ± s.d.

NS, not significant; TG, triglycerides; EC, esterified cholesterol.

All obese insulin-resistant women had increased body mass index (BMI; > 38 kg/m²) and waist-hip ratio (> 1.02). Insulin-resistant women were selected according to their insulin sensitivity using both the homeostasis model assessment (HOMA) method⁵ and the insulin suppression test,⁶ as explained below (Insulin resistance evaluation). All obese insulin-resistant women presented both HOMA levels above 2 and SSPG (steady-state plasma glucose) values, during the insulin suppression test, above 8 mmol/l.^{5,6} Insulin-resistant women were not glucose intolerant with fasting plasma glucose levels less than 6.1 mmol/l and plasma glucose level after a 75 g oral glucose load less than 7.8 mmol/l. Furthermore, they were normotriglyceridaemic since their fasting TG levels were within the normal limits of our laboratory (< 1.60 mmol/l), ranging from 0.69 to 1.47 mmol/l. No patient was taking any medication known to affect lipid metabolism.

All control subjects were in good health, with normal glucose tolerance and normal plasma lipid levels. They were not taking any medication.

Four weeks before the kinetic study, the subjects (obese and controls) were instructed to maintain their usual level of activity and to refrain from any strenuous exercise.

The protocol was approved by the Dijon University Hospital ethics committee and written informed consent was obtained before the study was started.

Experimental protocol

The kinetic study was performed in the fed state. Food intake, with a leucine-poor diet (1700 kcal/day, 55% carbohydrates, 39% fats and 7% proteins), was fractionated in to small equal portions which were provided every 2 h, starting 6 h prior to the tracer infusion up to the end of the study, in order to avoid important variations in apolipoprotein plasma concentration, as previously performed by other groups.^{7,8} To determine the kinetics of apolipoprotein A-I, the subjects received an intravenous injection of a 0.7 mg/kg bolus of L-[1-¹³C]leucine (99% ¹³C, Eurisotop, Saint Aubin, France), immediately followed by a 16 h constant infusion at 0.7 mg/kg/h. Blood samples (16 ml) were collected at hours 0, 0.25, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15 and 16. Blood was collected in tubes without anticoagulant but with a gel separator (Becton Dickinson, Meylan, France). Serum was separated by centrifugation and stored at 4°C. Sodium azide, an inhibitor of bacterial growth, and aprotinin (Sigma), a protease inhibitor, were added to plasma sample at final concentration of 500 and 17 mg/l, respectively.

Analytical procedures

Analytical procedures were performed as previously described in details.⁹⁻¹¹

Isolation of apolipoproteins. VLDL (density (*d*) < 1.006 g/ml) and HDL (1.070 < *d* < 1.21) were isolated from plasma by

sequential flotation ultracentrifugation, using a 50.4 rotor in a L7 apparatus (Beckman Instruments, Palo Alto, CA, USA) at 50 000 rpm for 6 h for VLDL and 21 h for HDL. HDL fractions were then dialysed against a 10 mmol/l ammonium bicarbonate buffer pH 8.2 containing 0.01% EDTA and 0.013% sodium azide. VLDL and HDL fractions were delipidated 1 h at -20°C using ten vols of diethylether-ethanol 3:1. Apolipoproteins from each lipoprotein fraction were isolated by preparative discontinuous sodium dodecyl sulphate-polyacrylamide gel electrophoresis on a 3 and 15% gel.^{12,13} Apolipoprotein bands were excised from polyacrylamide gels and hydrolysed in 6M HCl at 110°C for 16 h under nitrogen. Samples were then centrifuged to remove polyacrylamide. Supernatants were lyophilized in a Speed Vac (Savant Instrument, Farmingdale, NY, USA). Lyophilized samples were dissolved in 50% acetic acid and applied to an AG-50W-X8 200-400 mesh cation exchange resin (Bio-Rad, Richmond, VA, USA) and aminoacids were recovered by elution with 4N NH_4OH .⁸ They were converted to *N*-acetyl *O*-propyl (NAP) amino acid esters prior to analysis by GC/C/IRMS.^{14,15}

