Changes in Lipoprotein Kinetics Associated With Type 2 Diabetes Affect the Distribution of Lipopolysaccharides Among Lipoproteins

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Context: Lipopolysaccharides (LPSs) are inflammatory components of the outer membrane of Gram-negative bacteria and, in plasma, are mostly associated with lipoproteins. This association is thought to promote their catabolism while reducing their proinflammatory effects.

Objectives: Our aim was to determine the impact of lipoprotein kinetics on plasma LPS distribution and how it may affect patients with type 2 diabetes mellitus (T2DM).

Design: We performed a kinetic study in 30 individuals (16 T2DM patients, 14 controls) and analyzed the impact of changes in lipoprotein kinetics on LPS distribution among lipoproteins.

Results: Plasma LPS levels in T2DM patients were not different from those in controls, but LPS distribution in the two groups was different. Patients with T2DM had higher LPS-very low-density lipoprotein (VLDL; 31% ± 7% vs 22% ± 11%, $P = .002$), LPS-high-density lipoprotein (HDL; 29% ± 9% vs 19% ± 10%, $P = .015$), free (nonlipoprotein bound) LPS (10% ± 4% vs 7% ± 4%, $P = .043$) and lower LPS-low-density lipoprotein (LDL; 30% ± 13% vs 52% ± 16%, $P = .001$). In multivariable analysis, VLDL-LPS was associated with HDL-LPS ($P < .0001$); LDL-LPS was associated with VLDL-LPS ($P = .004$), and VLDL apolipoprotein (apo) B100 catabolism ($P = .002$); HDL-LPS was associated with free LPS ($P < .0001$) and VLDL-LPS ($P = .033$); free LPS was associated with HDL-LPS ($P < .0001$). In a patient featuring a dramatic decrease in VLDL catabolism due to apoA-V mutation, LDL-LPS was severely decreased (0.044 EU/mL vs 0.788 EU/mL in controls). The difference between T2DM patients and controls for LDL-LPS fraction was no longer significant after controlling for VLDL apoB100 total fractional catabolic rate.

Conclusions: Our data suggest that in humans, free LPS transfers first to HDL and then to VLDL, whereas the LPS-bound LDL fraction is mainly derived from VLDL catabolism; the latter may hence represent a LPS catabolic pathway. T2DM patients show lower LDL-LPS secondary to reduced VLDL catabolism, which may represent an impaired catabolic pathway. **(*J Clin Endocrinol Metab* 99: E1245–E1253, 2014)**

Lipopolysacharides (LPSs) are components of the outer membrane of Gram-negative bacteria (1). They are potent endotoxins, involved in the acute-phase response to bacterial infection, and induce a cytokine-mediated systemic inflammatory response that may be harmful and cause shock and severe multiple organ failure (2, 3). We

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Abbreviations: apo, apolipoprotein; BMI, body mass index; FCR, fractional catabolic rate; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LBP, LPS-binding protein; LDL, low-density lipoprotein; LPS, lipopolysaccharide; PR, production rate; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoprotein.

have previously shown in rodents that LPSs derived from Gram-negative bacteria caused metabolic endotoxemia, which triggered low-grade chronic inflammation and thus potentially increased the risk of diabetes and vascular disease (4–6). Similarly, in humans, fat and energy intakes are positively associated with metabolic endotoxemia, suggesting that LPS may bridge the gap between food intake and metabolic or cardiovascular disease in humans (7).

All classes of lipoproteins [chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high density lipoprotein (HDL)] bind LPS (8), and this binding attenuates the biological inflammatory response to LPS (9–11). Chylomicrons and VLDL have been shown to bind LPS and to reduce LPS-induced toxicity in mice (12). Similarly, the binding of LPS to LDL reduces their toxicity on endothelial cells (13). Finally, the ability of HDL to bind LPS and then to attenuate their toxicity has also been demonstrated (14, 15) through the reduction of fever, leukocytosis, and hypotension in adrenalectomized mice, thus preventing their death (14).

However, little is known about the distribution of LPS among the lipoproteins or the impact of lipoprotein metabolism on the distribution of LPS in humans. In one study performed in 10 individuals, HDL was shown to be the main LPS carrier holding 60% of LPS, whereas LDL and VLDL carried 25% and 12% of LPS, respectively (16). Among three ex vivo studies, two reported that LPS in humans was mainly located in VLDL and LDL (17, 18) and one reported, more precisely, that LDL was the major carrier for LPS (8). Thus, data on LPS distribution among lipoproteins are not only scarce, but also the relationship between LPS distribution and lipoprotein metabolism remains unknown. This latter point is critical because LPSs are mainly bound to lipoproteins. It is therefore of major importance to identify possible associations between lipoprotein kinetics and LPS distribution among the lipoproteins. This knowledge could also benefit patients with type 2 diabetes because this disease is associated with important abnormalities in lipoprotein kinetics that may significantly modify LPS distribution among lipoproteins and thus potentially modify the protection provided by some lipoproteins against LPS-induced metabolic inflammation.

