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Effect of atorvastatin on apolipoprotein B100 containing lipoprotein metabolism in type 2 diabetes

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List of non standard abbreviations :

VLDL (Very Low Density Lipoprotein), VLDLR (Very Low Density Lipoprotein Remnant), IDL (Intermediate Density Lipoprotein), LDL (High Density Lipoprotein), ApoB100 (Apolipoprotein B100), FCR (Fractional Catabolic Rate), PR (Production Rate), HMG CoA (Hydroxy Methyl Glutaryl Coenzyme A), HOMA (Homeostasis Model Assessment), MTP (Microsomal Triglyceride Transfer Protein), LPL (Lipoprotein Lipase), BMI (Body Mass Index).

Abstract

Seven hypertriglyceridemic patients with type 2 diabetes were treated with atorvastatin (40 mg/day) for two months. Kinetics of apolipoprotein B100 (apoB100) containing-lipoproteins were determined before and after atorvastatin treatment and compared to data obtained in 5 normolipidemic volunteers. ApoB100 metabolism was studied using stable isotopes and multicompartmental modeling.

Compared to normolipidemic obese subjects, type 2 diabetic patients had higher apoB100 concentration in very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and low density lipoproteins (LDL), (P<0.005). Kinetic analysis showed an increase in total apoB100 production rate (P<0.005) related to VLDL apoB100 overproduction (P<0.005). Patients were also characterized by a lower fractional catabolic rate (FCR) in VLDL (NS) or IDL (P<0.005) mainly related to a decrease in VLDL and IDL delipidation rate (P<0.005). Catabolism of LDL was also lower in diabetic patients (P<0.05).

Atorvastatin treatment significantly decreased, plasma triglycerides (P<0.05), total and LDL cholesterol (P<0.05), apoB100 in LDL, IDL and VLDL (P<0.05). Treatment significantly decreased total apoB100 production rate (P<0.05) but only for VLDL (P<0.05). Treatment normalized FCR in IDL and LDL (P<0.05). We concluded that atorvastatin improved lipid abnormalities in type 2 diabetic patients not only by increasing the clearance of apoB100 containing lipoproteins but also by decreasing VLDL production.

Atherosclerosis is the leading cause of morbidity and mortality in type 2 diabetic patients. Hypertriglyceridemia is an important lipid disorder associated with type 2 diabetes and may contribute to the development of atherosclerosis (Goldberg et al., 2001). Results of studies showing possible disturbances in LDL metabolism are inconsistent (Howard, 1987). Previous studies have underlined that the insulin resistance observed in type 2 diabetes is associated with an overproduction of apoB100 containing lipoproteins and reduced LDL and VLDL catabolism (Howard et al., 1987).

Atorvastatin is a strong competitive inhibitor of 3-hydroxy 3-methyl-glutaryl coenzyme A (HMG-CoA) reductase. In patients with familial hypercholesterolemia and combined hyperlipidemia, the cholesterol-lowering effects are related to reduction of LDL cholesterol and are explained either by an increase in LDL receptor activity or by reduction of LDL apoB100 production with atorvastatin as observed with other statins (Aguilar-Salinas et al., 1998). In addition to its cholesterol-lowering effect, atorvastatin also shows significant triglyceride-lowering properties in hypertriglyceridemic subjects (Lea et al., 1997) but conflicting results were reported concerning its effect on VLDL metabolism. Various effect have been already reported on VLDL production with atorvastatin : inhibiting effect in animal (Burnett et al., 1997) or cell models (Wilcox et al., 1999), stimulating effect (Forster et al., 1996) or no effect (Forster et al., 2002) in combined hyperlipidemic subjects and no effect in insulin resistant patients with no diabetes mellitus (Chang et al., 2002). However it appears that statins could be have an effect on VLDL production in situations with high VLDL production (Aguilar-Salinas et al., 1998).

The aim of the present study was to evaluate metabolic changes induced by atorvastatin in patients with type 2 diabetes and marked hypertriglyceridemia. This study focuses on the apoB100 containing lipoproteins (VLDL, IDL and LDL) in diabetic patients with combined dyslipidemia. We have studied apoB100 metabolism using endogenous labeling with deuterated leucine and multicompartmental modeling to analyze lipoprotein kinetics before and after atorvastatin treatment.