Determination of leucine enrichment by gas chromatograph/combustion/isotope ratio mass spectrometry (GC/C/IRMS). A Finnigan Mat Delta C isotope ratio mass spectrometer (Finnigan Mat, Bremen, Germany) coupled to an HP 5890 series II gas chromatograph (Hewlett Packard), was used to determine sample isotopic enrichment. The GC was equipped with a split/splitless injector and fitted with a BPX5 capillary column (30 m, 0.32 mm i.d., 25 μm film thickness, SGE, Ringwood, Vic, Australia) and a 2 m retention gap (RGK-1, SGE). Carrier gas was helium and the column head pressure was set at 14 psi. Injector temperature was 250°C for leucine analysis. The splitless mode injection was adopted. The solvent purging valve was opened 0.6 min after injection. The column was held isothermal at 50°C for 1 min after injection, then the temperature was programmed at $20^{\circ}\text{C}/\text{min}$ up to 135°C , at $2^{\circ}\text{C}/\text{min}$ from 135 to 150°C , at $15^{\circ}\text{C}/\text{min}$ from 150 to 290°C , and was held for 5 min at 290°C . The operation conditions of the ion source were as follows: source chamber pressure 1.4×10^{-6} mbar, ionising energy 80 eV, ion accelerating voltage 3 kV. Isotope abundance was expressed relatively to pulse peaks of reference gas. Data were analysed using the supplier software (Finnigan ISODAT).

Modelling

Apolipoprotein kinetics data were expressed as tracer/tracee ratios,^{9,16,17} $z(t)$, calculated as follows:

$$z(t) = \frac{e(t)}{e_i - e(t)}$$

where e_i is the tracer isotopic enrichment, $e(t) = a(t) - a_N$, and $a(t)$ and a_N are the isotope abundance of the labelled and the unlabelled species, respectively.¹⁸

Kinetic analysis of tracer-tracee ratios were made with the simulation analysis modelling SAAM II program¹⁹ (SAAM Institute Inc., Seattle, WA, USA). ApoA-I and B-100 data were analysed using a monoexponential function, assuming that the kinetics of these apolipoproteins are described by one single compartment. A more complex model would not significantly improve the fit of the data. The function used was $A(t) = Ap(1 - \exp[-k(t - d)])$, where $A(t)$ is the apolipoprotein enrichment at time t , Ap the enrichment at the plateau of the VLDL apo B100 curve, d the delay between the beginning of the experiment and the appearance of tracer in the apolipoprotein and k the fractional synthetic rate (FSR) of the apolipoprotein.^{3,20} It was assumed that the VLDL apo B100 tracer-tracee ratio at the plateau corresponds to the tracer-tracee ratio of the leucine precursor pool.²⁰ This estimation is made upon the assumption that apoB-100 and the great majority of apo A-I are synthesized by the liver, as previously demonstrated by Ikewaki *et al.*²⁰ VLDL apo B100 tracer-tracee ratio is widely used to estimate the isotopic enrichment of the hepatic protein synthetic precursor pool in human studies.^{21,22} In the steady state, the FSR equals the fractional catabolic rate (FCR).²³ The residence time (RT) is inversely related to the FCR: $RT = 1/\text{FCR}$.

Because the insulin-resistant patients were obese, the apoA-I production rate (PR) was calculated by multiplying the apoA-I concentration by the FSR of apoA-I. Thus, data were normalized to the plasma volume of each subject, as previously reported.^{10,11}

Analytical methods

Apolipoprotein and lipid assays. Concentrations of apoA-I were determined by immunoturbidimetry²⁴ with anti-apoA-I antibodies purchased from Boeringer Mannheim. ApoA-I standard was purchased from Boeringer Mannheim. ApoB concentrations were measured by immunoturbidimetry (Boeringer Mannheim). All chemical lipid assays were performed on a Cobas-Fara Centrifugal Analyser (Hoffmann-La Roche). Total cholesterol and unesterified cholesterol concentrations were measured by enzymatic methods using Boeringer Mannheim reagents. Triglyceride concentration was measured by enzymatic methods using Roche reagents.

Insulin resistance evaluation The insulin resistance level was estimated by using both the homeostasis model assessment (HOMA) method⁵ and the insulin suppression test.⁶ Insulin-stimulated glucose uptake was estimated by measuring the SSPG concentrations achieved during the last 60 min of a 180 min continuous infusion of somatostatin, insulin and glucose, during the insulin suppression test. Somatostatin, in this test, is used to suppress endogenous insulin production, and insulin and glucose are infused at a dose of 0.8 mU/kg/min and 6 mg/kg/min, respectively. The higher the SSPG level achieved during the last 60 min of

the continuous infusion, the more insulin resistant the patient is. Normal subjects have SSPG below 6.6 mmol/l.⁶

The HOMA was calculated with the following formula:⁵

$$\text{HOMA} = \frac{[\text{glucose}] \times [\text{insulin}]}{22.5}$$

where [glucose] is the fasting glucose concentration expressed in mmol/l and [insulin] is the fasting insulin concentration expressed in mU/l.

