Overproduction of VLDL<sub>1</sub> Driven by Hyperglycemia Is a Dominant Feature of Diabetic Dyslipidemia

Martin Adiels, Jan Borén, Muriel J. Caslake, Philip Stewart, Aino Soro, Jukka Westerbacka, Bernt Wennberg, Sven-Olof Olofsson, Chris Packard, Marja-Riitta Taskinen

Objective—We sought to compare the synthesis and metabolism of VLDL<sub>1</sub> and VLDL<sub>2</sub> in patients with type 2 diabetes mellitus (DM2) and nondiabetic subjects.

Methods and Results—We used a novel multicompartmental model to simultaneously determine the kinetics of apolipoprotein (apo) B and triglyceride (TG) in VLDL<sub>1</sub> and VLDL<sub>2</sub> after a bolus injection of [<sup>3</sup>H]<leucine and [<sup>2</sup>H]<glycerol and to follow the catabolism and transfer of the lipoprotein particles. Our results show that the overproduction of VLDL particles in DM2 is explained by enhanced secretion of VLDL<sub>1</sub> apoB and TG. Direct production of VLDL<sub>2</sub> apoB and TG was not influenced by diabetes per se. The production rates of VLDL<sub>1</sub> apoB and TG were closely related, as were the corresponding pool sizes. VLDL<sub>1</sub> and VLDL<sub>2</sub> compositions did not differ in subjects with DM2 and controls, and the TG to apoB ratio of newly synthesized particles was very similar in the 2 groups. Plasma glucose, insulin, and free fatty acids together explained 55% of the variation in VLDL<sub>1</sub> TG production rate.

Conclusion—Insulin resistance and DM2 are associated with excess hepatic production of VLDL<sub>1</sub> particles similar in size and composition to those in nondiabetic subjects. We propose that hyperglycemia is the driving force that aggravates overproduction of VLDL<sub>1</sub> in DM2. (Arterioscler Thromb Vasc Biol. 2005;25:1697-1703.)

Key Words: diabetes ■ dyslipidemia ■ VLDL ■ apolipoprotein B ■ triglycerides ■ compartmental modeling ■ kinetics ■ stable isotope

By 2025, >300 million people worldwide will have type 2 diabetes mellitus (DM2). Because atherosclerosis is an important complication of DM2, this will contribute significantly to an expected increase in cardiovascular disease worldwide. One important cardiovascular risk factor associated with DM2 is a dyslipidemia characterized by high levels of triglyceride (TG)-rich VLDL, low levels of HDL cholesterol, small, dense LDL, and impaired and prolonged postprandial hyperlipidemia. These abnormalities are present for years before DM2 is diagnosed clinically.

The discovery of heterogeneity within the major lipoprotein classes (VLDL, LDL, and HDL) has opened new avenues to identify specific perturbations of diabetic dyslipidemia. VLDL particles secreted from the liver vary in size and composition and can be classified by their density (0.94 to 1.06 g/mL), diameter (20 to 75 nm), and flotation [Svedberg flotation rate (Sf) 20 to 400]. VLDL can be separated into 2 main classes: large, buoyant VLDL<sub>1</sub> particles (Sf 60 to 400) and small, dense VLDL<sub>2</sub> particles (Sf 20 to 60). VLDL<sub>1</sub> particles contain more TG than VLDL<sub>2</sub> particles and are rich in apolipoprotein (apo) CHI and apoE. Large VLDL<sub>1</sub> particles are the major subclass of endogenous TG-rich lipoproteins and seem to be the major determinant of the plasma TG concentration in normolipidemic subjects. Although elevation of plasma TG is a consistent feature of diabetic dyslipidemia, little attention has focused on the VLDL subclass distribution in DM2. However, emerging data indicate a higher increase of VLDL<sub>1</sub> particles than of VLDL<sub>2</sub> particles in DM2.