We aimed to study in vivo the impact of lipoprotein metabolism on LPS distribution among lipoproteins by performing a lipoprotein kinetics study in 30 individuals [16 with type 2 diabetes mellitus (T2DM), 14 controls] featuring a wide range of plasma lipid profiles and lipoprotein kinetics data and by analyzing the relationship between lipoprotein kinetics and LPS distribution among VLDL, LDL, and HDL.

Materials and Methods

This prospective single-center study was approved by our regional ethics committee and written informed consent was obtained from all patients before study inclusion.

Subjects

The study was performed in 30 individuals, 16 patients with T2DM and 14 controls. Patients with T2DM were treated with oral glucose-lowering agents (metformin alone in four patients, metformin + sulfonylureas or glinides in 12 patients) and had stable glycated hemoglobin for at least 6 months. They had no overt cardiovascular disease, no macroalbuminuria, no renal impairment [glomerular filtration rate lower than 60 mL/mn], no history of alcohol and/or drug abuse, and no infection and were not treated with drugs known to affect lipid metabolism (corticoids, retinoids, antiproteases, estrogens, cyclosporine, or glitazones). No patients were being treated with hypolipidemic drugs (statins, fibrates, nicotinic acid, ezitimibe, or omega-3) or had been treated with such drugs during the 6 months prior to the study. Patients with a history of familial hyperlipidemia or plasma triglycerides above 400 mg/dL (4.5 mmol/L) were excluded.

All control subjects were in good health, had no family history of diabetes, and had normal body weight, waist circumference, and blood pressure. They showed normal plasma values of fasting blood glucose, fasting insulin, plasma lipids, C-reactive protein and homeostasis model assessment index of insulin resistance. They were not taking any medication. None of the women included in the study were taking oral contraceptives.

In vivo lipoprotein kinetics study

The in vivo kinetics study was performed as previously described in detail (19, 20).

Experimental protocol

The kinetics study was performed for each subject, in the fed state, with a primed bolus followed by a 16-hour constant infusion of L-[1-^{13}C]leucine, as previously reported (19, 21).

Isolation of apolipoproteins

VLDL, intermediate-density lipoprotein (IDL), LDL, and HDL were isolated from plasma by sequential flotation ultracentrifugation, using a 50.4 rotor in an L7 apparatus (Beckman Instruments) and then delipidated with diethylether-ethanol. Apolipoprotein (apo) B100 from VLDL, IDL, LDL, and apoA-I from HDL were obtained by preparative discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 3% and 15% gel and then hydrolyzed in 6 M HCl at 110°C for 16 hours under nitrogen vacuum to recover amino acids.

Determination of leucine enrichment by gas chromatograph to combustion to isotope ratio mass spectrometry

Amino acids were converted to N-acetyl O-propyl amino acid esters and analyzed with a Finnigan Mat Delta C isotope ratio mass spectrometer (Finnigan Mat) coupled to an HP 5890 series II gas chromatograph (Hewlett Packard), as previously described (20).

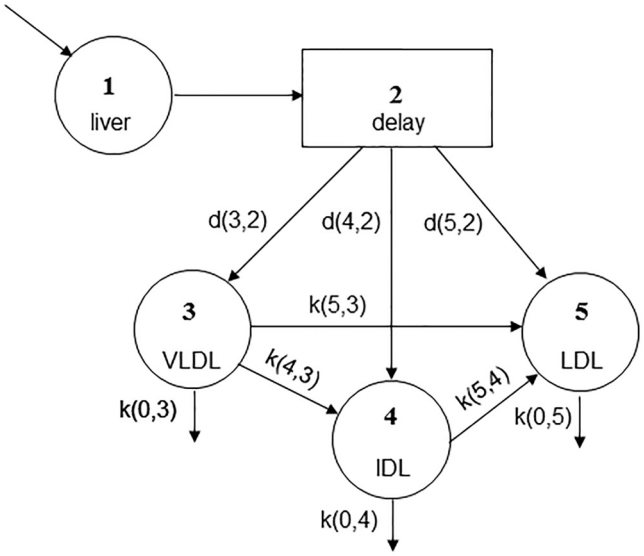

Figure 1. Multicompartmental model for kinetics analysis of apoB100 metabolism. A forcing function, corresponding to VLDL apoB100 plateau enrichment, was used to drive the appearance of leucine tracer into the different lipoprotein fractions (compartment 1). Compartment 2 is the delay compartment, representing the time required for the synthesis of apoB100 and its secretion into the plasma. Compartments 3, 4, and 5 represent plasma VLDL, IDL, and LDL apoB100, respectively. d(a,2), the fraction of apoB synthesized by the liver in compartment a; k(x,y), the fractional transfer coefficient from compartment y to x.