Plasma insulin was measured by radioimmunoassay (CIS Bio International, Gif sur Yvette, France).

Statistical analysis

Data are reported as mean \pm s.d. Statistical calculations were performed using the SPSS software package. The Spearman correlation coefficients were calculated in correlation analyses. The Mann–Witney *U*-test was used to compare clinical, biological and kinetic characteristics between patients and controls. A two-tailed probability level of 0.05 was accepted as statistically significant.

Results

Apolipoprotein and lipid concentrations

Clinical and glucose metabolism characteristics of the studied subjects are presented in Table 1. The insulin-resistant women were significantly overweight compared to control women (BMI = 40.5 \pm 3.9 vs 23.2 \pm 1.5 kg/m², *P* = 0.009). These patients had clinical features of insulin resistance such as significantly increased waist circumference (125 \pm 2 vs 75 \pm 6 cm, *P* = 0.009) and increased waist–hip ratio (1.04 \pm 0.01 vs 0.81 \pm 0.07, *P* = 0.009). Moreover, insulin resistance was confirmed in each subject by elevated HOMA (> 2) and SSPG values during the Insulin Suppression test (> 8 mmol/l). They were neither diabetic nor glucose intolerant as assessed by normal fasting and post-load glucose concentrations. Data for plasma apolipoprotein and lipid concentrations measured before and during the kinetic study are presented in Table 3. Since no significant variation was observed between measurements at four different infusion times (data not shown), it was considered that all subjects were in steady-state throughout the study. Each insulin resistant patient had a fasting triglyceride level within the normal range of our laboratory (< 1.60 mmol/l), ranging from 0.69 to 1.47 mmol/l. Compared to control subjects, the insulin-resistant subjects had significantly lower HDL cholesterol levels (*P* = 0.04) and significantly higher plasma triglycerides, during the fed state, and apoB concentrations. Mean HDL Triglycerides–Esterified Cholesterol ratio (HDL-TG–EC) was significantly higher in insulin-resistant patients than in controls (*P* = 0.016). ApoA-I concentrations were not different between insulin-resistant patients and controls.

Kinetic data

VLDL apoB-100 kinetic curves in insulin resistant patients and controls are shown in Figure 1. All the subjects (insulin-resistant and control) achieved plateau values for VLDL apoB-100 during the 16 h infusion period. Mean VLDL apoB-100 FCR was not significantly different between insulin resistant patients and controls (0.35 \pm 0.06 vs 0.32 \pm 0.08/h, NS).

The kinetic curves of apoA-I in insulin resistant patients and controls are shown in Figure 2. The isotopic enrichment (expressed as percentage of VLDL apo B100 plateau) increased more rapidly in insulin-resistant patients than in controls, indicating an increased FCR. As shown in Table 3, insulin resistant patients had a significantly higher apoA-I

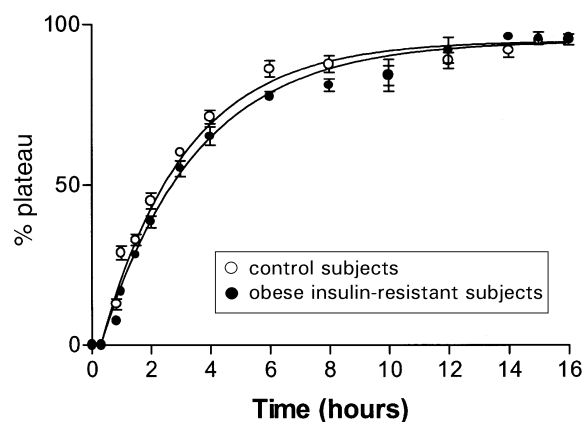


Figure 1 Kinetic curves of VLDL apoB-100 obtained during a primed constant infusion of L-[1-¹³C]leucine. [¹³C]leucine enrichment values, expressed as percentage of plateau, for control subjects and obese insulin-resistant subjects are shown. The curves were obtained by monoexponential modelling. Data are shown as mean \pm s.e.m.

