Dedicated to
Professor Jakub Gutenbaum
on his 70th birthday

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Kinetic analysis of LDL apoB transport and metabolism in non-steady states

by

Andrzej Weryński[1], Zbigniew Nahorski[2], Bo Angelin[3], Lars Berglund[4] and Mats Eriksson[3]

[1] Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences
[2] Systems Research Institute, Polish Academy of Sciences *and* University of Information Technology and Management
[3] Department of Medicine, Karolinska Institute, Huddinge University Hospital
[4] Department of Clinical Chemistry, Karolinska Institute, Huddinge University Hospital

Abstract: In this paper a novel kinetic analysis is used for evaluation of the low density lipoprotein (LDL) apoB fractional catabolic rate (FCR) after LDL apheresis which creates a nonsteady state. The formulae for calculation of FCR and LDL rate of appearance (Ra) as functions of time have been derived. Calculations for one normal and one familial hypercholesterolemic (FH) subjects showed that FCR as well as Ra exhibited variations which can be attributed to the circadian rhythm in LDL metabolism. LDL apheresis had a little impact on both Ra and FCR in normal as well as in FH subjects. However, Ra in FH subject was found to be much higher than that in the normal subject.

Keywords: low density lipoprotein (LDL), apheresis, kinetic modelling

1. Introduction

The kinetic analysis of radiolabelled LDL apoB transport and metabolism is a well established and broadly used method, especially for determination of LDL

apoB fractional catabolic rate (FCR). In this analysis a two compartment model comprising of the central compartment in which synthesis, represented by the rate of appearance (Ra), and catabolism (transport to environment) take place. It is also assumed that the peripheral compartment exchanges mass with the central compartment only and LDL is not synthesized there. It is also assumed that the physiological system is in the steady state, i.e. its rate coefficients are constant. To obtain good determination (so called identification) of the rate coefficients with a reasonable number of samples the sampling is performed rather frequently during the first day after injection of the radiolabelled LDL, when the decrease of the labelled LDL concentration (radioactivity) is most rapid, with less frequent sampling in the next days, and once a day sampling in the final days of the study. The rate coefficients (assumed to be constant) are calculated using Matthews analysis with application of the nonlinear regression.

The objective of this study is to use the kinetic analysis for evaluation of FCR after LDL apheresis which creates a nonsteady state and in which the Matthews method is, in principle, not applicable. The performed analysis should allow for comparison of possible FCR variations encountered in regular turnover studies with the post LDL variations reflecting a possible impact of LDL apheresis on FCR.

2. Methods

Theoretical basis. In 1957 Matthews (1957) published a widely known paper on evaluation of transport parameters from experiment with radioiodinated proteins. The important result of the paper was the statement that mamillary compartmental structure (Fig. 1) creates a suitable model for investigation of protein transport and metabolism in many cases. The mamillary compartmental model with a single output from the central compartment is identifiable, i.e. the rate coefficients between the central compartment and the peripheral ones as well as the rate coefficient describing output from the central compartment to the environment (often referred to as fractional catabolic rate, FCR) can be estimated using tracer disappearance data measured in the central compartment. The applicability of the Matthews method is, however, restricted to the situation in which all rate coefficients are constant (not variable in time). This condition is usually fulfilled by systems in the steady state. The limitation of the Matthews method (as well as generally compartmental models with constant parameters in general) to the steady state condition precludes its application to the study of dynamic features of the control system regulating protein synthesis and catabolism.

