In Vivo Evidence for Increased Apolipoprotein A-I Catabolism in Subjects With Impaired Glucose Tolerance

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The in vivo kinetics of the HDL apolipoproteins (apo) A-I and A-II were studied in six subjects with impaired glucose tolerance (IGT) and six control subjects with normal glucose tolerance (NGT), using a stable isotope approach. During a 12-h primed constant infusion of L-[ring-$^{13}C_6$]-phenylalanine, tracer enrichment was determined in apoA-I and apoA-II from ultracentrifugally isolated HDL. The rates of HDL apoA-I and apoA-II production and catabolism were estimated using a one-compartment model-based analysis. Triglycerides were higher in IGT subjects (1.33 ± 0.21 vs. 0.84 ± 0.27 mmol/l, $P < 0.05$), but were within the normal range. HDL cholesterol and apoA-I levels were significantly lower in subjects with IGT (1.07 ± 0.15 vs. 1.36 ± 0.14 mmol/l, $P < 0.05$; 0.94 ± 0.10 vs. 1.34 ± 0.07 g/l, $P < 0.01$). In IGT subjects, HDL composition was significantly altered, characterized by an increase in HDL triglycerides (4.9 ± 1.9 vs. 3.2 ± 1.0%, $P < 0.05$) and HDL phospholipids (34.7 ± 2.6 vs. 27.5 ± 5.8%, $P < 0.05$) and a decrease in HDL cholesteryl esters (10.1 ± 2.0 vs. 12.7 ± 2.9%, P < 0.05) and HDL apoA-I (31.5 ± 4.4 vs. 43.2 ± 2.4%, $P < 0.05$). The mean fractional catabolic rate (FCR) of HDL apoA-I was significantly higher in IGT subjects (0.34 ± 0.05 vs. 0.26 ± 0.03 day^{-1}, $P < 0.01$), while the HDL apoA-I production rate (PR), as well as the PR and FCR of HDL apoA-II, showed no differences between the two groups. There were significant correlations between HDL apoA-I FCR and the following parameters: HDL apoA-I ($r = –0.902$, $P < 0.001$), HDL cholesterol ($r = –0.797$, $P = 0.001$), plasma triglycerides ($r = 0.743$, $P < 0.01$), HDL triglycerides ($r = 0.696$, $P < 0.01$), and cholesterol ester transfer protein activity ($r = 0.646$, $P < 0.01$). We observed a strong positive association between increased apoA-I catabolism and insulin ($r = 0.765$, $P < 0.01$) and proinsulin ($r = 0.797$, $P < 0.01$) concentrations. These data support the hypothesis that the decrease in HDL cholesterol and apoA-I levels in IGT is principally the result of an enhanced apoA-I catabolism. The latter seems to be an early metabolic finding in IGT even when other lipid parameters, especially plasma triglycerides, still appear to be not or only weakly affected. *Diabetes* 47:1928–1934, 1998

Low concentrations of HDL cholesterol appear to be an outstanding lipoprotein predictor of cardiovascular diseases. A number of epidemiologic studies in nondiabetic people have demonstrated that HDL cholesterol and HDL apolipoprotein (apo) A-I concentrations inversely correlate with the incidence of coronary artery disease (CAD) (1–4). Furthermore, in both patients with NIDDM and those with impaired glucose tolerance (IGT), reduced HDL levels (HDL cholesterol levels are typically ~15–30% lower in these patients than in nondiabetic subjects) also have been found to be directly correlated with an increased risk of CAD (5–9). The true nature of the relationship between diabetic conditions and increased CAD still remains unclear, and the role of HDL has not been adequately proven. IGT, characterized by insulin resistance and hyperinsulinemia, has been suggested to be the transitional state between normal glucose tolerance (NGT) and NIDDM (10–12). In NIDDM, the altered insulin action influences hormone-sensitive enzymes that are directly involved in HDL metabolism, e.g., lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL), and cholesterol ester transfer protein (CETP) (13–15), and should have early consequences on the turnover of HDL particles, and conclusively, on the metabolism of HDL apoA-I and apoA-II. Recently, Frenais et al. (16) reported an increased catabolism of HDL apoA-I to be completely responsible for the lower HDL concentrations in NIDDM with a pronounced diabetic dyslipidemia (hypertriglyceridemia and low HDL cholesterol) and poor metabolic control when compared with nondiabetic subjects. However, only a few data are available on the earlier stages of the metabolic decompensation, particularly changes in lipoprotein metabolism, in IGT. To explore whether subjects with IGT but without a distinct hypertriglyceridemia already show alterations in their HDL metabolism, we assessed the in vivo kinetics of HDL apoA-I and apoA-II in IGT using endogenous labeling with L-[ring-$^{13}C_6$]-phenylalanine. The kinetic parameters for apoA-I and apoA-II were subsequently estimated by one-compartment model-based analysis, using the SAAM II program. To gain further insight into the metabolic etiology of low HDL in IGT, we looked for relationships between kinetic parameters of apoA-I and apoA-II metabolism and specific markers of an insulin resistant and/or hyperinsulinemic situation.