The different lipid abnormalities in the dyslipidemia associated with DM2 are metabolically closely linked. Strong evidence suggests that the increase in large VLDL<sub>1</sub> particles has consequences for the metabolism of other lipoproteins, including the accumulation of remnant particles, generation of small, dense LDL, lowering of HDL concentration, and changes in HDL composition. We propose that the increase in large VLDL<sub>1</sub> particles in DM2 initiates a sequence of lipoprotein changes, including increased amounts of smaller LDL and a decrease of HDL particles, that increase the risk of atherosclerosis.

In this study, we explored the mechanisms for the increase in VLDL<sub>1</sub> particles associated with DM2. We used a novel multicompartamental model that allows the kinetic parameters of TG and apoB100 in VLDL<sub>1</sub> and VLDL<sub>2</sub> to be simultaneously determined after a bolus of [<sup>3</sup>H]<leucine and [<sup>2</sup>H]<glycerol. 

Original received October 11, 2004; final version accepted May 23, 2005.

From the Department of Mathematical Sciences (M.A., B.W.), Chalmers University of Technology, Göteborg, Sweden; the Wallenberg Laboratory (M.A., J.B., S.-O.O.), Göteborg University, Sweden; the Department of Pathological Biochemistry (A.S., J.W., M.-R.T.), Glasgow Royal Infirmary, Scotland; and the Division of Cardiology (M.J.C., P.S., C.P.), Helsinki University Central Hospital, Biomedicum, Finland.

Correspondence to Jan Borén, MD, PhD, Wallenberg Laboratory, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden. E-mail Jan.Boren@wlab.gu.se

© 2005 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

DOI: 10.1161/01.ATV.0000172689.53992.25

1697
Methods

Subjects
A total of 30 men were included in the study, 20 control subjects and 10 DM2 subjects. The kinetics of VLDL1 and VLDL2 apoB and TG were reported for 17 of the control subjects previously. Because of technical errors, VLDL2 apoB and TG kinetics could not be estimated in 2 of the new control subjects. However, VLDL1 and VLDL2 apoB kinetics of these 2 control subjects were included in the study. All subjects underwent a physical examination and laboratory tests to exclude hepatic, renal, thyroid, and hematologic abnormalities. Subjects with coronary heart disease, diabetic retinopaties or microalbuminuria, total cholesterol >7.0 mmol/L, TG >5.0 mmol/L, or body mass index (BMI) >40 kg/m² were excluded. The control subjects had lipid values representative for a Western population, and all had normal glucose tolerance according to World Health Organization criteria. The diagnosis of DM2 was based on glucose tolerance test results in the diabetic range according to World Health Organization (1999) criteria or on the use of oral diabetes medication. The age of onset of DM2 was required to be at least 35 years. None of the subjects were using lipid-lowering treatment or insulin. The mean duration of DM2 was 6.6±4.7 years (range, 2 to 15). The study design was approved by the Helsinki University Central Hospital ethics committee, and each subject gave written consent. All samples were collected in accordance with the Helsinki Declaration. See supplement (online at http://atvb.ahajournals.org) for additional details.

ApoB and TG Turnover Protocol
The turnover protocol was performed with use of a bolus of [1H]leucine and [1H]glycerol. The particle composition and apoB mass of the VLDL1 and VLDL2 fractions were determined 30 minutes before and 0, 4, and 8 hours after the injection. The subjects fasted from 7:30 AM until 5 PM, when the last blood sample was taken.

Kinetic Analysis
See the online supplement at http://atvb.ahajournals.org for specific details. The outputs from the model are the fractional catabolic rate (FCR) of VLDL1 and VLDL2 (the fractional loss of mass due to direct catabolism from VLDL1 to VLDL2). In this model, we cannot distinguish unpaired, 2-sided D integrals for all subjects as well as separately for the DM2 and control subjects. For nonnormally distributed variables, values were logarithmically transformed before correlation analysis. Statistical analysis was performed with MatLab and Microsoft Excel.