Methods

Subjects

Seven patients with type 2 diabetes and dyslipidemia (Table 1) were included in the study. Insulin treatment was an exclusion criterion. Patients were treated with either sulfamides or biguanides for at least 3 months and no treatment change was allowed for the duration of the study. The insulin resistance was assessed by insulin sensitivity index (Homeostasis model assessment, HOMA) (Matthews et al., 1985). Five normolipidemic obese subjects matched for age and body mass index with the diabetic patients were also studied as controls. The study subjects were instructed by a dietician to follow weight-maintenance diet, composed of 45% carbohydrate, 35% fat and 20% protein, for at least 1 week prior to study commencement. The experimental protocol was approved by the ethical committee of Nantes University Hospital, and written informed consent was obtained before the study was started.

Experimental protocol

Diabetic patients were treated for two months with atorvastatin (40 mg/day). Kinetic studies of apoB100 metabolism were performed before and after treatment.

The endogenous labeling of apoB100 was carried out by constant infusion of [D_3]-leucine in subjects fasted overnight for 12 h prior to the study, and who remained fasting during the entire procedure (Maugeais et al., 2001). Each subject received intravenously a prime of 10 μmol.kg^{-1} of tracer

immediately followed by a constant tracer infusion (10 μmol.kg^{-1}.h^{-1}) for 14 h. Venous blood samples were drawn into EDTA tubes (Venoject, Paris, France) at baseline and at 15, 30, 45 min, 1, 1.5, 2, 2.5 h, and then hourly until 14 h. Sodium azide, an inhibitor of bacterial growth, and Pefabloc SC (Interchim, Montluçon, France), a protease inhibitor, were added to blood samples at a final concentration of 1.5 and 0.5 mmol/L, respectively.

Analytical procedures

Isolation and measurement of enrichment of lipoprotein containing apoB100.

VLDL1 (Sf 400-60) and VLDL2 (Sf 60-20) were separated by density gradient ultracentrifugation (Gaw et al., 1992) using a swinging bucket rotor at 40000 rev./min for 24 h at 10°C (RPS 40T, Hitachi). IDL (1.006<d<1.020 g/mL) and LDL (1.020<d<1.063 g/mL) were separated by standard sequential ultracentrifugation methods (Havel et al., 1955) using a fixed-angle rotor at 40000 rev./min for 22 h at 10°c (CP70, Hitachi).

Isolation and measurement of leucine enrichment in apoB100 have been described previously (Maugeais et al., 2001). Briefly, apoB100 in lipoproteins was isolated by sodium dodecylsulfate polyacrylamide gel electrophoresis. Apolipoprotein bands were dried under a vacuum and then hydrolyzed. The amino acids were purified by cation exchange chromatography, then esterified and derivatized. Electron-impact gas chromatography-mass spectrometry was performed on a 5891 A gas chromatograph connected with a 5971 A quadrupole mass spectrometer. The isotopic ratio was determined by selected

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ion-monitoring at m/z of 282 and 285. Calculations of apoB100 kinetic parameters were based on the tracer-to-tracee mass ratio (Cobelli et al., 1992).

Measurements of lipids and apoB100

Cholesterol and triglyceride levels were measured using commercially available enzymatic kits (Boehringer Mannheim GmbH, Mannheim, Germany) at three different sampling times. Apolipoprotein AI concentration in plasma was measured by immunonephelemetry (Behring, Rueil Malmaison, France) at the same sampling times. ApoB100 concentrations were obtained in lipoprotein fractions by combining selective precipitation and mass spectrometry (Beghin et al., 2000). The percentage recovery of cholesterol, triglycerides and apolipoprotein B100 after centrifugation was higher than 85%

Modeling

Kinetic analysis of tracer-to-tracee ratios was achieved using computer software for simulation, analysis and modeling (Barrett et al., 1998).