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Received for publication 2 March 1998 and accepted in revised form 24 August 1998.

apo, apolipoprotein; CAD, coronary artery disease; CETP, cholesterol ester transfer protein; FCR, fractional catabolic rate; FFA, free fatty acids; FSD, fractional standard deviation; FSR, fractional synthetic rate; HTGL, hepatic triglyceride lipase; IGT, impaired glucose tolerance; LpA-I, subclass of HDL particles that contains only apoA-I; LpA-I:A-II, subclass of HDL particles that contains both apoA-I and apo A-II; LPL, lipoprotein lipase; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PR, production rate; TPLA, total postheparin lipase activity; WHO, World Health Organization.

RESEARCH DESIGN AND METHODS

Subjects. All subjects consented to participate in the study, which was approved by the local medical ethics committee. The participants were six nonobese normotriglyceridemic IGT subjects (triglycerides <1.7 mmol/l) and six healthy normolipidemic subjects with NGT. Their basic clinical and biochemical characteristics are given in Table 1. In all subjects, there was no evidence of thyroid, liver, cardiovascular, hematologic, or renal abnormalities, and none were taking medications. All of the subjects were nonsmokers. The diagnosis of IGT was based on two consecutive oral glucose tolerance tests (OGTTs) according to World Health Organization (WHO) guidelines and criteria (two 2-h post-glucose values between 7.8 and 11.1 mmol/l) (17). Plasma glucose was determined by the hexokinase method, using a commercial test kit from Boehringer-Mannheim (Mannheim, Germany); plasma insulin and proinsulin were assayed by enzyme immunoassay (BioSource, Ratingen, Germany).

Experimental protocol. The study protocol has been previously published and was only slightly modified (18). In brief, 1 week before the study, subjects were placed on an isocaloric diet with a moderate fat content (47% carbohydrate, 37% fat, 16% protein, and 200 mg cholesterol/1,000 kcal; four meals per day). Then they were admitted to a metabolic ward for 2 days. Patients and control subjects fasted overnight and during the turnover procedure. For measuring the metabolism of multiple human apolipoproteins in vivo, the proteinogenic tracer amino acid L-[ring-$^{13}C_6$]-phenylalanine (isotopic purity 99.0%; Cambridge Isotope Laboratories, Woburn, MA) was administered as a primed constant infusion. The tracer dose was prepared by dissolving L-[ring-$^{13}C_6$]-phenylalanine (10 mg/l) in 0.9% NaCl. The solutions were demonstrated to be sterile and free from pyrogens. An intravenous catheter was inserted into a superficial vein of each arm, one for tracer administration and the other for blood sampling. After a priming bolus of 550 µg/kg L-[ring-$^{13}C_6$]-phenylalanine, a constant infusion of 12 µg · kg^{-1} · min^{-1} L-[ring-$^{13}C_6$]-phenylalanine was continued for 12 h. Before the priming bolus and during the tracer infusion, >30 blood samples were obtained. Blood (10–20 ml) was collected into vacuum tubes containing EDTA at a final concentration of 0.1%. Plasma was recovered after centrifugation at 4°C. Sodium azide (0.01%) and aprotinin (200 KU/ml) were added immediately. Samples were stored at 4°C. These plasma aliquots were used to determine plasma lipids and concentrations of apoB-100, apoA-I, and apoA-II, as well as plasma enrichments of free phenylalanine and tyrosine, to ascertain a tracer and precursor steady state.

Determination of CETP activity and total postheparin lipase activity. For determination of CETP activity, plasma aliquots were shock-frozen in liquid nitrogen and stored in cryovials at –80°C. The CETP activity test kit (WAK-Chemie Medical, Bad Soden, Germany) includes a donor and an acceptor particle. Fluorescent cholesteryl linoleate [*N*-(7-Nitrobenz-2-oxa-1,3-diazole)-23,24-dinor-5-cholen-22-amine-3β-ol (NBD-CE)] is present in self-quenched state when contained within the core of the donor particle. The CETP-mediated transfer is determined by the increase in fluorescence intensity as the cholesteryl linoleate is removed from the donor to the acceptor in the presence of a CETP-containing plasma sample. CETP activity is expressed as nanomoles per milliliter per hour (19).