Modeling

^{13C}Leucine enrichment was initially expressed in δ-percentage and converted into tracer to tracee ratio prior to modeling (20, 22). The data were analyzed with the Simulation Analysis and Modeling II program (SAAM Institute, Inc) (23).

The kinetics of apoB100 in the VLDL, IDL, and LDL fractions were derived by a multicompartmental model, as shown in Figure 1, with each compartment representing a group of kinetically homogenous particles. This model has been used in many kinetics studies performed with the constant infusion of a stable isotope (19, 24) and has been shown to give a statistically better fit of tracer to tracee ratio data than more complex multicompartmental models (24, 25). Because the experiment was carried out under steady-state conditions, the fractional synthesis rate was equal to the fractional catabolic rate (FCR). The direct FCR of VLDL apoB100 was the constant k(0,3) and the indirect FCR of VLDL apoB100 was k(4,3) + k(5,3). The total VLDL apoB100 FCR was the sum of direct and indirect FCRs of VLDL apoB100. The direct FCR of IDL apoB100 and the FCR from IDL to LDL (indirect FCR of IDL apoB100) were equal to k(0,4) and k(5,4), respectively. The total IDL apoB100 FCR was the sum of direct and indirect FCRs of IDL. The LDL apoB FCR was k(0,5).

ApoA-I data were analyzed using the following monoexponential function: $A(t) = Ap(1 - \exp[-k(t-d)])$, where A(t) is the apolipoprotein enrichment at time t, Ap the enrichment at the plateau of the VLDL apoB100 curve, d the delay between the beginning of the experiment, and the appearance of tracer in the apolipoprotein and k the fractional synthetic rate of the apolipoprotein (26, 27).

The production rates (PRs) of each lipoprotein fraction (VLDL apoB100, IDL apoB100, LDL apoB100, and HDL apoA-I) were calculated as the product of its total FCR and its pool size divided by body weight. The pool sizes were calculated by multiplying the apolipoprotein (apoB100 or ApoA-I) concentration in the lipoprotein fraction (VLDL, IDL, LDL, or HDL) by the estimated plasma volume (4.5% of body weight) (28). In obese subjects [body mass index (BMI) ≥ 30 kg/m,^{2}] plasma volume was corrected as previously reported by many authors (29–31).

Analytical procedures

Fasting plasma glucose, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were measured on a Dimension analyzer with dedicated reagents (Dade Behring). ApoB and apoA1 were measured by immunoturbidimetry.

Plasma and lipoprotein LPS contents were assayed as followed. Briefly, lipoprotein fractions and plasma were diluted in LPS-free water 10 times, heated to 70°C for 15 minutes, and immediately cooled to +4°C. Two subsequent dilutions (1:20 and 1:40 final dilutions) were performed in a Limulus amaebocyte lysate free water and LPS concentrations assessed in duplicates using the Endosafe detection system, based on the Limulus amaebocyte lysate kinetic chromogenic methodology (Charles River), as previously described (4). Serum lipopolysaccharide-binding protein (LBP) was measured by an ELISA (HyCult Biotech).

Statistical analysis

Data are reported as mean ± SD. Statistical calculations were performed using the SPSS software package. The distribution of each variable was analyzed using the Kolmogorov-Smirnov test. The Mann-Whitney *U* test was used to compare clinical, biological, and kinetic characteristics between groups. Percentages between groups were compared by using the Yates' χ^2 test. The correlation analysis has been performed by calculating the Pearson correlation coefficient (r). After controlling for covariates, means were compared by analysis of covariance. Multivariable analyses were performed by stepwise multivariable linear regression to analyze the independent association between VLDL-LPS, LDL-LPS, HDL-LPS, and free LPS, on the one hand, and several variables, on the other hand. For each multivariable analysis, the independent variables selected were those that were associated with the dependent variable (VLDL-LPS, LDL-LPS, HDL-LPS, or free LPS) in univariate analysis (correlation) with values of $P < 0.15$, the kinetics of the lipoprotein itself (for instance, VLDL apoB100 PR, and VLDL apoB100 total FCR when studying VLDL-LPS), and other potentially confounding variables (gender, age, BMI, diabetes, and plasma LBP). Among the selected variables, variables were entered into the model for a significance of F value less than 0.05 and removed from the model for a significance of F value less than 0.10. Data that were not normally distributed were log transformed before introduction into the statistical model. A two-tailed level of $P = .05$ was accepted as statistically significant.