The model used (figure 1) is adapted from a minimal model previously developed for apoB100 metabolism in normolipidemic subjects (Maugeais et al., 2001), but taking into account heterogeneity in VLDL as reported in type 2 diabetics (Tilly-Kiesi et al., 1996), e.g. large (VLDL1) and small VLDL (VLDL2), VLDL remnants (VLDLR) and shunt between VLDL and LDL. In this model apoB100 enters into plasma through VLDL secretion and direct production of IDL and LDL. ApoB100 direct removal occurs from VLDL1 (k(0,10)), VLDL2 (k(0,20)), VLDLR (k(0,11)), IDL (k(0,30)) and LDL (k(0,40)). ApoB100 transfer to higher density lipoproteins occurs by delipidation for VLDL1 (k(20,10), k(11,10)), VLDL2 (k(30,20), k(40,20)) and IDL (k(40,30)). The use of more complex

models did not provide significant improvement in the fitting from F test and Akaike information criterion (Pont et al., 1998). A forcing function, corresponding to the time course of plasma leucine enrichment, was used to drive the appearance of leucine tracer into apoB100 of the different lipoprotein fractions (Maugeais et al., 2001).

For comparison between the three groups (controls and diabetics subjects before and after treatment) the VLDL1, VLDL2 and VLDLR data were presented as VLDL delipidation rate and VLDL fractional catabolic rate (FCR), which represents the sum of delipidation and direct removal rate. The VLDL delipidation rate was calculated as VLDL2 delipidation flux divided by total VLDL mass. The VLDL direct removal was calculated as flux of VLDL1, VLDL2 and VLDLR direct removal divided by total VLDL mass.

As all our patients are obese, pools of apoB100 in plasma or in VLDL, IDL and LDL were calculated by multiplying the apoB100 concentration by 0.037 (L/kg) assuming a plasma volume of 3.7% of body weight (Dagher et al., 1965). The apoB100 production rate (PR) in mg/kg/h represents the product of FCR and pool size of apoB100 in lipoprotein fractions.

Statistical analysis

Results are reported as mean ± standard deviation. The Mann-Whitney-test, performed with Statview F-4.5 (Abacus Concept, Berkeley, CA, USA) was used. A two-tailed probability level of 0.05 or less was accepted as statistically significant.

Results

Lipoproteins, lipids and apolipoproteins

Concentrations of lipids and apoB100 in plasma and in lipoprotein fractions are shown in Tables 1 and 2 for control subjects and diabetic patients before and after atorvastatin treatment. Patients before treatment compared to controls showed hypertriglyceridemia (340±167mg/dL, $p<0.05$) and hypercholesterolemia (269.7±31mg/dL, $p<0.05$). These diabetic patients showed higher total apoB100 concentration than controls ($P<0.005$). This difference was explained by a higher concentration in the three studied lipoproteins. In VLDL, higher ($P<0.005$) apoB100 content in diabetic patients was related to an increased content in both VLDL1 ($P<0.005$) and VLDL2 ($P<0.005$). No difference was observed in apolipoprotein AI between diabetic patients and controls.

Treatment with atorvastatin significantly decreased triglyceridemia (246.4±116.4 vs 340.0±167.0 mg/dL, $P<0.05$) and cholesterolemia (189.3±49.4 vs 269.7±31.0 $mg.dL^{-1}$, $P<0.05$). Concentration of apoB100-VLDL decreased by 30% ($P<0.05$). This decrease was significant in VLDL1 ($P<0.05$) and VLDL2 ($P<0.05$). Treatment also significantly decreased IDL and LDL apoB100 concentration, respectively by 30% ($P<0.05$) and 40% ($P<0.05$).

Routine biochemical and hematological examinations were unaffected by atorvastatin administration (not shown), with no change in Body mass index, fasting plasma glucose, HOMA or hemoglobin A1c concentrations.

Lipoproteins kinetics

Representative curves for a control (subject C1), and for a diabetic patient (subject D2) before and after atorvastatin treatment are shown in figure 2. Model fitted lines and experimental points showed close agreement. Values of B100 masses in each compartment calculated with the model from steady state were in close agreement with chemically measured values (differences between predicted and measured values were of 5 to 20%, data not shown). Methods provided identified values ± standard deviation as obtained by iterative least squares fitting for individual kinetic parameters. Standards deviation were less than 30% for most of the parameters (data not shown).