Results

Characterization of Subjects
The characteristics of DM2 and control subjects are summarized in Table I (available online at http://atvb.ahajournals.org). The DM2 subjects had mild dyslipidemia, with significantly higher total plasma TG (1.85±0.42 versus 1.35±0.48 mmol/L), lower HDL (0.96±0.30 versus 1.37±0.22 mmol/L), and higher plasma glucose (176±39 versus 106±10 mg/dL) than controls. There were no significant differences in the age, weight, BMI, or cholesterol levels between the 2 groups.

Concentrations and Compositions of VLDL Subclasses
There were no significant differences in the relative composition of VLDL1 or VLDL2 in DM2 and control subjects (Table II, available online at http://atvb.ahajournals.org). As expected, VLDL1 particles had higher TG content than did VLDL2 particles (65% versus 42%). DM2 subjects had larger apoB and TG pools of both VLDL1 and VLDL2 than did control subjects (Table). ApoB and TG pool sizes showed a strong, linear correlation in both VLDL1 and VLDL2 (Figure 1A).

Kinetic Parameters for VLDL Subclass Metabolism
In each subject, the FCR, FDCR, FTR, and production rate were determined for both apoB and TG (Table). The production rates of VLDL1 apoB and VLDL1 TG were both significantly higher in DM2 than in control subjects (Table), and more apoB and TG were transferred from VLDL1 to VLDL2 in DM2 subjects (P<0.01). Thus, the total production of apoB and TG was increased in DM2 subjects, despite the fact that VLDL2 apoB and TG direct production rates were comparable in diabetic and control subjects. Interestingly, for both VLDL1 and VLDL2, the TG-apoB ratio of newly produced particles was similar in the 2 groups (Table). Strong, linear correlations were found between apoB and TG production rates in both VLDL1 and VLDL2 (Figure 1B).

There was no significant difference between DM2 and control subjects in either apoB or TG FTR from VLDL1 to VLDL2 (VLDL1 FTR), direct catabolic rate from VLDL1 (VLDL1 FDCR), total catabolic rate from VLDL1 (VLDL1 FCR), total catabolic rate from VLDL2 (VLDL2 FCR), or total VLDL catabolic rate (VLDL FCR).

Determinants of Production and Clearance Rates
In all subjects, the VLDL1 apoB and TG production rates were correlated significantly with plasma glucose (P<0.001), insulin (apoB, P<0.01; TG, P<0.05), and HOMA-IR (P<0.001) (Figure 2). Notably, VLDL1 TG production was correlated with free fatty acids (FFAs). VLDL1 TG production was correlated significantly with HOMA-IR (P<0.05), but VLDL1 apoB production rate was not correlated with any of the parameters plasma glucose, insulin, HOMA-IR, FFAs, or BMI. In all subjects, neither apoB (VLDL1 or VLDL2) nor TG FCRs were correlated with plasma glucose, insulin,
HOMA-IR, FFAs, or BMI. However, VLDL₁ TG and apoB FCR were correlated inversely with plasma glucose in DM2 subjects ($P<0.05$).

In a regression analysis of VLDL₁ TG production, plasma glucose explained 51% of the variation; HOMA-IR, 37%; insulin, 16%; and FFAs, 14%. Including all parameters (except HOMA-IR, which is a product of plasma glucose and insulin) in the multivariate regression model explained 55% of the variation; however, only plasma glucose remained significant (Table III).

For VLDL₁ apoB production, explanatory variables in the linear (log-log) regression were HOMA-IR (36% of the variation), plasma glucose (35%), insulin (22%), and FFAs (10%; $P=0.09$). Altogether, these variables explained 42% of the variability of VLDL₁ apoB production, but plasma glucose was the only parameter remaining significant. Thus, the contribution of FFAs as a predictor of VLDL₁ apoB production was minor. The significant correlation between VLDL₂ TG production and HOMA-IR explained 16% of the variation (Table III).

### Determinants of VLDL₁ and VLDL₂ Pool Sizes

In all subjects, the VLDL₁ apoB and TG pool sizes were correlated with their respective production rates and FCRs (Figure 3). The pool sizes also were correlated with insulin and HOMA-IR ($P<0.01$), as well as with plasma glucose ($P<0.05$).