Total postheparin lipase activity (TPLA) that includes HTGL and LPL activities was also measured in the subjects after a 12-h overnight fast and 10 min after intravenous injection of heparin (20 U/kg body wt). The postheparin lipase activity was measured using a commercially available fluorometric rate assay (WAK-Chemie Medical). The lipase substrate is 1-trinitrophenyl-amino-dodecanoyl-2-pyrendecanoyl-3-*O*-hexadecyl-sn-glycerol, in which the trinitrophenyl group quenches the pyrene fluorescence intramolecularly upon its hydrolysis. The gradual increase in pyrene fluorescence reflects the time-dependent progress of lipolysis and, under substrate saturation conditions, TPLA. This lipase assay is continuous and does not require separation of substrate and reaction products. The total lipolytic enzyme activity is expressed as micromoles per liter per second (20).

Lipoprotein isolation. VLDLs (d < 1.006 g/ml), LDLs (1.006 < d < 1.063 g/ml), and HDLs (1.063 < d < 1.210 g/ml) were isolated from 0.5 ml plasma by sequential ultracentrifugation using an Optima TLX table-top ultracentrifuge with fixed angle rotor TLA-120.2 at 120,000 r/min (Beckman Instruments, Palo Alto, CA) (21,22). Lipid and protein constituents in plasma and lipoprotein fractions were measured as previously described (21). Individual apolipoproteins were isolated from the plasma lipoproteins by preparative SDS-polyacrylamide gradient gel electrophoresis (5–15%) using a Tris-glycine buffer system. Immunoblotting served for definite identification of separated apolipoproteins. The stained electrophoretically separated apolipoprotein bands were excised from polyacrylamide gels and hydrolyzed in 12 N hydrochloric acid at 115°C for 24 h. The hydrochloric acid was subsequently evaporated, and the samples were reconstituted in 0.5 ml of 50% acetic acid. The free amino acids were isolated from plasma or protein hydrolysates by cation exchange chromatography using Dowex AG-50W-X8 (H^+, 100–200 mesh) resin obtained from Bio-Rad (Richmond, CA). The amino acids were purified and derivatized, and isotopic enrichment was analyzed by gas chromatography/mass spectrometry as previously published (23–24). Because of the nonnegligible masses associated with stable isotope tracers, it is necessary to transform enrichment data to tracer-to-tracee ratios. Data in this format are analogous to the specific activity obtained from radiotracer experiments. For calculation of isotopic enrichment and tracer-to-tracee ratios, see study by Cobelli et al. (25).

Kinetic analysis. The tracer-to-tracee ratio curves of VLDL apoB-100, HDL apoA-I, and HDL apoA-II were fitted to a monoexponential function using the SAAM II program (SAAM Institute, Seattle, WA), as published by Ikewaki et al. (26,27). The function used was defined as $A(t) = A_p(1 - e^{[-k(t-d)]})$, where $A(t)$ is the tracer-to-tracee ratio at time t, A_p is the mean tracer-to-tracee ratio plateau value of the precursor pool for the apolipoprotein of interest, d is the lag time or delay time, and k is the fractional synthetic rate (FSR). This approach was based on the following assumptions: *1*) The isotopic enrichment of VLDL apoB-100 at plateau was used as the estimate of the precursor pool enrichment for both apoA-I and apoA-II synthesis. Because it is a protein that turns over quickly, isotopic enrichment of VLDL apoB-100 reaches a plateau earlier than other liver proteins, such as fibrinogen and albumin, in primed constant-infusion experiments (23,24). This enrichment plateau indicates that its isotopic enrichment is nearly the same as liver amino acyl-tRNA, the real precursor of protein synthesis. Therefore, enrichment of VLDL apoB-100 has been proposed as a surrogate measure of the isotopic enrichment of the hepatic amino acyl-tRNA pool (28). This estimation is made upon the assumption *2*) that apoB-100 and the vast majority of HDL apoA-I and all apoA-II are synthesized by the liver or that, in the case of apoA-I, the enrichment of hepatic and intestinal tissues is similar. This model considers synthesis (including the delay of tracer appearance) and catabolism of apoA-I and apoA-II from total HDL. At steady state, the FSR equals the fractional catabolic rate (FCR). The HDL apoA-I and apoA-II production rates (PRs) were determined by the formula PR = (FSR × HDL apoA-I/A-II concentration × plasma volume) × (body weight)$^{-1}$. Plasma volume of all subjects (all BMIs were <27 kg/m^2) was assumed to be 4.5% of their body weight.

Statistical analysis. Statistical analysis was performed using the SPSS/PC software package. Descriptive data were expressed as arithmetic means ± SD. Because the data did not fit a normal distribution, nonparametric tests were used, i.e., Mann-Whitney U tests for comparison of numerical variables between groups and Spearman's rank correlation coefficients for correlation analysis. Additionally, partial correlations were calculated using sex, age, and BMI as covariates. Pooled data were given for correlation analysis because of the strong homogeneity of the relationships between most parameters found when analyzing NGT and IGT subjects separately. Fractional standard deviation (FSD) of k was calculated using SAAM statistics.