At baseline a dramatic increase of total apoB100 production was observed in type 2 diabetic patients compared to controls (240% of controls, P<0.005) (Table 3). This was related to a higher apoB100-VLDL production (*P*<0.005), with no change for other lipoproteins. VLDL FCR was lower (67% of the controls but not significantly) related to decreased delipidation rate (one third of the controls, *P*<0.005). IDL FCR was also decreased (*P*<0.005) related to a lower delipidation rate (*P*<0.05). In contrast to low LDL apoB100 FCR (57% of the controls, *P*<0.05), the diabetic patients had no consistent perturbations in LDL-apoB100 production compared to controls (increased in 2 subjects, with no change in two subjects and decreased in 3 subjects).

Treatment with atorvastatin significantly reduced overall production of apoB100 by 20% (0.91±0.41 vs 1.17±0.59 mg/kg/h, P<0.05) (Table 3). This decrease was related to VLDL-apoB100 production (*P*<0.05) with no significant change in direct production for IDL and LDL. The most dramatic change induced by atorvastatin treatment was the normalization of IDL and LDL FCR (2 fold increase compared to baseline, *P*<0.05) with no change of VLDL FCR or

delipidation rate. The increase of IDL FCR was related to significant increase of delipidation rate to LDL (P<0.05).

Discussion

This study is the first kinetic analysis of effect of atorvastatin on apoB100 metabolism in type 2 diabetes. Analyses were performed using stable isotopes taking into account heterogeneity of VLDL and comparisons were made to matched controls. The model used in this study is consistent as shown by a low coefficient of variation for most of the individual parameters. The principal conclusion of this study is that in type 2 diabetic atorvastatin lowered triglyceridemia by lowering apoB100 VLDL production and cholesterolemia essentially by stimulating LDL uptake.

Effect of diabetes on apoB100 metabolism

Studied type 2 diabetic patients showed hypertriglyceridemia related to an increased VLDL concentration (both VLDL1 and VLDL2). This was linked to a higher VLDL production rate in all the patients and lower delipidation rate of VLDL resulting in a low total FCR of VLDL in most of the subjects. Previous kinetic studies have shown increased VLDL production rate in type 2 diabetes (Kissebah et al., 1982, Cumming et al., 1995). In rat hepatocytes insulin inhibits apoB100 secretion by stimulating degradation of newly synthesized apoB100 (Sparks et al., 1990). Then, hepatic VLDL-apoB100 overproduction in type 2 diabetes could result from impaired action of insulin. In animal models, resistance to insulin action increased intracellular stability of nascent apoB100 and enhanced expression of MTP (microsomal triglyceride transfer protein), which facilitated the assembly and secretion of apoB100-containing lipoprotein particles (Taghibiglou et al., 2000).

The observed decrease in VLDL delipidation rate compared to controls results in normal (Cummings et al., 1995) or lower (Kissebah et al., 1982) FCR of VLDL. The decrease of VLDL delipidation rate reported in type 2 diabetes (Taskinen et al., 1990) is related to insulin resistance (Eckel et al., 1989). Another possible cause could be the physical changes of VLDL making these lipoproteins a poor substrate for LPL (lipoprotein lipase) (Saheki et al., 1993).

Hypercholesterolemia was related to a high concentration of LDL in all the patients. This high concentration of LDL apoB100 was induced by a low FCR of LDL in most of the patients compared to controls. This decrease of LDL FCR already reported (Howard et al., 1987) could be explained by an insulin stimulating effect on LDL receptor activity (Taskinen et al., 1990) and a glycosylation of LDL particles that can alter binding to these receptors (Hiramatsu et al., 1985).

In type 2 diabetic patients, compared to controls, VLDL was less channeled towards conversion into LDL than direct removal (data not shown) as previously reported in other studies (Taskinen et al., 1990, Howard et al., 1987). This is related to the decreased VLDL delipidation rate discussed above. Such a situation could explain the lack of high concentrations of LDL frequently observed (Howard et al., 1987) in spite of low LDL catabolism. Both perturbations with contradictory effects on LDL concentrations could explain the inconsistent increase of LDL amount reported in type 2 diabetes.

Effect of atorvastatin on apoB100 metabolism in diabetic subjects

Atorvastatin treatment lowered VLDL total concentration by lowering VLDL production rate with no change in FCR. The low VLDL delipidation rate did not increase with treatment. This process, catalyzed by LPL (Olivecrona et

al., 1995), appears to be associated to insulin resistance which was not improved by the treatment as seen by the HOMA index which did not change after treatment.