Results

Baseline characteristics

The clinical and biological characteristics of the 14 control subjects and the 16 patients with T2DM are presented in Table 1. Plasma LPS levels in patients with T2DM were not different from those in controls, but LPS distribution was significantly different between the two groups. Indeed, patients with T2DM had a lower proportion of

Table 1. Clinical, Biological, and Lipoprotein Kinetics Data of Patients With Type 2 Diabetes and Control Subjects

Characteristics	Patients With Type 2 Diabetes (n = 16)	Control Subjects (n = 14)	*P* Value
Age, y	55.8 ± 9.2	29.6 ± 11.5	<.001
Sex ratio, male/female	5/11	9/5	NS
BMI, kg/m^2	31.8 ± 4.0	22.4 ± 1.8	<.001
Duration of diabetes, y	9.0 ± 4.2		
Waist circumference, cm	104 ± 12	77 ± 8	<.001
Fasting glycemia, mmol/L	9.67 ± 3.20	4.68 ± 0.26	<.001
Fasting insulin, μU/mL	9.6 ± 5.7	4.4 ± 1.8	.011
HOMA-IR	3.93 ± 2.14	0.91 ± 0.35	<.001
HbA_{1c}, %	7.4 ± 1.5		
Triglycerides, mmol/L	2.66 ± 0.95	0.76 ± 0.24	<.001
LDL-cholesterol, mmol/L	3.46 ± 1.16	2.90 ± 0.70	NS
HDL-cholesterol, mmol/L	1.03 ± 0.28	1.47 ± 0.28	.001
Plasma LPS, EU/mL	0.92 ± 0.66	0.94 ± 0.66	NS
VLDL-LPS, %	31 ± 7	22 ± 11	.002
LDL-LPS, %	30 ± 13	52 ± 16	.001
HDL-LPS, %	29 ± 9	19 ± 10	.015
Free LPS, %	10 ± 4	7 ± 4	.043
LBP, μg/mL	16.19 ± 3.12	14.82 ± 2.17	NS
VLDL apoB100 pool, mg	714 ± 362	119 ± 98	<.001
VLDL apoB100 total FCR, pool/d	4.27 ± 1.42	8.01 ± 2.43	<.001
VLDL apoB100 direct FCR, pool/d	1.33 ± 1.09	2.16 ± 2.66	NS
VLDL apoB100 indirect FCR, pool/d	2.94 ± 1.06	5.84 ± 2.91	.002
VLDL apoB100 PR, mg/kg · d	32.02 ± 15.27	12.65 ± 5.58	<.001
IDL apoB100 pool, mg	536 ± 252	100 ± 99	<.001
IDL apoB100 total FCR, pool/d	2.92 ± 1.72	10.86 ± 8.12	<.001
IDL apoB100 direct FCR, pool/d	0.42 ± 0.93	1.47 ± 2.21	NS
IDL apoB100 indirect FCR, pool/d	2.50 ± 1.53	9.39 ± 6.61	<.001
IDL apoB100 PR, mg/kg · d	15.11 ± 6.19	11.21 ± 6.01	NS
LDL apoB100 pool, mg	3160 ± 736	1884 ± 675	<.001
LDL apoB100 FCR, pool/d	0.49 ± 0.14	0.51 ± 0.14	NS
LDL apoB PR, mg/kg · d	16.69 ± 3.34	13.70 ± 2.64	.017
Plasma apoA-I, g/L	1.27 ± 0.23	1.33 ± 0.17	NS
HDL apoA-I FCR, pool/d	0.28 ± 0.07	0.19 ± 0.04	.001
HDL apoA-I PR, mg/kg · d	14.80 ± 4.49	11.46 ± 2.11	.034

Abbreviations: HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostasis model assessment index of insulin resistance; NS, not significant.

LDL-LPS (30% ± 13% vs 52% ± 16%, $P = .001$) and higher proportion of VLDL-LPS (31% ± 7% vs 22% ± 11%, $P = .002$), HDL-LPS (29% ± 9% vs 19% ± 10%, $P = .015$), and free (nonlipoprotein bound) LPS (10% ± 4% vs 7% ± 4%, $P = .043$), compared with controls.