Likewise, in control subjects, the VLDL₁ apoB and TG pool sizes were correlated significantly with their respective production rates (apoB, $r=0.49$, $P<0.05$; TG, $r=0.59$, $P<0.05$) and FCRs (apoB, $r=-0.80$, $P<0.001$; TG, $r=0.79$, $P<0.001$). The significant correlation between VLDL₁ apoB
A strong correlation was observed in the subgroups VLDL1 (apoB, \( r = -0.54, P < 0.01 \)) but positively with VLDL1 production (apoB, \( r = 0.43, P < 0.05 \); TG, \( r = 0.49, P < 0.01 \)).

Importantly, these correlations remained significant in analyses of the 2 subgroups. Thus, in control subjects, plasma TG was correlated negatively with VLDL1 FCRs (apoB, \( P < 0.001; \) TG, \( P < 0.001 \)) and VLDL2 FCRs (apoB, \( P < 0.001; \) TG, \( P < 0.01 \)). In DM2 subjects, the respective correlations were significant but less strong (\( P < 0.05 \)).

In a multivariate analysis of plasma TG, wherein VLDL1 apoB (or TG) production, their FCRs, VLDL2 apoB (or TG) direct production, and their FCRs were independent variables, only the VLDL1 apoB (or TG) production and VLDL1 FCR remained significant.

**Discussion**

This study shows that the overproduction of VLDL particles in DM2 is entirely accounted for by enhanced secretion of VLDL1 particles due to increased production of both VLDL1 apoB and TG. Diabetes per se did not influence the direct production of VLDL2 apoB and TG. The production rates of VLDL1 TG and apoB were closely related, as were the pool sizes of VLDL1 TG and apoB (Figure 1). Importantly, there was no significant difference in particle composition of either VLDL1 or VLDL2 between DM2 and control subjects, and the TG-apoB ratio of newly synthesized particles was very similar in both groups (Table II). These findings demonstrate that DM2 and insulin resistance are associated with excess hepatic production of VLDL1 particles that are similar in size and composition to those of nondiabetic subjects.

The correlations between TG and apoB pool sizes in both VLDL1 and VLDL2 and between the actual production rates of both VLDL1 and VLDL2 TG and apoB reveal a close coordination between TG and apoB metabolism in VLDL species. Previous studies reported that VLDL particles in subjects with DM2 are TG enriched and larger than in nondiabetic subjects.8–10 However, in those studies, the VLDL subclasses were not analyzed separately. Therefore, a relatively larger increase in VLDL1 pools than in VLDL2 pools in DM2 subjects would be erroneously reflected as an increase in the fractional TG content in VLDL particles.

In humans, the mean residence time of VLDL1 apoB is the main determinant of apoB pool size and of plasma TG concentration.11 We consistently observed a significant correlation between TG and apoB pool sizes and the respective FCRs, indicating that the lipolytic rate was the major determinant of pool size over a wide range of plasma TG levels. The production rates of VLDL1 TG and apoB were also related to pool size, but the relation was less strong than for FCR. Our findings are in good agreement with earlier studies showing that both the FCR and the secretion rate of VLDL TG contribute to plasma TG variation in nondiabetic subjects and that the VLDL TG FCR is a more powerful determinant than the secretion rate.12

**Determinants of Plasma TG**

Plasma TG showed significant correlations with plasma glucose (\( P < 0.05 \)), insulin (\( P < 0.01 \)), and HOMA-IR (\( P < 0.01 \)) in all subjects. However, these correlations were not significant in the analyses of the subgroups. In all subjects, plasma TG was correlated negatively with VLDL1 FCR (apoB, \( r = -0.83, P < 0.001 \); TG, \( r = -0.75, P < 0.001 \)) and with VLDL2 FCR (apoB, \( r = -0.67, P < 0.001 \); TG, \( r = -0.54, P < 0.01 \)).