RESULTS

Biochemical characteristics and HDL composition. Baseline characteristics, plasma concentrations of lipids, and HDLs for the six IGT subjects and the six control subjects are presented in Tables 1 and 2. The HbA_{1c} level in both groups was <5%. Fasting values for plasma glucose, free fatty acids (FFA), insulin, and proinsulin, and additionally, the 2-h post values (2-h after 75-g oral glucose challenge) for glucose, FFA, insulin, and proinsulin were significantly increased in IGT subjects. These values were accepted as highly indicative of an insulin-resistant/hyperinsulinemic state in the IGT subjects. Plasma triglycerides were higher by 58% in IGT subjects (P < 0.05), but were within the normal range. The total cholesterol, apoB-100, and apo A-II plasma levels were not different in IGT and NGT subjects. Plasma apoA-I concentrations and HDL cholesterol levels were significantly decreased in IGT subjects. When focusing on all HDL constituents, HDL apoA-I and HDL cholesteryl esters were found to be significantly lower in IGT subjects, whereas the HDL triglycerides and HDL phospholipids were significantly increased in IGT subjects. Free cholesterol and apoA-II in HDL were not different in the two groups. Furthermore, the HDL particle composition was substantially altered in IGT subjects, leading to particles enriched with triglycerides and phospholipids but depleted in their cholesterol and apolipoprotein content (Table 2).

CETP activity and TPLA. The rate of cholesterol ester transfer in plasma was significantly higher by 34% in the IGT subjects (P < 0.05) when compared with control subjects.

TABLE 1
Baseline characteristics of NGT and IGT subjects

	NGT subjects	IGT subjects
Age (years)	39 ± 12	41 ± 12
Sex (M/F)	3/3	3/3
BMI (kg/m^2)	23.6 ± 1.2	24.2 ± 1.4
Waist-to-hip ratio	0.85 ± 0.05	0.90 ± 0.08
Fasting plasma glucose (mmol/l)	4.55 ± 0.76	6.38 ± 0.79*
2-h postglucose (mmol/l)‡	4.92 ± 0.52	9.36 ± 1.02*
Fasting insulin (pmol/l)	45 ± 13	68 ± 11†
2-h postinsulin (pmol/l)	88 ± 28	370 ± 110*
Fasting proinsulin (pmol/l)	0.67 ± 0.49	2.38 ± 1.51†
2-h postproinsulin (pmol/l)	2.30 ± 0.73	11.14 ± 5.69*
FFA (mmol/l)	0.29 ± 0.11	0.71 ± 0.18*
2-h post-FFA (mmol/l)	0.02 ± 0.01	0.04 ± 0.01*
HbA_{1c} (%)	<5	<5
Triglycerides (mmol/l)	0.84 ± 0.27	1.33 ± 0.21†
Total cholesterol (mmol/l)	4.87 ± 0.60	4.93 ± 1.09
HDL cholesterol (mmol/l)§	1.36 ± 0.14	1.07 ± 0.15†
ApoA-I (g/l)	1.41 ± 0.09	1.08 ± 0.11†
ApoA-II (g/l)	0.23 ± 0.03	0.22 ± 0.02
ApoB-100 (g/l)	1.09 ± 0.21	1.24 ± 0.39
CETP activity (nmol · ml^{-1} · h^{-1})	25.4 ± 5.3	34.1 ± 5.5†
TPLA (μmol · l^{-1} · s^{-1})‖	1.18 ± 0.09	1.12 ± 0.15

Data are means ± SD (the values of glucose and insulin are based on the mean of two consecutive OGTTs). *P < 0.01 (Mann-Whitney test) for the difference between IGT and NGT subjects. †P < 0.05, ‡2-h postprandial values after 75-g oral glucose challenge. §Sum of free and esterified cholesterol. ‖TPLA.

TPLA was somewhat, but not significantly, lower in the IGT subjects than in the control subjects.

HDL apoA-I and apoA-II kinetics. Plasma steady-state conditions of plasma free L-[$^{13}C_6$]-phenylalanine were achieved within 20–50 min in all study subjects (mean enrichment plateau values: 7.12 ± 0.62% in IGT vs. 7.32 ± 0.87% in NGT, NS). The enrichment levels of plasma free amino acid tracers remained in a steady state throughout the course of the primed constant infusion (data not shown). Steady-state conditions of endogenously formed L-[$^{13}C_6$]-tyrosine were reached within 30–60 min in all subjects (mean enrichment plateau values: 1.02 ± 0.25% in IGT vs. 1.09 ± 0.39% in NGT, NS). The latter indicates intracellular steady-state enrichment conditions of phenylalanine as a precursor for the protein synthesis. The plateau of isotopic enrichment of the rapidly metabolized VLDL apoB-100 was reached within 5–7 h in all subjects (mean enrichment plateau values: 5.11 ± 0.42% in IGT vs. 5.86 ± 0.21% in NGT, P < 0.05) and remained in a steady state throughout the course of the primed constant infusion (data not shown). This strongly supports the modeling of a steady-state tracer input. Representative tracer-to-tracee ratio curves in HDL apoA-I and A-II are shown in Fig. 1 for one person from each group. The kinetic parameters are presented in Table 3. In all cases, the FSD of the k value was <0.1, indicating satisfactory fit to the isotopic enrichment data. The HDL apoA-I pool size was significantly decreased by 25% (P < 0.05) in IGT subjects when compared with NGT subjects. The HDL apoA-I FCR in IGT subjects was significantly increased by 31% (P < 0.01). On the other hand, the HDL apoA-I PR in IGT subjects did not differ from that of normal control subjects. Furthermore, the HDL apoA-II FCR, PR, and pool size showed no differences between the two groups. Thus, in IGT subjects, the decrease below normal in HDL apoA-I pools was exclusively the result of an increased catabolism of HDL apoA-I.