The limitation of compartmental models with constant parameters was realized by investigators working on the glucose - insulin system, and modifications of one compartment (Steele, 1959) and two compartment model (Radziuk et al., 1974) were proposed, enabling the calculation of variable rates of synthesis and

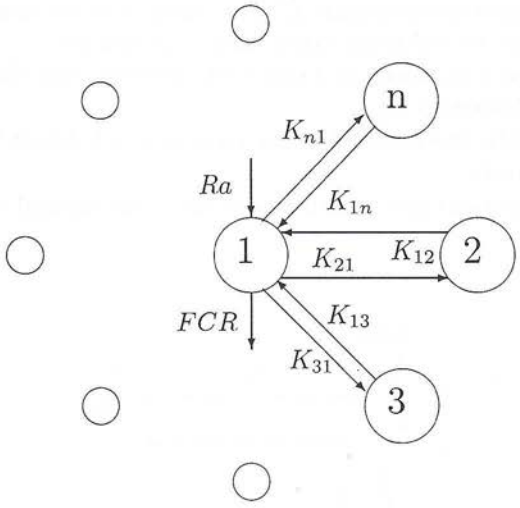

Figure 1. General mamillary compartment model. $K_{12} \ldots K_{1n}$ represent mass transport coefficients from the peripheral compartments $2, \ldots n$ to the central compartment 1. $K_{21} \ldots K_{n1}$ represent rate coefficients from the central compartment 1 to the peripheral compartments $2 \ldots n$. FCR, the fractional catabolic rate, represents mass transport coefficient from the central compartment to the environment.

catabolism.

In this paper we utilized the general idea presented in the reports by Steele (1959) and Radziuk et al. (1974) to develop a modification of the Matthews method suitable for this problem. Appendix I analyzes the general mamillary compartmental model shown in Fig. 1, in which rate coefficients between the central and peripheral compartments are variable or constant and the input to the central compartment (appearance or synthesis rate, Ra) as well as the output rate coefficient from the central compartment to the environment (fractional catabolic rate, FCR) can vary in time.

Evaluation of LDL synthesis and fractional catabolic rates in non-steady-state conditions. The Matthews model restricted to two compartments (Fig. 2) is a widely accepted model for the investigation of LDL transport and metabolism in the steady state (Langer et al., 1972; Packard et al., 1976). This model has been also applied for ^{125}I LDL apoB turnover studies, as was reported previously (Eriksson et al., 1989; Ericsson et al., 1991). Since theoretical analysis has shown that significant increase of FCR as well as Ra may

be expected after LDL apheresis (Weryński et al., 1990) we applied the two compartment model to investigate LDL synthesis and catabolism in non-steady state condition in the following experimental situations:

(i) Disturbance was caused by rapid LDL apheresis (see Experimental procedure and Appendix II).

(ii) Labelled LDL was injected to the patient as a bolus at the termination of LDL apheresis.

Under these conditions the formulas (17) and (18) derived in the Appendix I are applicable.

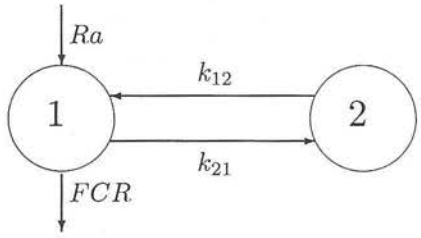

Figure 2. Two compartment model of the LDL apoB transport and metabolism. 1 and 2 represent plasma compartment and extravascular compartments. K_{12}, K_{21} and FCR are the mass transport coefficients between compartments and the fractional catabolic rate, respectively. Ra represents LDL apoB appearance (synthesis) rate.

Calculations. The radioactivity and the specific activity data were smoothed using a moving average technique, i.e. every data point taken for calculation of Ra and FCR using (17) and (18) was calculated as an arithmetic average from three adjacent radioactivity or specific activity data points. The derivatives for a particular time point t_k were calculated using the central difference approximation. The integrals in (17) and (18) were calculated using the linear interpolation between data points. Calculation of the rate constants (microparameters), which may be interpreted as mass transport parameters between the central and the peripheral compartments, K_{12} and K_{21}, as well as FCR in the steady state, $FCR(0)$, were performed using the well known formulas for the two compartment model (Godfrey, 1983) as well as the estimated parameters (macroparameters) of the two exponential function, representing the radioactivity decay curve.