Atorvastatin decreased production rate of VLDL apoB100 in spite of insulin resistance. The production rate of VLDL is controlled by liver cholesterol availability and particularly from de novo synthesis (Watts et al., 1995) through possible effect on translocation, intracellular apoB100 degradation, ACAT activity and MTP activity (Huff et al., 1997). Statins through their effects on HMG-CoA reductase inhibition decrease the intrahepatic free cholesterol concentration. It has been previously reported in cellular models that atorvastatin but not simvastatin decreased the production of VLDL apoB100 (Wilcox et al., 1999). This effect could be only observed for the most potent statins because it was shown in animals that the magnitude of decrease in hepatic VLDL apoB100 secretion is determined by the extent of HMG-CoA reductase inhibition (Burnett et al., 1999). The mechanisms possibly involved a defect of translocation of apoB100 into the lumen of the endoplasmic reticulum, increasing by this way the amount of apoB100 degraded within the cell (Mohammadi et al., 1998). Atorvastatin could decrease cholesterol synthesis and availability of cholesterol and cholesterol ester for the normal assembly of apoB100 containing lipoprotein particles. Using lovastatin (Cuchel et al., 1997, Arad et al., 1990) and Pravastatin (Vega et al., 1990), it was shown a decrease in VLDL production. However, it has recently been reported no atorvastatin effect on VLDL production rate in moderate combined hyperlipidemic subjects (Forster et al., 2002) and insulin resistant patients with no diabetes and less marked VLDL overproduction (Chan et al., 2002). These apparently conflicting

results could come from studying patients with different metabolic profiles, and specially different level of VLDL production (Illingworth, 1991, Aguilar-Salinas et al., 1998).

Opposite results were found for effect of treatment on delipidation rate of IDL and VLDL. Delipidation process of IDL is catalyzed by hepatic lipase (Olivecrona et al., 1995) and was normalized by treatment. As discussed above, activity of lipoprotein lipase appears to be modulated by insulin resistance activity but hepatic lipase could be independent of insulin action and essentially modulated by changes of IDL composition. Whatever the mechanisms of the contradictory effects of atorvastatin on delipidation process, our results suggest that delipidation rates of VLDL and IDL are not regulated by the similar pathways, as previously reported (Peinado-Onsurbe et al., 1992).

The main change induced by the treatment concerned LDL. Treatment with a statin is reported to decrease LDL production rate and/or to increase LDL catabolism. For example, in combined hyperlipidemia (Cuchel et al., 1997), lovastatin decreased LDL production rate without change of catabolism. In another form of familial combined hyperlipidemia (Aguilar-Salinas et al., 1997), pravastatin increased LDL FCR with no change of production rate. The same result was reported in normolipidemic volunteers during simvastatin treatment (Malmendier et al., 1989). This inconsistency could result from the dose used (and potency of the statin used) and from the studied pathological situation (Aguilar-Salinas et al., 1998). In the present study atorvastatin normalized LDL concentration by normalizing FCR through stimulating LDL receptor activity as previously demonstrated with simvastatin (Malmendier et al., 1989).

In conclusion, our kinetic data demonstrated the various effects of atorvastatin on dyslipidemia in type 2 diabetes and underlined the usefulness of this treatment even in case of increase of triglycerides and moderate increase of LDL cholesterol.

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Footnotes :

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Legends for Figures :

Figure 1 : Model of apolipoprotein B100 containing-lipoprotein metabolism. Details are described in “Methods”.

Figure2 : Time course of enrichment of plasma leucine (-) and apolipoprotein B100 in representative control (subject C1, A) and representative diabetic (subject D2) before (B) and after atorvastatin treatment (C) for VLDL1 (□), VLDL2 (*), IDL (O) and LDL (+).