Correlation analysis. As illustrated in Fig. 2, the HDL apoA-I FCR correlated negatively with HDL apoA-I levels (r = –0.902, P < 0.001) and positively with CETP activity (r = 0.646, P < 0.001). Furthermore, the HDL apoA-I FCR correlated with several parameters of HDL composition: HDL cholesterol (r = –0.797, P = 0.001), HDL cholesteryl ester mass percentage (r = –0.778, P = 0.001), HDL apoA-I mass percentage (r = –0.870, P < 0.001), HDL triglycerides (r = 0.696, P < 0.01), and HDL triglyceride mass percentage (r = 0.836, P < 0.001). In addition, the HDL apoA-I FCR correlated in a direct manner with plasma triglycerides (r = 0.743, P < 0.01), fasting insulin (r = 0.765, P < 0.01), fasting proinsulin (r = 0.797, P = 0.001), 2-h postinsulin (r = 0.814, P < 0.01), and 2-h postproinsulin (r = 0.786, P < 0.01).

Then, to compensate for possible effects of age, BMI, and sex, partial coefficients were calculated with age, BMI, and sex as covariates (Table 4). Statistical significance remained in all parameters discussed above. A significant positive correlation was also established between CETP activity and plasma triglycerides (r = 0.614, P < 0.05), HDL triglycerides (r = 0.595, P < 0.05), 2-h postinsulin (r = 0.643, P < 0.05), and 2-h postproinsulin (r = 0.608, P < 0.05). The HDL apoA-II PR correlated

TABLE 2
HDL composition in NGT and IGT subjects

	NGT subjects		IGT subjects	
n	6		6	
HDL triglycerides (mmol/l)	0.12 ± 0.04	(3.2 ± 1.0)	0.21 ± 0.07*	(4.9 ± 1.9)*
HDL free cholesterol (mmol/l)	0.21 ± 0.06	(2.47 ± 0.61)	0.23 ± 0.02	(2.72 ± 0.15)
HDL cholesteryl ester (mmol/l)	1.05 ± 0.08	(12.67 ± 2.92)	0.81 ± 0.07*	(10.07 ± 1.95)†
HDL phospholipids (mmol/l)	1.20 ± 0.36	(27.5 ± 5.8)	1.46 ± 0.24	(34.7 ± 2.6)†
HDL apoA-I (g/l)	1.34 ± 0.07	(43.2 ± 2.36)	0.94 ± 0.10†	(31.5 ± 4.41)†
HDL apoA-II (g/l)	0.24 ± 0.03	(8.0 ± 0.7)	0.22 ± 0.01	(7.3 ± 0.6)
HDL residual protein (g/l)	0.10 ± 0.06	(3.3 ± 2.5)	0.26 ± 0.06	(8.7 ± 1.6)*

Data are concentrations ± SD (mass percentage ± SD). Mean lipoprotein mass composition of HDL, i.e., the mass percentage of each lipid and protein constituent from the total lipoprotein mass (%) (21). HDL residual protein consists of other HDL protein constituents, e.g., apolipoproteins E and CI–CIII. *P < 0.05; †P < 0.01 (Mann-Whitney test) for the difference between IGT and NGT subjects.