[Figure 1. A. Measured apoB pool size plotted vs measured TG pool size. There was a strong, linear correlation between the apoB and TG pools in both VLDL1 and VLDL2. The VLDL1 pools were significantly larger in DM2 than in controls (\( P < 0.01 \)), although data for all subjects plot on the same line. The same strong correlation was observed in the subgroups VLDL1 (controls, \( r = 0.97, P < 0.001 \); DM2, \( r = 0.97, P < 0.001 \)) and VLDL2 (controls, \( r = 0.95, P < 0.001 \); DM2, \( r = 0.92, P < 0.001 \)). B, ApoB production plotted vs TG production. There was a strong, linear correlation between apoB and TG production in both VLDL1 and VLDL2 and a significantly higher production of both VLDL1 apoB and VLDL2 TG in DM2 than in control subjects (apoB, \( P < 0.01 \); TG, \( P < 0.001 \)). The TG-apoB ratio of VLDL1 and VLDL2 (ie, the slope of the lines) showed no significant difference between DM2 and control subjects. Kinetics of VLDL1 and VLDL2 apoB and TG were reported for 17 of the 20 control subjects previously.7 Control subjects: VLDL1 (□) and VLDL2 ( ), DM2 subjects: VLDL1 ( ), and VLDL2 ( ). Abbreviations are as defined in text.]

and TG pool sizes and their respective FCRs was also found in the DM2 subjects (apoB and TG, \( r = -0.78, P < 0.01 \)).

The VLDL2 apoB and TG pool sizes displayed similar correlations as the VLDL1 pools. In all subjects, the VLDL2 apoB and TG pool sizes were correlated with their respective FCRs (\( P < 0.01 \)), insulin, HOMA-IR, and plasma glucose (all \( P < 0.05 \)). The VLDL1 TG pool size was also correlated with the direct production rate (\( P < 0.05 \)).

**Figure 1. A. Measured apoB pool size plotted vs measured TG pool size. There was a strong, linear correlation between the apoB and TG pools in both VLDL1 and VLDL2. The VLDL1 pools were significantly larger in DM2 than in controls (\( P < 0.01 \)), although data for all subjects plot on the same line. The same strong correlation was observed in the subgroups VLDL1 (controls, \( r = 0.97, P < 0.001 \); DM2, \( r = 0.97, P < 0.001 \)) and VLDL2 (controls, \( r = 0.95, P < 0.001 \); DM2, \( r = 0.92, P < 0.001 \)). B, ApoB production plotted vs TG production. There was a strong, linear correlation between apoB and TG production in both VLDL1 and VLDL2 and a significantly higher production of both VLDL1 apoB and VLDL2 TG in DM2 than in control subjects (apoB, \( P < 0.01 \); TG, \( P < 0.001 \)). The TG-apoB ratio of VLDL1 and VLDL2 (ie, the slope of the lines) showed no significant difference between DM2 and control subjects. Kinetics of VLDL1 and VLDL2 apoB and TG were reported for 17 of the 20 control subjects previously.7 Control subjects: VLDL1 (□) and VLDL2 ( ). DM2 subjects: VLDL1 ( ) and VLDL2 ( ). Abbreviations are as defined in text.**
profoundly affect lipolytic rate. However, our DM2 subjects had good glycemic control and only moderately elevated plasma TGs. Defective VLDL removal might be more critical in diabetic subjects with poor glycemic control or more severe hypertriglyceridemia.9,10

In contrast, direct production of VLDL₂ apoB and TG was comparable in DM2 and control subjects. Thus, the current data provide additional evidence that pathways for VLDL₁ and VLDL₂ metabolism in the liver are independently regulated.2,11 Previously, we showed that insulin infusion suppressed VLDL₁ apoB production in normal healthy subjects but had no effect on direct VLDL₂ apoB production.13 In contrast, insulin failed to suppress VLDL₁ apoB production in men with DM2.14 These data suggest that VLDL₁ metabolism is profoundly dysregulated in DM2.