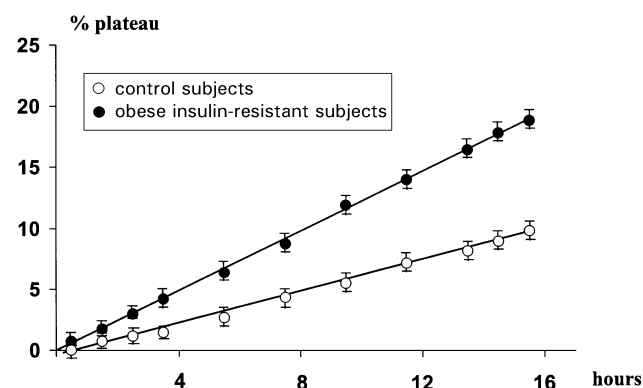


Figure 2 Kinetic curves of HDL apoA-I obtained during a primed constant infusion of L-[1-¹³C]leucine. [¹³C]leucine enrichment values, expressed as percentage of VLDL apo B100 plateau, for control subjects and obese insulin-resistant subjects are shown. The curves were obtained by monoexponential modelling. Data are shown as mean \pm s.e.m.

Table 3 Kinetic parameters of apoA-I in insulin resistant patients and controls

	Plasma ApoA-I (mg/dl)	ApoA-I FCR (/day)	ApoA-I RT (days)	ApoA-I PR (mg/l/day)
<i>Insulin-resistant patients</i>				
1	116	0.311	3.22	361
2	109	0.380	2.63	413
3	169	0.275	3.64	464
4	73	0.365	2.74	268
5	127	0.249	4.02	316
Mean ± s.d.	119 ± 34	0.316 ± 0.056	3.25 ± 0.59	364 ± 77
<i>Controls</i>				
1	115	0.238	4.20	275
2	141	0.238	4.20	336
3	112	0.245	4.08	274
4	116	0.151	6.61	175
5	127	0.181	5.51	230
Mean ± s.d.	122 ± 12	0.210 ± 0.040	4.92 ± 1.11	258 ± 60
Patients vs controls P-value	NS	0.009	0.009	NS

NS, not significant; FCR fractional catabolic rate; RT, residence time; PR, production rate.

fractional catabolic rate than controls (0.316 ± 0.056 vs 0.210 ± 0.040 /day, $P = 0.009$). Thus, insulin resistant patients had a significant lower plasma residence time for apoA-I than controls (3.25 ± 0.59 vs 4.92 ± 1.11 days, $P = 0.009$; Table 3).

Correlations

Spearman correlation coefficients analysis in both control and insulin-resistant populations are shown in Table 4.

Insulin resistance indexes were associated with lipid levels. Indeed HOMA was negatively correlated with HDL-C ($r = -0.71$, $P = 0.022$) and positively correlated with plasma TG concentration in the fasting state ($r = 0.63$, $P = 0.048$), plasma TG concentration in the fed state ($r = 0.66$, $P = 0.037$)

and with HDL-TG-EC ratio ($r = 0.79$; $P = 0.008$). HOMA was also correlated with the waist-hip ratio ($r = 0.78$, $P = 0.008$), with the waist circumference ($r = 0.78$, $P = 0.008$) and the BMI ($r = 0.64$, $P = 0.043$).

ApoA-I fractional catabolic rate was positively correlated with fasting insulin concentration ($r = 0.91$, $P < 0.001$), post-load insulin concentration ($r = 0.92$, $P < 0.001$), post-load glucose ($r = 0.85$, $P = 0.002$), HOMA ($r = 0.87$, $P = 0.001$), TG in the fed state ($r = 0.69$, $P = 0.026$) and HDL-TG-EC ($r = 0.73$; $P = 0.016$). ApoA-I fractional catabolic rate was also positively correlated with the BMI ($r = 0.65$, $P = 0.041$), the waist circumference ($r = 0.81$, $P = 0.004$) and the waist-hip ratio ($r = 0.71$, $P = 0.021$). ApoA-I fractional catabolic rate was negatively correlated with plasma HDL-cholesterol ($r = -0.72$, $P = 0.019$).

ApoA-I production rate was not correlated with plasma triglycerides, or with HDL-cholesterol or with HDL-TG-EC.