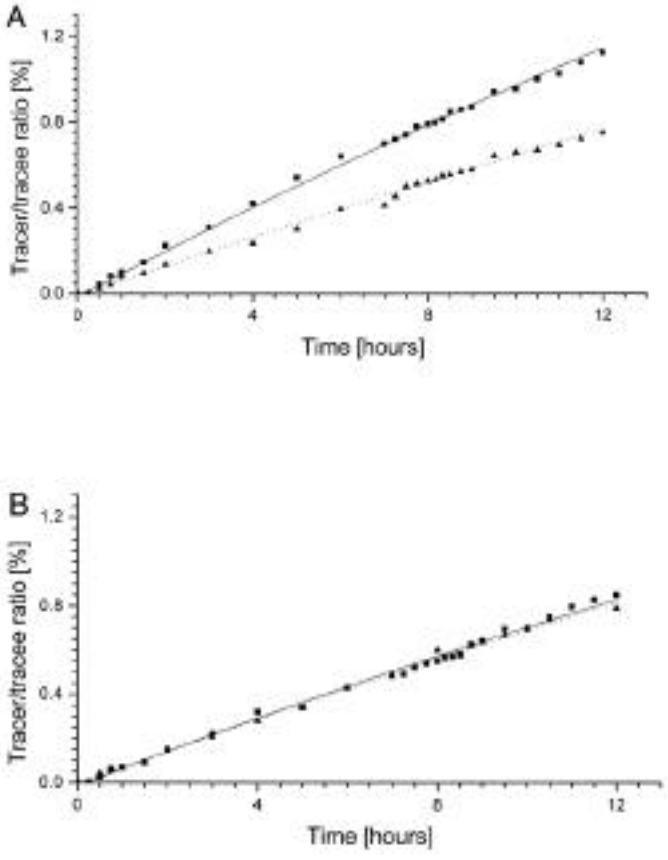

FIG. 1. Plots showing representative HDL apoA-I (*A*) and apoA-II (*B*) L-[ring-$^{13}C_6$]-phenylalanine enrichment curves in an IGT patient (■) and a control subject (▲) during the course of the primed constant infusion. Symbols represent observed data; lines indicate computer-derived fits as calculated by the monoexponential function described in METHODS.

significantly with HDL apoA-II levels ($r = 0.560$, $P < 0.05$). On the other hand, no correlation was found between TPLA and HDL apoA-I PR or HDL apoA-II FCR and plasma lipids and proteins or between HDL apoA-I PR or HDL apoA-II FCR and HDL composition.

DISCUSSION

This article describes the first stable isotope study on the kinetics of HDL apoA-I and apoA-II in normotriglyceridemic patients with IGT. We confirm reports of reduced HDL cholesterol levels in IGT subjects, but our data demonstrate an altered composition of HDL particles and a substantially increased in vivo catabolism of HDL apoA-I in these patients. We also show relationships between early markers of an insulin-resistant/hyperinsulinemic state and the parameters of in vivo HDL turnover.

ApoA-I and apoA-II are the major protein moieties of human HDL particles, comprising ~90% of total HDL protein mass, and may be regarded as kinetic markers for HDL (29). ApoA-I and apoA-II define two subclasses of HDL particles that contain either both apoA-I and A-II (LpA-I:A-II) or only apoA-I (LpA-I). There are several turnover studies reporting the metabolic fate of apoA-I to be separate from that of apoA-II (26,27,30–33). The apoA-I FCR appears to be the most important factor determining LpA-I levels, while the rate of apoA-II production is the major determinant of distribution of apoA-I in LpA-I and LpA-I:A-II (34). Tilly-Kiesi et al. (35) reported similar FCR of apoA-I in older men and women showing no differences of apoA-I catabolism between LpA-I and LpA-I:A-II. Brinton et al. (36,37) have carried out turnover studies on a large number of subjects with a wide range of HDL cholesterol concentrations (0.5–3.0 mmol/l) that confirm a strong inverse correlation between HDL cholesterol and the apoA-I FCR. This was true in both normo- and hypertriglyceridemic subjects. Furthermore, the same authors (38) found insulin sensitivity, lipolytic enzyme activity, and plasma triglycerides to be major correlates of HDL particle size, which was hypothesized to be the primary determinant of HDL catabolism. Recently, Frenais et al. (16) showed that in hypertriglyceridemic patients with NIDDM, the reduced HDL concentrations are due to a significantly higher catabolism of HDL apoA-I. The latter was significantly correlated with plasma triglyceride levels. Furthermore, they found the kinetics of apoA-I in HDL subclasses to be similar, and consequently modeled the HDL apoA-I by using a one-compartment model-based analysis.

The focus of the present work was on apoA-I and apoA-II of total HDL. For the calculation of apoA-I and apoA-II kinetic parameters, we used the method of endogenous labeling of apolipoproteins by the essential proteinogenic amino acid L-[$^{13}C_6$]-phenylalanine. Available evidence exists suggesting that the early steady-state labeling of endogeneously formed plasma free L-[$^{13}C_6$]-tyrosine can be used as a reliable indicator of intrahepatic steady-state conditions of phenylalanine enrichment, supporting the modeling of a steady-state input (18). The plateau tracer-to-tracee ratio of VLDL apoB-100 serves as an estimate of the precursor pool enrichment for apoA-I and apoA-II synthesis (23,26,27). The appearance rate of L-[$^{13}C_6$]-phenylalanine in apoA-I and apoA-II was calculated by a one-compartmental model that is explicitly defined by a monoexponential function, as described in METHODS. Recent reports provide evidence that this approach is a reasonable method for determining the apoA-I and apoA-II turnover rates in studies using a primed constant infusion. The current model generated satisfactory fits to the isotopic enrichment data that could be obtained