Kinetics data

The kinetics of VLDL apoB100, IDL apoB100, LDL apoB100, and HDL apoA-I in controls and patients with T2DM are shown in Table 1. Patients with T2DM showed a higher VLDL apoB100 PR and a lower total FCR for VLDL apoB100 and IDL apoB100, which was due to significantly lower indirect FCRs for VLDL apoB100 and IDL apoB100. Patients with type 2 diabetes showed a significantly higher HDL apoA-I FCR.

Correlations (Supplemental Table 1)

VLDL-LPS correlated positively with HDL-LPS ($r = 0.740$, $P < .001$), with free LPS ($r = 0.693$, $P < .001$) and LDL-LPS ($r = 0.395$, $P = .031$). No significant correlations were found between VLDL-LPS, on the one hand, and VLDL apoB100, IDL apoB100, LDL apoB100, or HDL apoA-I kinetics, on the other hand. LDL-LPS correlated positively with VLDL-LPS ($r = 0.395$, $P = .031$), free LPS ($r = 0.385$, $P = .036$), VLDL apoB100 total FCR ($r = 0.447$, $P = .013$), VLDL apoB100 indirect FCR ($r = 0.396$, $P = .030$), and IDL apoB100 indirect FCR ($r = 0.362$, $P = .048$). HDL-LPS correlated positively with VLDL-LPS ($r = 0.740$, $P < .001$) and free LPS ($r = 0.871$, $P < .001$). No significant correlations were found between the kinetics of HDL-LPS, on the one hand, and VLDL apoB100, IDL apoB100, LDL apoB100, or HDL apoA-I kinetics, on the other hand. Free LPS correlated positively with HDL-LPS ($r = 0.871$, $P < .001$), VLDL-LPS ($r = 0.693$, $P < .001$) and LDL-LPS ($r = 0.385$, $P = .036$). No significant correlations were found between free LPS kinetics, on the one hand, and VLDL apoB100, IDL apoB100, LDL apoB100, or HDL apoA-I kinetics on the other hand.

Table 2. Multivariable Analyses With VLDL-LPS as Dependent Variable Performed in the Whole Studied Population (Including Both Patients With Type 2 Diabetes and Controls)

Variables	Coefficient	SD	β	t	*P* Value
HDL-LPS	0.554	0.095	.740	5.83	<.0001

Variables selected for multivariable analysis: LDL-LPS, HDL-LPS, free LPS, VLDL apoB100 PR, VLDL apoB100 total FCR, age, gender, BMI, diabetes, and LBP. $r^2 = 0.55$.

Multivariable analyses

In a multivariable analysis, the only variable significantly associated with VLDL-LPS was HDL-LPS (β = .740, $P < .0001$), whereas LDL-LPS, free LPS, VLDL apoB100 PR, VLDL apoB100 total FCR, age, gender, BMI, LBP, and diabetes were not (Table 2).

In multivariable analysis, LDL-LPS was significantly associated with VLDL apoB100 total FCR ($\beta = .510$, $P = .002$) and with VLDL-LPS ($\beta = .464$, $P = .004$) but not with HDL-LPS, free LPS, VLDL apoB100 PR, IDL apoB100 indirect FCR, IDL apoB100 total FCR, LDL apoB100 PR, LDL apoB100 FCR, age, gender, BMI, LBP, or diabetes (Table 3). When VLDL apoB100 total FCR was replaced in the model by VLDL apoB100 indirect FCR, both VLDL apoB100 indirect FCR and VLDL-LPS, but not the other variables, were significantly associated with LDL-LPS.

In a multivariable analysis, HDL-LPS was significantly associated with free LPS ($\beta = .592$, $P < .0001$) and VLDL-LPS ($\beta = .322$, $P = .033$) but not with LDL-LPS, apoA-I FCR, apoA I PR, age, gender, BMI, LBP, or diabetes (Table 4).

In a multivariable analysis, the only variable significantly associated with free LPS was HDL-LPS ($\beta = .819$, $P < .0001$), whereas VLDL-LPS, LDL-LPS, apoA-I FCR, apoA-I PR, VLDL apoB100 total FCR, VLDL apoB100 PR, IDL apoB100 total FCR, IDL apoB100 total PR, LDL apoB100 PR, LDL apoB100 FCR, age, gender, BMI, LBP, and diabetes were not (Table 5).

Table 3. Multivariable analyses with LDL-LPS, as dependent variable performed in the whole studied population (including both patients with type 2 diabetes and controls)

Variables	Coefficient	SD	β	t	*P* Value
VLDL apoB100 total FCR	0.063	0.018	0.510	3.43	=0.002
VLDL-LPS	0.957	0.307	0.464	3.12	=0.004

Variables selected for multivariable analysis: VLDL-LPS, HDL-LPS, free LPS, VLDL apoB100 PR, VLDL apoB100 total FCR, IDL apoB100 total FCR, IDL apoB100 indirect FCR, LDL apoB100 PR, LDL apoB100 FCR, age, gender, BMI, diabetes, and LBP. $r^2 = 0.42$.