Table 1 : Clinical characteristics of controls (C) and diabetics (D) before (Diabetics I) and after treatment (Diabetics II)

Subjet	age y	sex	BMI kg/m²	FPG mmol/L	HbA1c %	HOMA	TC mg/dL	TG mg/dL	LDL-C mg/dL	HDL-C mg/dL	AI mg/dL	B100 mg/dL
Diabetics I												
D1	47	F	35,2	7,8	7,1	2,6	263,0	670,0	135,8	43,5	146,0	99,43
D2	57	F	28,4	12,4	10,2	3,7	289,0	408,0	150,6	80,3	149,0	92,10
D3	58	M	32,0	6,1	7,6	3,1	272,0	248,0	185,1	35,3	93,0	117,43
D4	57	F	33,2	12,3	8,9	10,0	264,0	169,0	192,1	41,1	93,0	123,13
D5	50	F	35,7	9,4	7,2	7,0	321,0	235,0	207,4	52,7	151,0	120,78
D6	65	F	30,1	7,6	7,8	4,0	260,0	373,0	150,2	37,1	134,0	92,32
D7	50	M	27,4	10,9	8,1	6,4	219,0	277,0	133,2	39,3	125,0	86,74
mean	54,86		31,7	9,50	8,13	5,26	269,71	340,00	164,93	47,03	127,29	104,56
sd	6,20		3,2	2,46	1,10	2,66	30,96	166,99	29,52	15,70	25,12	15,40
Diabetics II												
D1	47	F	35,2	7,2	7,3	2,6	155,0	428,0	46,2	38,6	147,0	50,89
D2	57	F	28,4	17,0	11,0	11,1	197,0	378,0	61,5	86,6	146,0	65,91
D3	58	M	32,0	5,3	7,0	3,2	192,0	266,0	102,9	42,5	115,0	69,28
D4	57	F	33,2	12,5	0,2	11,5	143,0	127,0	61,6	68,3	84,0	64,19
D5	50	F	35,7	9,3	7,5	5,5	273,0	148,0	161,1	51,5	153,0	98,25
D6	65	F	30,1	8,3	8,2	4,2	137,0	193,0	69,1	34,8	127,0	52,77
D7	50	M	27,4	13,8	8,2	3,7	228,0	185,0	109,8	55,6	126,0	73,58
mean	54,9		31,7	10,5	7,1	6,0	189,3*	246,4*	87,4*	54,0	128,3	67,8*
sd	6,2		3,2	4,1	3,3	3,8	49,4	116,4	39,9	18,3	23,9	15,8
Controls												
C1	46	F	30,0	4,4	4,8	1,2	185,0	99,8	122,5	33,3	122,0	68,0
C2	48	M	31,6	4,4	4,9	1,7	139,0	105,0	64,4	28,0	110,0	51,8
C3	47	M	29,6	5,7	4,3	3,9	198,0	78,0	107,9	36,4	111,0	57,9
C4	40	M	29,6	5,9	5,3	1,8	222,0	64,0	121,2	69,2	211,0	61,0
C5	51	M	33,5	6,2	5,0	2,1	234,0	117,0	129,8	36,0	116,0	73,2
Mean	46,4		30,9	5,3##	4,9#	2,1#	195,6 #	92,8 #	109,2##	40,6	134,0	62,4##
sd	4,0		1,7	0,8	0,4	1,0	37,1	21,4	26,2	16,3	43,3	8,4

FPG : Fasting blood glucose, HOMA, Homeostasis model assessment, TG : Triglycerides. TC : Total Cholesterol

* comparison between diabetic before and after treatment, * : p<0.05

comparison between diabetic and controls, # : p<0.05,# :p<0.005

Table 2 : Lipoprotein apolipoprotein B100 concentration (mg/L) in controls (C) and diabetics (D) before (Diabetics I) and after (Diabetics II) atorvastatin treatment .

	B-VLDL	B-VLDL1	B-VLDL2	B-IDL	B-LDL
Diabetics I					
D1	243,2	145,1	98,2	51,2	699,8
D2	176,3	93,3	83,0	63,5	681,2
D3	172,8	88,4	84,4	80,4	921,1
D4	125,0	59,9	65,1	84,8	1021,5
D5	124,3	53,3	71,0	96,0	987,5
D6	166,2	93,0	73,2	76,1	680,9
D7	129,6	69,6	60,0	57,5	680,3
Mean	162,5	86,1	76,4	72,8	810,3
sd	42,4	30,6	13,0	16,0	158,5
Diabetics II					
D1	145,2	62,0	83,2	34,4	329,3
D2	132,6	69,1	63,5	55,9	470,6
D3	124,3	64,3	60,0	53,7	514,8
D4	101,1	46,5	54,6	50,5	490,3
D5	101,3	47,1	54,2	75,4	805,8
D6	112,7	62,2	50,5	48,8	366,2
D7	95,2	52,2	43,0	32,8	607,8
Mean	116,1*	57,6*	58,4*	50,2 *	512,1 *
sd	18,6	9,0	12,7	14,3	159,3
Controls					
C1	30,0	13,5	16,5	43,6	606,2
C2	27,1	12,2	14,9	48,2	442,6
C3	42,5	19,1	23,4	21,0	515,7
C4	35,5	16,0	19,5	41,5	533,0
C5	47,2	21,3	26,0	48,6	478,5
Mean	36,5 ##	16,4 ##	20,1 ##	40,6 ##	515,2 ##
sd	8,4	3,8	4,6	11,4	61,7