What forces drive the overproduction of VLDL₁ particles in DM2 and insulin resistance? VLDL₁ assembly and secretion are complex multiplicative processes in which substrate availability and insulin play key roles.3,15,16 Substantial evidence indicates that hepatic fatty acid availability regulates VLDL TG production.17 Insulin downregulates apoB via the phosphatidylinositol-3-kinase pathway,18 and insulin resistance results in diminished phosphatidylinositol-3-kinase that may increase VLDL secretion.19 Another site of insulin action is the microsomal TG transfer protein (MTP) gene that contains an insulin response element in the promoter.20 It is noteworthy that increased MTP mRNA expression is associated with enhanced synthesis of VLDL in animal models of insulin resistance.21 Recent studies indicate that insulin regulates transcription of the genes coding for MTP and the insulin receptor through the mitogen-activated protein kinase (extracellular signal–regulated kinase) cascade.22 Thus, insulin signaling influences the assembly process of VLDL at multiple levels, and disturbed insulin signaling results in increased VLDL production. Therefore, the associations of fasting insulin and HOMA index with VLDL₁ apoB and TG production rates are consistent with the seminal role of insulin resistance as a driving force for increased VLDL₁ production. Overall, these data confirm and extend earlier studies reporting a relation between both plasma insulin and HOMA–IR and VLDL apoB production rate in nondiabetic subjects.12,23

An unexpected finding was the strong, positive correlation between plasma fasting glucose and VLDL₁ apoB (A) and VLDL₁ TG (B) production and between HOMA–IR and VLDL₁ apoB (C) and VLDL₁ TG (D) production. VLDL₁ production was correlated well with both plasma glucose and HOMA–IR. Control subjects: VLDL₁ production vs plasma glucose (apoB, r = 0.29, NS; TG, r = 0.26, NS) and vs HOMA–IR (apoB, r = 0.42, NS; TG, r = 0.27, NS). DM2 subjects: VLDL₁ production vs plasma glucose (apoB, r = 0.49, NS; TG, r = 0.73, P < 0.05) and vs HOMA–IR (apoB, r = 0.24, NS; TG, r = 0.12, NS). VLDL₁ control subjects (▫) and VLDL₁, DM2 subjects (יו). Abbreviations are as defined in text.
plasma insulin levels seems to promote lipogenesis through concomitant upregulation of both SREBP-1c and ChREBP. The synergistic action of SREBP-1c and ChREBP directs the conversion of excess glucose to fatty acids and enhances esterification but attenuates fatty acid oxidation. This process results in excess availability of TGs, which likely are both channeled into VLDL secretion and stored as liver fat. Our finding that FFAs were correlated with VLDL1 TG production is consistent with the concept that hepatic fatty acid availability regulates VLDL TG production. Furthermore, the results indicate that plasma FFAs seem to increase primarily VLDL1 production. This concept highlights increased DNL as a potential cause for both overproduction of VLDL and fatty livers. Finally, moderate hyperglycemia has been shown to increase the secretion of VLDL TG in the setting of excess fatty acid availability in human volunteers. We propose that hyperglycemia is the driving force that aggravates overproduction of VLDL1 particles under conditions of insulin resistance in DM2.

Several studies have reported an association between BMI and VLDL production. Recognizing the heterogeneity of DM2 and the impact of obesity, we tried to recruit subjects with moderate overweight, and only 2 control subjects and 4 with DM2 had a BMI ≥30 kg/m². Although the 2 groups had similar mean BMIs, the range was wider in the DM2 group. This experimental design dilutes the impact of BMI on the kinetics parameters and probably explains why BMI was not correlated with VLDL1 apoB and TG production rates.