Table 4. Multivariable analyses with HDL-LPS as dependent variable performed in the whole studied population (including both patients with type 2 diabetes and controls)

Variables	Coefficient	SD	β	t	*P* Value
Free LPS (log)	0.136	0.033	0.592	4.13	<.0001
VLDL-LPS	0.430	0.191	0.322	2.25	.033

Variables selected for multivariable analysis: VLDL-LPS, LDL-LPS, free LPS apoA-I FCR, apoA-I PR, age, gender, BMI, diabetes, and LBP. $r^2 = 0.72$.

Overall, data from the multivariable analyses indicate the following: 1) that VLDL-LPS is associated with HDL-LPS and LDL-LPS, 2) that LDL-LPS is associated with VLDL-LPS and VLDL catabolism, 3) that HDL-LPS is associated with VLDL-LPS and free LPS, and 4) that free LPS is associated with HDL-LPS. All these links are summarized in Figure 2.

Reduced VLDL catabolism is associated with significant reduction of LDL-LPS: evidence from an apoA-V mutated patient

To certify that VLDL catabolism is an important factor controlling LDL-LPS concentration, we studied the distribution of LPS among lipoproteins in a patient with a functional deficiency of the lipoprotein lipase (enzyme involved in VLDL catabolism) due to a mutation in the *apoA-V* gene. This patient whose lipoprotein kinetics had been previously studied by our group and published in collaboration with other groups (32) showed a dramatic reduction in VLDL catabolism (VLDL apoB100 FCR: 0.51 pool/d vs 4.27 ± 1.42 pool/d in patients with type 2 diabetes and vs 8.01 ± 2.43 pool/d in controls). In this patient with severe decrease in VLDL catabolism, the LPS concentration in the LDL faction was very low (0.044 EU/mL vs 0.276 EU/mL in patients with persons with diabetes and vs 0.788 EU/mL in controls). The ratio of LDL-LPS to VLDL-LPS was very low in the *ApoA-V*-mutated patient 0.23 as compared with a mean 1.55 value in the population of the study. These data clearly indicate that a profound reduction of lipoprotein lipase activity, leading

Table 5. Multivariable analyses with free LPS as dependent variable performed in the whole studied population (including both patients with type 2 diabetes and controls)

Variables	Coefficient	SD	β	t	*P* Value
HDL-LPS	3.558	0.470	0.819	7.57	<.0001

Variables selected for multivariable analysis: VLDL-LPS, LDL-LPS, HDL-LPS, apoA-I FCR, apoA-I PR, VLDL apoB100 total FCR, VLDL apoB100 PR, IDL apoB100 total FCR, IDL apoB100 total PR, LDL apoB100 PR, LDL apoB100 FCR, age, gender, BMI, diabetes, an LBP. $r^2 = 0.67$.

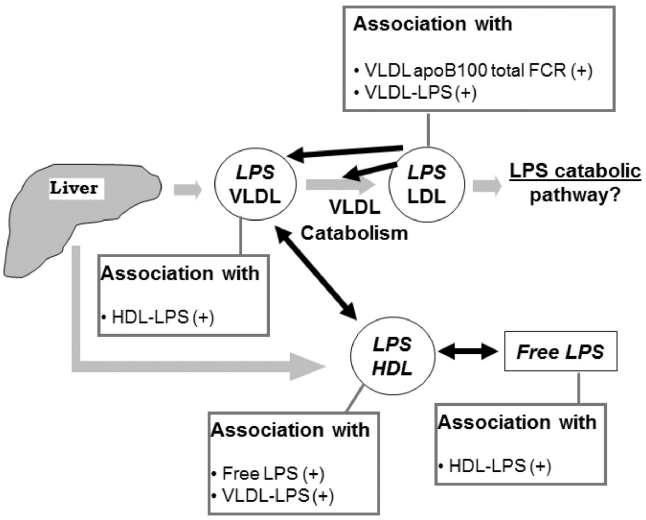

Figure 2. Summary of the associations between LPS lipoprotein fractions and lipoprotein kinetics (data from multivariate analyses). The + symbol indicates a positive association.

to a dramatic decrease in VLDL catabolism, reduces significantly the concentration of LPS in the LDL particles.