Participants. The study comprised of two participants, one normal control A.K. and one patient, L.M., with heterozygous familial hypercholesterolemia (FH). The diagnosis of FH was confirmed by determination of high affinity

Table 1. Basic data on the participants

	Age [years]	Weight [kg]	Relative body weight* [%]	Plasma lipids Cholesterol [mmol/l]	Plasma lipids Trigliceride [mmol/l]	Clinical remarks
L.M.	47	86	122	9.7	3.1	FH, IHD#
	48	90	128	8.7	3.4	
A.K.	25	75	82	3.7	0.4	normal

*Calculated as $\frac{\text{body weight (kilograms)}}{\text{height (centimeters) - 100}}$

#IHD - ischemic heart disease

iodine-125-labelled LDL degradation in isolated mononuclear blood cells incubated in lipoprotein-deficient serum for three days: the high affinity iodine-125-labelled LDL degradation in the FH patient was only 6% of that in controls (Langer et al., 1972). The clinical data for the subjects are shown in Table 1. The participants were not markedly obese although L.M. had gained in weight between the first and second turnover study. The patient who had evidence of two myocardial infarctions before he was thirty years old, has been on regular treatment with LDL apheresis since 1983. His medication with the beta blocking agents was kept unchanged during the studies.

The participants had no clinical or laboratory evidence of thyroid, hepatic or renal disease or diabetes mellitus.

The ethical aspects of the study were approved by the ethical committee of Karolinska Institute, and informed consent was obtained prior to the studies.

Experimental procedure. The participants were given a standarized diet of the natural type during the study (Einarsson et al., 1985). About 35% of the energy content was supplied as fat, most of which contained saturated fatty acids. The major part of the carbohydrates, which accounted for 45% of the calories, was supplied as starch. The energy intake, calculated from standard food tables, was adjusted to keep the body weight constant. The daily intake of cholesterol was about 0.5 mmol (200 mg). Potassium iodide, 200 mg daily, was given orally 5 days before and during the studies to suppress uptake of radioiodine by the thyroid.

After an overnight fast, about 100 ml blood was drawn into EDTA - containing vacuum tubes, and plasma was obtained after 1000 speed centrifugation at 3 000 rpm in the cold. LDL were prepared by sequential ultracentrifugation, labelled with ^{125}I, and reinjected into the subjects within 5 days of initial sampling (Langer et al., 1972; Kesaniemi and Grundy, 1982). Before the injection of the labelled LDL the participants were treated with LDL apheresis. This treatment was performed in the Transfusion Unit after an overnight fast.

The apheresis was performed using an MA-01 system (Kanegafuchi Chemical Industrial Company Ltd, Osaka, Japan). The plasma separator consisted of polysulfone hollow fibers (Sulflux, Kanegafuchi) with an average pore diameter of 0.2 m and an effective surface area of 0.5 m^2 in a 140 ml chamber. Two columns, each containing 150 ml of cellulose beads covalently bound to dextran sulphate (Liposorber, Kanegafuchi) were used for specific adsorption of apoB-containing lipoproteins (Mabuchi et al., 1987, Eriksson et al., 1989). The treated plasma volume in the patient L.M. was 1100 ml in the first treatment session, and 2895 ml in the second. The treated plasma volume in A.K. was 1900 ml. Before LDL-apheresis the blood was drawn for lipoprotein quantitation (see below). At the end of the LDL apheresis session, labelled LDL (30-60 Ci; see below) were injected and blood samples were drawn at 10 and 20 minutes, and then 2, 4, 6, 8 and 10 hours after injection. Thereafter, daily fasting blood samples were collected (at 8 am) for 13 days. All blood samples were collected in EDTA - containing tubes.

To estimate the rate coefficients in the steady state a standard LDL apoB turnover study (Matthews, 1957; Langer et al., 1972) was performed in the patient L.M. one month before the first treatment with LDL apheresis described above. Briefly, LDL labelled with ^{125}I were injected, and blood samples were then collected (at 8 am) for 12 days. The plasma radioactivity decay curve was used in calculations of the rate coefficients by the standard kinetic analysis (Godfrey, 1983) using Matthews' two compartment model. The plasma volume was calculated as 4.5% of the body weight and corrected for overweight with factors $b = \frac{4500+ac}{(100+c)\times 45}$, where a represents the plasma volume per kilogram adipose tissue (9.7 ml for males), $c = RBW - 100$ is the excess of relative body weight, and $RBW = \frac{\text{body weight in kilograms}}{\text{height in centimeters}-100} \times 100$ (Alexander et al., 1962).