* Comparison between diabetics before and after treatment, * : $p<0.05$

Comparison between diabetics at baseline and controls, # : $p<0.05$, ## :$p<0.005$

Table 3 : Kinetic data of apoB100 containing lipoproteins in controls and diabetic patients before (Diabetics I) and after treatment (Diabetics II).

	VLDL			IDL				LDL		
	TPR	FCR	DR	TPR	DPR	FCR	DR	TPR	DPR	FCR
Diabetics I										
D1	0,65	0,06	0,01	0,12	0,04	0,05	0,00	0,08	0,09	0,003
D2	0,77	0,11	0,07	0,29	0,07	0,11	0,05	0,48	0,00	0,017
D3	1,55	0,22	0,05	0,46	0,15	0,14	0,01	0,31	0,29	0,008
D4	0,67	0,13	0,08	0,35	0,10	0,10	0,10	0,56	0,10	0,013
D5	1,91	0,39	0,12	0,37	0,14	0,10	0,10	0,71	0,03	0,018
D6	0,70	0,11	0,10	0,55	0,00	0,19	0,11	0,48	0,06	0,018
D7	0,65	0,13	0,05	0,28	0,08	0,13	0,00	0,13	0,16	0,005
Mean	0,99	0,16	0,07	0,35	0,08	0,12	0,05	0,39	0,10	0,012
Sd	0,52	0,11	0,04	0,14	0,05	0,04	0,05	0,23	0,10	0,006
Diabetics II										
D1	0,43	0,07	0,05	0,29	0,01	0,20	0,15	0,28	0,04	0,020
D2	0,68	0,13	0,07	0,29	0,04	0,13	0,09	0,34	0,08	0,018
D3	1,11	0,22	0,07	0,53	0,07	0,24	0,04	0,25	0,20	0,012
D4	0,63	0,16	0,15	0,61	0,03	0,30	0,27	0,56	0,11	0,029
D5	1,44	0,37	0,07	0,50	0,02	0,17	0,11	0,49	0,13	0,016
D6	0,52	0,12	0,10	0,33	0,06	0,17	0,17	0,45	0,00	0,031
D7	0,48	0,13	0,12	0,24	0,04	0,19	0,19	0,68	0,26	0,029
Mean	0,75 *	0,17	0,09	0,40	0,04	0,20*	0,14*	0,44	0,12	0,022*
Sd	0,38	0,10	0,03	0,14	0,02	0,06	0,08	0,16	0,09	0,007
Controls										
C1	0,37	0,34	0,29	0,41	0,04	0,25	0,20	0,38	0,00	0,017
C2	0,28	0,28	0,23	0,45	0,06	0,25	0,19	0,34	0,05	0,021
C3	0,27	0,17	0,14	0,27	0,00	0,35	0,26	0,48	0,24	0,025
C4	0,25	0,19	0,17	0,37	0,07	0,24	0,20	0,57	0,32	0,029
C5	0,35	0,20	0,18	0,31	0,00	0,17	0,17	0,33	0,03	0,014
Mean	0,30 ##	0,24	0,20##	0,36	0,04	0,25##	0,20#	0,42	0,13	0,021 #
Sd	0,05	0,07	0,06	0,07	0,03	0,06	0,03	0,10	0,14	0,006

TPR: Total production rate (mg/kg/h), DR : Delipidation rate (h^{-1}), DPR : Direct production rate (mg/kg/h),

FCR : Fractional catabolic rate (h^{-1})

* Comparison between diabetics before and after treatment, * : $p<0.05$

Comparison between diabetics at baseline and controls, # :$p<0.05$, ## : $p<0.005$