Reduced VLDL catabolism is the main factor for decreased LDL-LPS in type 2 diabetes

To determine whether the decreased VLDL catabolism observed in patients with T2DM was implicated in the reduced LDL-LPS fraction, we compared the LDL-LPS fraction in patients with type 2 diabetes with that in controls by analysis of covariance controlling for VLDL apoB100 total FCR. After controlling for VLDL apoB100 total FCR, the difference between patients with T2DM and controls for the LDL-LPS fraction was no longer statistically significant, suggesting that reduced VLDL catabolism is the main factor responsible for the decreased LDL-LPS fraction in type 2 diabetes.

Discussion

We report here for the first time a precise analysis of LPS distribution among lipoproteins in normolipidemic controls and in patients with type 2 diabetes. First, our data strongly suggest that in humans the nonlipoprotein fraction of LPS transfers to HDL and then to VLDL, whereas the LPS-bound LDL fraction is mainly derived from VLDL catabolism. This newly uncovered mechanism may be a catabolic pathway for LPS. Second, we showed that LPS distribution among lipoproteins is significantly modified in patients with type 2 diabetes, with a higher proportion of LPS in VLDL, in HDL, and in free LPS and a lower proportion of LPS in LDL than was the case in controls. These data suggest impaired LPS catabolism specific to type 2 diabetic patients. Third, the reduced VLDL catabolism observed in type 2 diabetes might be the main factor responsible for the lower proportion of LPS in LDL in patients with diabetes.

Previous studies showed that a large proportion of LPS is bound to lipoproteins, such as VLDL and LDL (17, 18) and particularly to LDL (8). Furthermore, our results are also in line with ex vivo data, which showed that LDL particles display high LPS-binding capacity (18). However, in this study, we have further increased our understanding about LPS metabolism by showing that the proportion of LPS bound to LDL in type 2 diabetes patients is lower than that in healthy controls. It is noticeable that our data and data from others (8, 17, 18) are different from those reported by Levels et al (16), who found, in a smaller population, that 60% of LPS was bound to HDL, 25% was bound to LDL, and 12% was bound to VLDL. These discrepancies could be due to the different lipoprotein separation and LPS assay procedures used. Therefore, to further support our discovery that type 2 diabetes is associated with modified LPS-bound lipoprotein metabolism, we used a state-of-the-art experimental procedure using lipoprotein tracers to determine the possible relationship between LPS distribution among lipoproteins and lipoprotein kinetics. We analyzed individuals, including patients with type 2 diabetes and normal subjects, with a wide range of lipoprotein kinetics. Data from the multivariable analysis show a strong positive association between HDL-LPS and free LPS, suggesting, in vivo, a bilateral LPS transfer between the free compartment and HDL lipoproteins. The transfer of LPS between the free compartment and HDL particles has also been suggested by in vitro data (15). In addition, in multivariable analyses, we showed a strong association between HDL-LPS and VLDL-LPS, suggesting that the LPS transfer between HDL and VLDL also occurs in vivo.

In an ex vivo study, it was observed that the LPS fluorescence signal shifted with time from HDL to VLDL, suggesting LPS transfer from HDL to VLDL (16). We cannot totally exclude the possibility that HDL could also deliver LPS directly to the liver through scavenger receptor class B member 1. However, the absence of any association between HDL-LPS and HDL catabolism indicates that the direct catabolism of HDL-LPS by scavenger receptor class B member 1 is not likely to be significant. Furthermore, data from the literature indicate that LPS binding protein and phospholipid transfer protein may be implicated in the transfer of LPS between lipoproteins (33). However, this hypothesis needs to be validated. We did not find any association between the kinetics of either apoB100 or apoA-I and LPS distribution among VLDL, HDL, and the free fraction. This suggests that the distribution of LPS among VLDL, HDL, and the free fraction and also LPS

transfer between the free fraction, HDL, and VLDL are not likely to be influenced significantly by lipoprotein kinetics. Altogether, our data suggest that in vivo free LPSs transfer to HDL and then to VLDL.

Regarding the LDL-LPS fraction, we showed that the VLDL catabolic pathway is an important determinant of LDL-LPS in the overall population. More precisely, we showed that the LDL-LPS fraction correlated significantly not only with VLDL apoB100 total FCR but also with VLDL apoB100 indirect FCR and IDL apoB100 indirect FCR. Indirect catabolism of VLDL apoB100 represents the catabolism of VLDL to IDL and the indirect catabolism of IDL apoB100 represents IDL catabolism to LDL. Thus, our data indicate that the catabolic pathway VLDL to IDL and then to LDL is an important determinant of LDL-LPS. This is reinforced by the data obtained in a patient with a mutation in the an *ApoA-V* gene, showing, in this situation of dramatic reduction of VLDL catabolism, a significant reduction in LPS-LDL, which suggests the transfer of LPS from VLDL to LDL through the catabolism of the VLDL. We cannot exclude the possibility that some VLDL-LPS might be directly removed by the liver via direct hepatic catabolism of VLDL particles. However, we know from in vivo kinetics studies in humans that direct catabolism of VLDL by the liver only moderately affects total VLDL catabolism, which is mainly represented by the VLDL-IDL-LDL cascade (34, 35).