Table 4 Spearman correlation coefficients analysis (r -values) in both control and insulin-resistant populations

	r	P-value
<i>HOMA</i>		
TG (fasting state)	0.63	0.048
TG (fed state)	0.66	0.037
HDL-C	-0.71	0.022
HDL-TG/EC	0.79	0.008
Waist circumference	0.78	0.008
Waist-hip	0.78	0.008
BMI	0.64	0.043
<i>ApoA-I FCR</i>		
Fasting insulin	0.91	< 0.001
Post-load insulin	0.92	< 0.001
HOMA	0.87	0.001
TG (fed state)	0.69	0.026
HDL-TG-EC	0.73	0.016
HDL-cholesterol	-0.72	0.019
Post-load glucose	0.85	0.002
Waist-hip	0.90	< 0.001
Waist circumference	0.81	0.004
BMI	0.65	0.041

Discussion

The abnormalities of HDL cholesterol metabolism are likely to play an important role in the development of atherosclerosis in insulin-resistant patients. Our study was designed to explore the kinetics of apoA-I, the major apolipoprotein of HDL particles, in obese women at an early stage of insulin resistance, before the onset of glucose intolerance and increased fasting triglycerides. Our results demonstrate that such patients, at an early stage of insulin resistance, already have a significant alteration of apolipoprotein A-I metabolism with a 50% increase of apoA-I catabolism. Mean HDL cholesterol was significantly decreased in insulin resistant patients and below the normal value (1.20 mmol/l) for women. This indicates that the first abnormality of lipid metabolism in insulin resistant patients, concerns HDL cholesterol.

Our study is the first apoA-I kinetic study, using stable isotopes, in insulin-resistant subjects at an early stage of

insulin resistance, before the onset of impaired glucose tolerance and increased fasting TG levels. Indeed, in the radioisotope kinetic study of apoA-I performed by Brinton *et al*, the subgroup of six patients considered as 'normotriglyceridaemic' had a mean fasting triglyceride level of 1.46 ± 38 mmol/l, which is more elevated than in our patients.⁴ Moreover, three of these six subjects presented fasting triglycerides ranging from 1.64 to 2.06 mmol/l, above the upper limit of the normal range of triglycerides in most laboratories, indicating that these subjects were not strictly normotriglyceridaemic. Furthermore, one, among these six patients, was diabetic on oral hypoglycaemic treatment.⁴ In our study, we decided to select insulin-resistant patients without impaired glucose tolerance and showing fasting triglyceride levels in the normal range. Our five obese women were truly insulin resistant, as demonstrated by significantly increased HOMA (normal values < 2),⁵ SSPG (normal values < 6.6 mmol/l),⁶ plasma fasting and post-load insulin values. However, they were neither diabetic nor glucose intolerant as assessed by normal fasting and post-load glucose concentrations. Furthermore, our insulin-resistant subjects had fasting triglyceride levels which were in the normal range of our laboratory and not significantly different from those in controls. In our study, we have been able to compare the kinetic data found in insulin-resistant subjects with those obtained in sex- and age-matched controls. So far, no apoA-I kinetic data in such patients at an early stage of insulin resistance are available.

We chose to study the metabolism of apoA-I in the post-prandial state, since this state is the most frequent for humans during a 24 h period. Moreover, many data suggest that atherosclerosis could be a post-prandial phenomenon.²⁵ The daily food intake was divided into small equal portions taken by the subjects every 2 h, as previously performed by several groups.^{7,8} With this protocol plasma lipid and apolipoprotein values remained constant throughout the kinetic study.

HDL apoA-I turnover rate was studied by a 16 h primed-constant infusion of L-[1-¹³C]leucine. Long-term kinetic studies in control subjects, have indicated that apoA-I is secreted into both fast and slow turning-over plasma pools.²⁶ However, under our study conditions, kinetic heterogeneity of HDL apoA-I was not apparent. A kinetic study in control subjects has shown that no more information could be obtained by separation of HDL2 apoA-I and HDL3 apoA-I.³ In that study, kinetic curves obtained with HDL2 apoA-I and HDL3 apoA-I were shown to be very similar, indicating a fast rate of interconversion between these subclasses of lipoproteins.³ Thus, our approach is a reasonable method for determining apoA-I turn-over rate and is now widely used.^{3,27,28}

The kinetic parameters obtained for apoA-I in control subjects are in good agreement with results obtained in previous studies using stable isotopes³ or radioiodinated apoA-I.^{29,30} The true plateau value for apoA-I, corresponding to the proapoA-I plateau, has been shown to be about 80% of

VLDL apo B100 plateau²⁸ for control subjects, indicating that apoA-I precursor enrichment is well approximated by VLDL apo B100 plateau. We can suppose that it is about the same in insulin-resistant subjects. Thus, we can assume that the choice of the VLDL apo B100 plateau in the kinetic parameters calculations has a negligible impact on the comparison between patients and controls.