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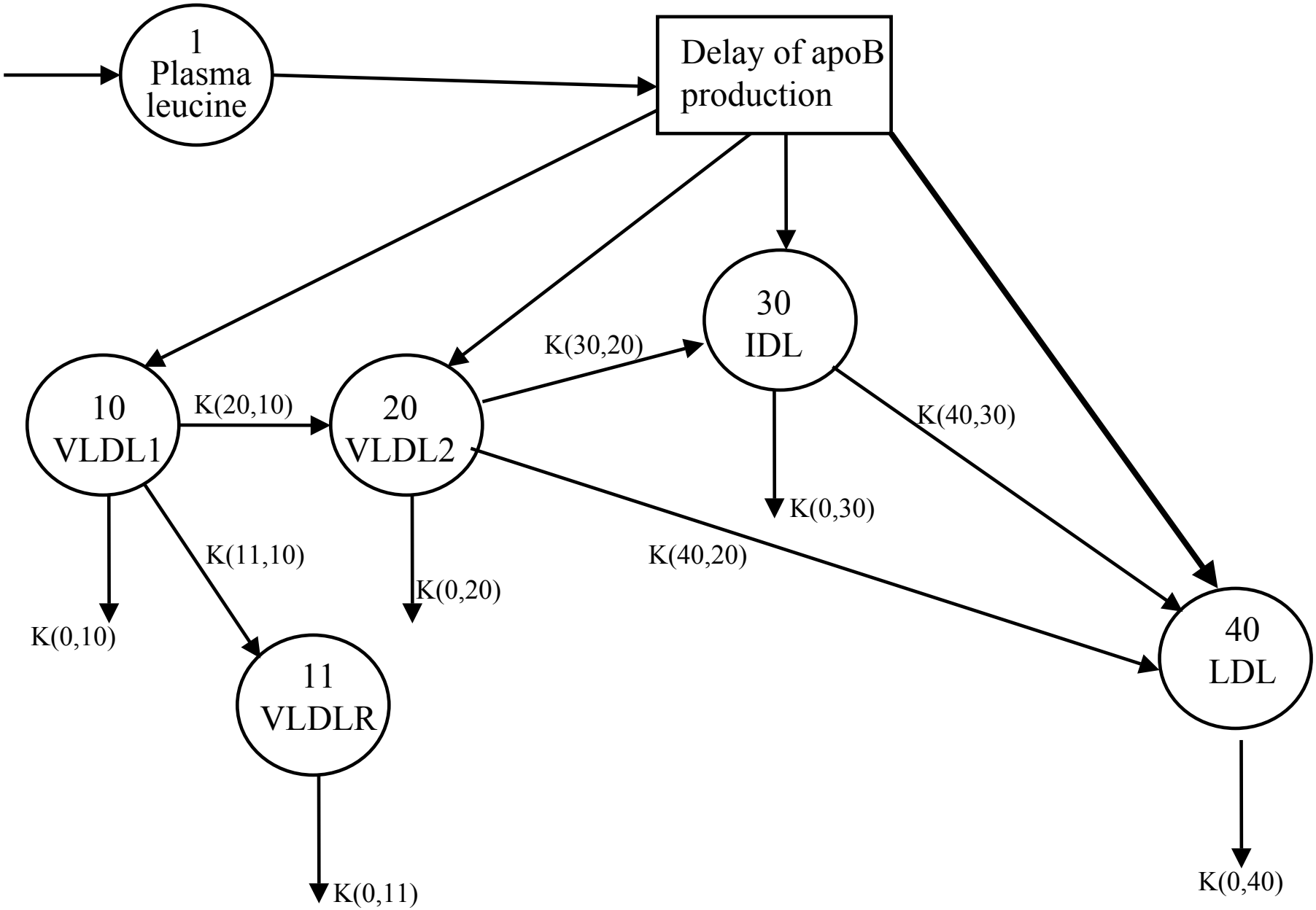
1
Plasma
leucine
Delay of apoB
production
10
VLDL1
K(20,10)
20
VLDL2
K(30,20)
30
IDL
K(40,30)
K(0,30)
K(40,20)
K(11,10)
K(0,20)
K(0,10)
11
VLDLR
40
LDL
K(0,11)
K(0,40)

Figure 2