TABLE 3
HDL kinetics in NGT and IGT subjects

	NGT subjects	IGT subjects
n	6	6
Plasma volume (liters)	3.30 ± 0.51	3.19 ± 0.50
HDL apoA-I FCR (day^{-1})	0.26 ± 0.03	0.34 ± 0.05*
HDL apoA-I pool size (mg/kg)	64.42 ± 5.54	48.08 ± 8.84†
HDL apoA-I PR ($mg \cdot kg^{-1} \cdot day^{-1}$)	16.57 ± 1.12	16.19 ± 1.11
HDL apoA-II FCR (day^{-1})	0.19 ± 0.02	0.17 ± 0.03
HDL apoA-II pool size (mg/kg)	11.00 ± 1.23	9.94 ± 0.67
HDL apoA-II PR ($mg \cdot kg^{-1} \cdot day^{-1}$)	2.03 ± 0.20	1.64 ± 0.30

Data are means ± SD. *$P < 0.01$, †$P < 0.05$ (Mann-Whitney test) for the difference between IGT and NGT subjects. The pool size is expressed as milligrams per kilogram body weight.

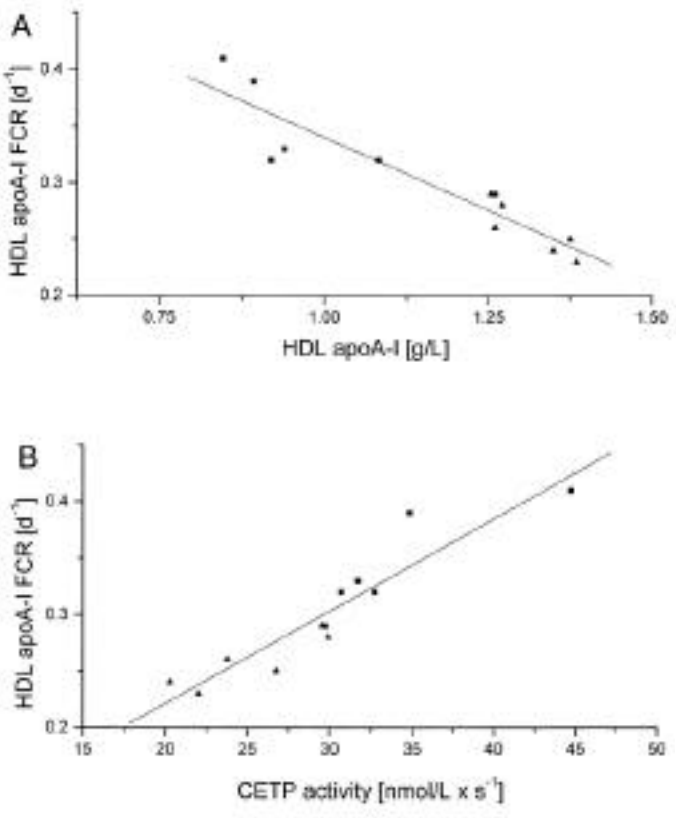

FIG. 2. Relationship between HDL apoA-I FCR and HDL apoA-I concentration (*A*) and CETP activity (*B*) in 12 subjects with IGT (■) or NGT (▲).

from HDL apoA-I and apoA-II. The direct comparison of our data to the work of Frenais et al. (16) on HDL apoA-I kinetics in NIDDM is possible because they used the same mathematical approach.

In the present study, participants were selected as IGT subjects according to the WHO criteria, as described in METHODS.

TABLE 4
Partial correlation analysis in both NGT and IGT subjects taking into account age, sex, and BMI

HDL apoA-I FCR	
vs. HDL apoA-I	–0.962 (0.000)
vs. HDL apoA-1 %*	–0.881 (0.001)
vs. triglycerides	0.534 (0.037)
vs. HDL triglycerides	0.754 (0.009)
vs. HDL triglyceride %*	0.865 (0.001)
vs. HDL cholesterol	–0.665 (0.025)
vs. HDL cholesterol %*	–0.680 (0.022)
vs. fasting insulin	0.735 (0.012)
vs. fasting proinsulin	0.635 (0.033)
vs. CETP activity	0.617 (0.023)
HDL apoA-II PR	
vs. HDL apoA-II	0.671 (0.024)

Data are *r* (*P*). *n* = 12. *Mean lipoprotein mass composition, i.e., the mass percentage of each lipid and protein constituent from the total lipoprotein mass (%) (21).