In addition, the strong independent association between VLDL apoB100 indirect FCR and LDL-LPS suggests that the VLDL-IDL-LDL catabolic cascade is likely to be the main catabolic pathway for VLDL-LPS. Moreover, in the multivariable analysis, we found no association between HDL-LPS and LDL-LPS, suggesting that direct LPS transfer between HDL and LDL is probably minor and that most of the LPS transfer between HDL and LDL is indirect through VLDL. Interestingly, it has been shown in vitro that LDL-LPS complexes compete with LDL particles for LDL receptor-mediated uptake (8). These in vitro data and our in vivo findings strongly suggest that the VLDL-IDL-LDL pathway is a catabolic pathway for LPS. In addition, because the proportion of LPS in the LDL fraction in healthy individuals is high, we also suggest that the LDL catabolic pathway is important for the catabolism of LPS and that this pathway may play a significant role in reducing the inflammatory potential of the endotoxin.

Interestingly, several clinical studies have indicated that statin therapy may be beneficial in patients with endotoxemia. In a retrospective study, Liappis et al (36) reported significantly lower mortality in patients undergoing statin therapy than in patients without statin therapy. In a prospective observational study in patients with acute bacterial infections, previous treatment with statins significantly reduced the risk of severe sepsis (2.4% vs 19%) and admission to an intensive care unit (3.7% vs 12.2%) (37). The mechanisms by which statins reduce mortality and morbidity in patients with endotoxemia remain unclear. However, our data suggest that the increased LDL catabolism induced by statins may promote the LPS-LDL catabolic pathway and thus reduce the consequences of endotoxemia, notably with regard to inflammation. The ability of statin treatment to reduce the overall inflammatory tone in patients with endotoxemia suggests that the LPS-LDL catabolic pathway is probably a significant catabolic pathway for LPS in plasma. This is in line with many results from clinical trials indicating that the clinical benefit of statin therapy may also be attributed to mechanisms independent of their cholesterol-lowering effects (38, 39).

The influence of VLDL kinetics appears to be important for LPS distribution in the LDL fraction. The multivariable analysis showed that the association between LPS-LDL and VLDL catabolism was independent of diabetes, thus reinforcing the hypothesis that VLDL catabolism is probably an important predictor for LPS distribution in the LDL fraction.

In our population of patients with type 2 diabetes, who showed major abnormalities in lipoprotein kinetics including decreased catabolism of VLDL and IDL, we found significant differences in LPS distribution among lipoproteins with, more particularly, a markedly lower proportion of LDL-LPS than was the case in controls. Furthermore, we showed that the difference in LDL-LPS between patients with T2DM and controls was no longer statistically significant after controlling for VLDL apoB100 total FCR, indicating that reduced VLDL catabolism was the main cause of the decreased LDL-LPS fraction in type 2 diabetes. The major reason for the decrease in LDL-LPS in patients with type 2 diabetes is the dramatic reduction in VLDL catabolism in this population. The slowing of VLDL and IDL catabolism in type 2 diabetes with a reduced LDL-LPS fraction may represent an impaired LPS catabolic pathway that could contribute to overall metabolic inflammation as reported in type 2 diabetic patients (40). We believe that this impaired LPS catabolic pathway, which is secondary to lower VLDL and IDL catabolism and thus leads to LPS retention in the VLDL fraction, could promote inflammation.

In conclusion, our data suggest that substantial LPS transfer occurs in vivo between free LPS and HDL and between HDL and VLDL, whereas LPS-LDL is mainly derived from VLDL catabolism and may represent a catabolic pathway for LPS. In the future, it would be interesting to determine whether new agents to accelerate LDL catabolism (proprotein convertase subtilisin/kexin type 9

inhibitors, for instance) might be helpful in patients with endotoxemia. In addition, T2DM patients showed a significantly lower proportion of LPS bound to LDL as a result of reduced VLDL catabolism. This may represent an impaired LPS catabolic pathway. Further studies are needed to clarify whether the impaired LPS catabolic pathway in patients with diabetes is likely to promote low-grade inflammation.

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