Lipoprotein preparation and radiolabeling. Solid KBr was added to plasma to a density of 1.019 g/ml. This solution was centrifuged in an ultacentrifuge (L5-75, Beckman Instruments, Inc., Palo Alto, CA) for 18 h at 50.000 rpm using a 60 Ti rotor (Beckman Instruments, Inc.). The top fraction was removed by slicing the tube with a tube slicer (Beckman Instruments, Inc.). The infranatent was adjusted to the density 1.063 g/ml by adding solid KBr, and thereafter centrifuged for 18 h at 59.000 rpm in the same rotor. The top fraction containing LDL was washed once by ultracentrifugation at d=1.063 g/ml for 18 h at 59.000 rpm.

The isolated LDL were dialyzed extensively against 0.15 M NaCl with 0.01% EDTA, pH 7.0. The entire isolation procedure was performed at 4^0C.

The isolated LDL were iodinated with ^{125}I Na (obtained from the Radiochemical Centre, Amersham, UK) using the method of McFarlane (1958) as modified for lipoproteins (Bilheimer et al., 1972). Unbound ^{125}I was removed by chromatography on a Sephadex G-25 column (Pharmacia, Sweden) followed by extensive dialysis against 0.15 M NaCl with 0.01% EDTA, pH 7.0, overnight.

The specific activity of the LDL was 150-300 cpm/ng protein; < 2% of the radioactivity was soluble in 10% trichloroacetic acid and < 10% could be extracted with chloroform/methanol (2:1; vol/vol).

The labelled ^{125}I-LDL was sterilized by passage through two 0.45-μm filters (Millipore Continental Water Systems, Bedford, MA). Approximately 30-60 Ci (1.1-2.2 MBq) of LDL (0.5-2mg) were diluted with 5% human albumin and 0.15 NaCl to a total volume of 5 ml, and reinjected into the participants immediately after LDL apheresis.

Lipoprotein quantification and measurement of LDL specific radioactivity. Lipoprotein quantitation using a standarized procedure (Carlsson, 1973) was performed on all occasions when blood was drawn. 4 ml plasma was ultracentrifuged (LKB, Ultraspin 70) for 18 h at 35 000 rpm using a 50 AT rotor (LKB, Sweden). The tubes were sliced and the supernatant as well as the infranatant fractions were analyzed for cholesterol and triglycerides content using standard enzymatic techniques (Nycomed Pharma AS, Norway, Boehringer-Mannheim, Germany).

A portion of the infranatant was treated with phosphotungstic acid/magnesium chloride in order to precipitate apoB containing lipoproteins.

In another portion of the infranatant, the density was adjusted to 1.063 by addition of Kbr. The sample was then ultracenrifuged for 18 h at 35 000 rpm using a 50 Ti rotor. In the supernatant, LDL specific activity was calculated after determination of the protein content (Lowry, 1951) as well as measurement of the radioactivity. It was confirmed by agarose gel electrophoresis (Noble, 1968) that the isolated, radiolabelled fraction corresponded to LDL.

3. Results

The steady state LDL apoB turnover study in the patient L.M. resulted in values of the rate coefficients between the central and the peripheral compartment of $K_{12} = 0.419$ day^{-1} and $K_{21} = 0.135$ day^{-1}. The steady state fractional catabolic rate $FCR(0)$ was 0.217 day^{-1}. These values of K_{12} and K_{21} were used in the subsequent calculations of nonsteady state Ra and FCR in the same patient. In the subject A.K. the values of K_{12} and K_{21} were adopted as average values from 45 LDL turnover studies (Weryński et al., 1999). The nonsteady state was the result of LDL apheresis treatment performed in the patient L.M. about one month after steady state turnover study; the apheresis study was repeated one year later. The LDL apheresis study in subject A.K. was performed only once.