The significantly greater values of basal and 2-h post levels of FFA, insulin, and proinsulin are considered reliable indicators of an insulin resistant state or, in the case of proinsulin, of abnormalities of insulin secretion in these patients (10,11,39). Plasma triglycerides were significantly higher in IGT subjects when compared with normal control subjects but, notably, were still in the normal range. HDL cholesterol and apoA-I concentrations were significantly lower in IGT subjects. The apoA-II levels were not affected in IGT subjects. The HDL apoA-I FCR was significantly increased in IGT subjects when compared with control subjects. On the other hand, the apoA-I PR, apoA-II FCR, and apoA-II PR were not different in IGT subjects. The increased HDL apoA-I FCR was positively correlated with triglyceride concentrations and negatively correlated with HDL cholesterol and apoA-I. Furthermore, there was a direct correlation between increased HDL apoA-I FCR and an altered HDL composition. Thus, the metabolic basis of low HDL cholesterol and apoA-I in IGT subjects appears to be an increased apoA-I catabolism. This increase was selective, as the HDL apoA-II kinetics in IGT subjects was normal.

In the IGT subjects studied, the composition of their HDL particles is substantially altered. HDL particles were significantly enriched in triglycerides and phospholipids and, on the other hand, depleted in their cholesteryl ester and protein content. HDL apoA-I FCR, but not HDL apoA-II FCR or the PR of HDL apoA-I and apoA-II, was positively correlated with HDL triglyceride mass percentage and negatively correlated with HDL cholesteryl ester and HDL apoA-I mass percentage.

The more triglyceride-enriched the HDL particles are, the more susceptible they are to HTGL or LPL action (40–42). HTGL is involved in reverse cholesterol transport, and its activity is negatively correlated with plasma HDL cholesterol. On the other hand, a positive correlation exists between HTGL activity and fasting insulin levels in insulin-resistant NIDDM patients (14,43). In contrast, low HDL cholesterol and high triglycerides in diabetic patients are associated with decreased LPL activity (42,44–46).

In the present study, the total lipase activity in IGT subjects, i.e., the sum of LPL and HTGL activities, tends to be decreased. However, the parameter TPLA may be altered by two opposite effects: upregulated HTGL and downregulated LPL. First, these effects are possibly not clearly expressed under the present conditions, and second, they may superimpose each other. No correlations were found between TPLA and plasma lipids and parameters of HDL constitution and kinetics, respectively.

On the other hand, increased CETP activity has been demonstrated in subjects with low HDL levels (40). An accelerated cholesteryl ester transfer may be associated with an enrichment of HDL with triglycerides (40,47,48) and has been reported in insulin-resistant obese subjects (49), as well as in both IDDM (50) and NIDDM (51) patients.

A new finding is the higher CETP activity in nonobese IGT subjects. CETP activity was directly correlated with HDL apo A-I FCR. Moreover, although all of our patients were in the normal range for plasma triglycerides, there is a strong direct correlation between CETP activity and triglycerides in plasma and in HDL, and with insulin and proinsulin after glucose challenge. Consequently, we assume that an increased CETP activity in conjunction with modulated lipoprotein and hepatic lipase activities transfers cholesteryl esters from

large HDL particles to LDL and VLDL, followed by a change in HDL composition. As indicated above, the conformational change in HDL favors its catabolism. The extent of these kinetic alterations in IGT appears to be between normal and diabetic conditions, as described by others (16,33). Additionally, there is evidence that alterations in HDL composition lead to changed binding of apoA-I to the particles itself (52). It is hypothesized that apoA-I is weakly bound to cholesteryl ester-depleted HDL particles, and as a consequence, the apoA-I pool is an easily dissociable one that is much more rapidly catabolized.

In conclusion, our data suggest that in IGT, the increase in HDL apoA-I FCR caused the low HDL concentrations. The HDL apoA-I FCR is strongly associated with an alteration in HDL particle composition and, on the other hand, with markers of the insulin resistance in IGT. The heterogeneous influence of insulin as both an activator and inhibitor of the concerted action of lipolytic enzymes and transfer proteins may be the metabolic basis of reduced HDL levels, expressed in terms of its increased catabolism. Increased CETP activity appears to be the most important modulator of HDL composition and, consequently, of HDL catabolism in IGT. Because of the limited number of IGT patients studied and the known pathophysiological heterogeneity of dysbalanced insulin action, these results may not comprehensively reflect the insulin-resistant population in general. Further studies on HDL composition and metabolism should differentiate between variations in CETP secondary to changes in triglycerides and triglyceride-rich lipoproteins, and variations in CETP directly due to insulin action. However, considering the responsibility of a disturbed direct insulin action on CETP for a significant influence on HDL metabolism in IGT, we speculate that alterations of the HDL apoA-I kinetics are possibly an early event in IGT even under normotriglyceridemic conditions.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (DFG project no. Ju-343/1-1, Lipoprotein Kinetics in the Metabolic Syndrome).

The authors wish to thank Uta Buro, Baerbel Zeiler, and the staff of the Metabolic Ward for their expert technical assistance.

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