A potential weakness of the study is that sampling was performed for 8 hours only, which is not optimal for catching the tails of VLDL1 and VLDL2 curves. A longer sampling period would likely give more information on glycerol and TG kinetics in the liver, especially in subjects with more severe hypertriglyceridemia, and it is possible that the lack of a relation with VLDL2 kinetics might have been reversed if we had more data on the kinetics at later time points. Nevertheless, we were able to calculate the production, transfer, and catabolic rates. Another possible flaw is the use of the population mean to model plasma glycerol. In 6 subjects (both DM2 and controls), however, we measured plasma glycerol enrichment and modeled VLDL1 and VLDL2 metabolism by using both the population mean and the glycerol enrichment data. For the production and catabolic rates, the average difference in the calculated parameters with these 2 approaches was in the range of 2.5% to 8%; VLDL1 FCR and production rates were within 10% in both control and diabetic subjects. Thus, we considered the use of population means satisfactory for modeling plasma glycerol kinetics in all subjects.
References


Overproduction of VLDL<sub>1</sub> Driven by Hyperglycemia Is a Dominant Feature of Diabetic Dyslipidemia

Martin Adiels, Jan Borén, Muriel J. Caslake, Philip Stewart, Aino Soro, Jukka Westerbacka, Bernt Wennberg, Sven-Olof Olofsson, Chris Packard and Marja-Riitta Taskinen

Arterioscler Thromb Vasc Biol. 2005;25:1697-1703; originally published online June 9, 2005;
doi: 10.1161/01.ATV.0000172689.53992.25

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/25/8/1697

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2005/06/09/01.ATV.0000172689.53992.25.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/
SUPPLEMENTARY DATA

Overproduction of VLDL₁ Driven by Hyperglycemia Is a Dominant Feature of Diabetic Dyslipidemia, by Adiels et al.

Subjects
One subject in each group was taking acetylsalicylic acid. Five patients with DM2 were treated with diet alone, two with diet and sulfonylurea, and three with a combination of diet, sulfonylurea, and metformin. One patient with DM2 was using antihypertensive therapy (felodipine). Medications were continued throughout the study period.

Isolation of Lipoproteins
VLDL₁ and VLDL₂ were isolated from plasma by cumulative flotation gradient ultracentrifugation as detailed previously. Briefly, 8.4 ml of plasma was mixed with 0.6 ml of 1.019 g/ml density NaCl solution and used to prepare total VLDL by ultracentrifugation (100,000 rpm, 2 h 45 min, at 23°C) in a tabletop centrifuge (TLA-100.3 Fixed Angle Rotor, Beckman Instruments, Palo Alto, USA). Total VLDL was aspirated from the top in 2 ml, and its density was increased to 1.118 g/ml by the addition of solid NaCl (170 mg/ml of VLDL solution). A 2 ml aliquot of this preparation was layered over a 0.5 ml cushion of d 1.182 g/ml NaBr solution and a six-step discontinuous salt gradient of d 1.0988-1.0588 g/ml was constructed above it as described by Lindgren et al. The Ti40 SW rotor was subjected to centrifugation at 39,000 rpm for 1 h and 21 min at 23°C and decelerated without braking. VLDL₁ was removed in the top 1.0 ml of solution which was replaced with 1.0 ml of d 1.0588 g/ml solution before continuing centrifugation. VLDL₂ was then isolated from the top 0.5 ml of the gradient following centrifugation at 18,500 rpm for 15 h 29 min at 23 °C.

Biochemical Analyses
ApoB and TG concentrations in the lipoprotein fractions were measured in samples obtained at 0, 4, and 8 h and prepared as described. ApoB and TG pool sizes were calculated as the product of plasma volume (4.5% of body weight) and the plasma
concentration of apoB and TG in VLDL₁ and VLDL₂; the leucine and glycerol content of each pool were determined as described.² Biochemical analyses were performed as described.² The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated from the fasting glucose and serum insulin concentrations as follows: fasting insulin (µl/ml) x fasting glucose (mmol/l)/22.5.³ Hemoglobin A₁c was measured by high-pressure liquid chromatography using the fully automated glycosylated Hemoglobin Analyzer System (BioRad).