As pointed out by Egusa *et al*,³¹ comparison of kinetic data between obese and lean subjects presents difficulties. Indeed, fat mass may have a different contribution to lipoprotein metabolism than fat-free mass. Since lipoproteins are metabolically active in the plasma compartment, we expressed the production rates adjusted to plasma volume instead of body weight, as previously reported.^{10,11}

Our results indicate that insulin resistance significantly influences HDL metabolism since the insulin resistance index (HOMA) was negatively correlated with HDL cholesterol levels. Furthermore, apoA-I fractional catabolic rate was positively correlated with clinical (waist-hip ratio, waist circumference) and biological markers of insulin resistance (plasma fasting and post-load insulin levels, HOMA).

Our insulin-resistant women had a significant acceleration of apoA-I catabolism compared to control women, leading to a significant reduction of plasma apoA-I residence time. Although the difference was not statistically significant apoA-I production rate tended to be increased in insulin-resistant women compared to controls. This is likely to explain why plasma apoA-I levels were not significantly different between obese subjects and controls. We may think that the increased production rate of apoA-I could be a response to its accelerated catabolism. These kinetic modifications, observed in insulin resistant subjects, reflect an important acceleration of apoA-I 'turn-over' rate, with a significant reduction of plasma apoA-I residence time, which may contribute to the increased cardiovascular risk observed in insulin resistant subjects. Moreover, HDL cholesterol level was significantly negatively correlated with apoA-I fractional catabolic rate, but not with apoA-I production rate, indicating that the acceleration of apoA-I catabolism is the main factor responsible for the decrease of HDL-cholesterol level insulin resistant subjects. Thus, obese women, at an early stage of insulin resistance with still normal fasting plasma triglycerides and without impaired glucose tolerance already have an important alteration of apoA-I metabolism with a 50% increase of apoA-I catabolism, leading to a significant reduction of its plasma residence time.

ApoA-I fractional catabolic rate was positively correlated with TG in the fed state. The correlation between apoA-I fractional catabolic rate and the plasma triglyceride concentration suggests that the slightly more elevated TG concentration in insulin-resistant subjects compared to controls, which is observed mostly in fed state, could contribute to increase apoA-I catabolism. As previously reported in insulin resistance,³² we observed an enrichment of HDL particles in triglycerides in our patients. In the present study, the sig-

nificantly inverse correlation between apoA-I residence time and the HDL TG-EC ratio argue for an increased HDL catabolism partly promoted by the HDL enrichment in triglycerides. Pietzsch *et al* have shown that subjects with impaired glucose tolerance (IGT) had an increased apoA1 FCR, and found a positive correlation between apoA1 FCR and HDL-triglycerides.³³ These data support our results. It has been shown that HDL with a higher triglyceride content are a better substrate for hepatic lipase leading to their faster catabolism.³³ An increased CETP (cholesteryl ester transfer protein) activity has been reported in insulin-resistant obese subjects leading to enrichment of HDL with triglycerides.³⁴ Moreover, Pietzsch *et al* have found a significant positive correlation between CETP activity and apoA-I FCR in patients with IGT.³³ Although CETP activity has not been measured in our present study, we may hypothesize that triglyceride enrichment of HDL, possibly promoted by an increased activity of CETP, is likely to be an important factor leading to increase the catabolism of HDL.

In conclusion, the results of our kinetic study indicate that women at an early stage of insulin resistance (before the onset of impaired glucose tolerance and increased fasting triglycerides) already have a significant alteration of the metabolism of apoA-I, the main apolipoprotein of HDL particles. The first kinetic abnormality observed in the obese insulin-resistant women is a significant 50% increase of apoA-I catabolism. This increased apoA-I catabolism could be promoted by an increased triglyceride content of HDL particles, as suggested by our results. Further studies are needed to show that the same mechanisms apply for obese insulin-resistant men. Acceleration of apoA-I catabolism in insulin-resistant patients compromises HDL metabolism and is consistent with an increased cardiovascular risk.

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