**Kinetic Analysis**

Measured apoB and TG pool sizes in VLDL₁ and VLDL₂ were converted into leucine and glycerol equivalents.² The injected amount of D3-leucine and D5-glycerol, the leucine and glycerol pool sizes in VLDL₁ and VLDL₂, and the enrichment curves of leucine and glycerol in plasma, VLDL₁, and VLDL₂ were used as the data set for a multi-compartmental model that allowed simultaneous modeling of apo B and TG kinetics as described.² Each apoB compartment had a corresponding TG compartment, and the size of a particle in a compartment was estimated from the TG mass divided by the apoB mass. Flux of apoB from one compartment to another (and hence particle flow) was accompanied by an equivalent TG flux (dependant on the TG/apoB ratio). Thus, a fraction of the TG was removed from a compartment when a particle (as traced by apoB leucine) reached the destination compartment. The rest of the TG was deemed to be removed by hydrolysis.

**REFERENCES**


### Table I. Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=20)</th>
<th>DM2 Subjects (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age, years</td>
<td>50 ± 9</td>
<td>25–59</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83 ± 9</td>
<td>70–102</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.2 ± 2.6</td>
<td>22.1–30.1</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>6.2 ± 2.6</td>
<td>2.0–11.0</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>106 ± 10</td>
<td>91–130</td>
</tr>
<tr>
<td>Hemoglobin A₁₀, %</td>
<td>7.4 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.62 ± 0.73</td>
<td>0.46–2.96</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.35 ± 0.48</td>
<td>0.67–2.63</td>
</tr>
<tr>
<td>Chol, mmol/L</td>
<td>5.11 ± 0.90</td>
<td>3.76–7.90</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.37 ± 0.22</td>
<td>0.96–1.68</td>
</tr>
<tr>
<td>FFA, µmol/L</td>
<td>555 ± 86</td>
<td>352–667</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>103 ± 21</td>
<td>66–140</td>
</tr>
</tbody>
</table>

* p<0.01, † p<0.001 versus controls.
<table>
<thead>
<tr>
<th>Table II. Compositions of VLDL₁ and VLDL₂ Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Composition (% of Total)</td>
</tr>
<tr>
<td>Control Subjects ($n=20$)</td>
</tr>
<tr>
<td>VLDL₁</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>FC</td>
</tr>
<tr>
<td>CE</td>
</tr>
<tr>
<td>PL</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>ApoB</td>
</tr>
<tr>
<td>VLDL₂</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>FC</td>
</tr>
<tr>
<td>CE</td>
</tr>
<tr>
<td>PL</td>
</tr>
<tr>
<td>Total protein</td>
</tr>
<tr>
<td>ApoB</td>
</tr>
</tbody>
</table>

FC indicates total free cholesterol; CE, cholesterol esters; PL, phospholipids.
Table III. Multivariate regression analysis of predictors of VLDL\textsubscript{1} and VLDL\textsubscript{2} apoB and TG productions

<table>
<thead>
<tr>
<th></th>
<th>VLDL\textsubscript{1} TG, $r^2=0.55$</th>
<th>VLDL\textsubscript{1} apoB, $r^2=0.42$</th>
<th>VLDL\textsubscript{2} TG, $r^2=0.17$</th>
<th>VLDL\textsubscript{2} apoB, $r^2=0.08$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coef. ± SD</td>
<td>$p$</td>
<td>Coef. ± SD</td>
<td>$p$</td>
</tr>
<tr>
<td>P-Glucose</td>
<td>0.93 ± 0.25</td>
<td>&lt; 0.001</td>
<td>0.68 ± 0.28</td>
<td>0.023</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.14 ± 0.14</td>
<td>NS</td>
<td>NS</td>
<td>0.23 ± 0.18</td>
</tr>
<tr>
<td>FFA</td>
<td>0.38 ± 0.33</td>
<td>NS</td>
<td>0.33 ± 0.36</td>
<td>NS</td>
</tr>
</tbody>
</table>

The multivariate regression was performed on all subjects. Coef., Standard coefficient; SD, standard deviation; NS, not significant ($p>0.05$).