The calculated time courses of LDL apoB synthesis, Ra, and fractional catabolic rate, FCR, after LDL apheresis in the two subjects are presented in Figs. 3 and 4.

During the initial two days after LDL apheresis FCR exhibited large variations. In the final posttreatment days the FCR values - averaged between

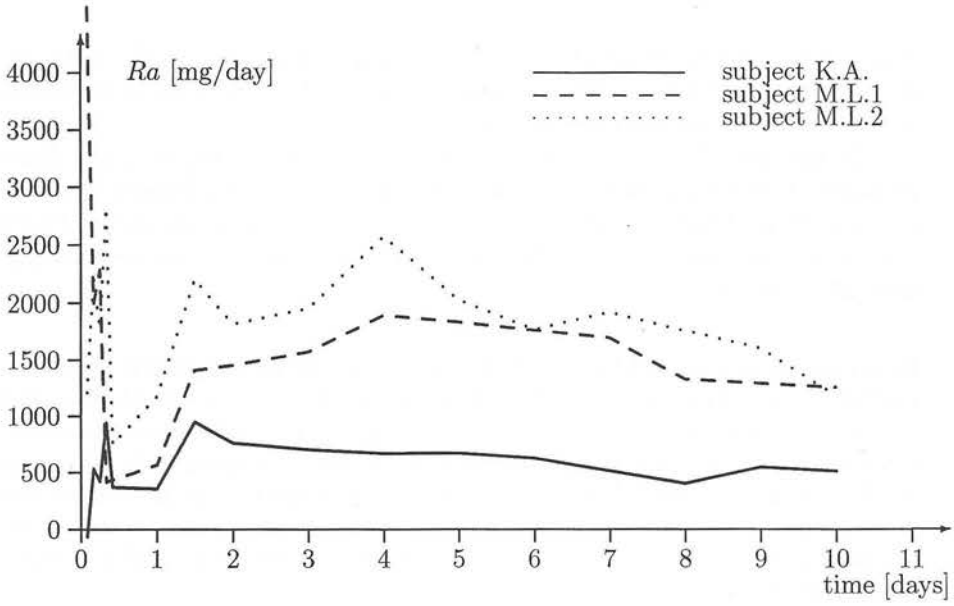

Figure 3. Evolution of the LDL apoB synthesis rate Ra in the post LDL apheresis period. A.K. – control subject. L.M.1 – FH patient, first treatment. L.M.2 – FH patient, second treatment.

the 4th and the 11th posttreatment days - were 0.32 day^{-1} in the normal subject, and 0.25 day^{-1} (first treatment) and 0.27 day^{-1} (second treatment) in the FH patient. These values for the FH patient are only slightly higher than the FCR value obtained for this patient during the steady state LDL apoB turnover study ($FCR(0) \approx 0.22$ day^{-1}). In contrast to the similar behaviour of FCR time courses the time courses of Ra were much more different. In the FH patient Ra reached the maximal values of 1900 mg/day (first treatment) and 2600 mg/day (second treatment), in both cases on the 4th day posttreatment. In the normal subject Ra reached its maximum of 900 mg/day at 1.5 day posttreatment. Thus, there were clear differences in Ra between the FH patient and the control subject, whereas the findings were similar in the two studies performed in the patient L.M.

The steady state LDL apoB synthesis rate, $Ra(0)$, calculated from the steady state LDL apoB turnover study in the patient L.M., as $Ra(0) = m_{1s}FCR(0)$, where m_{1s} is the steady state LDL apoB mass in blood plasma, was $Ra(0) =$ 1460 mg/day. The time averaged value of Ra and Ra_{av} calculated using Ra time course after the first LDL apheresis treatment of the patient L.M. (L.M.1, Fig. 3) was found to be $Ra_{av} = 1510$ mg/day which, surprisingly, is practically equal to $Ra(0)$. Ra_{av} calculated using the Ra time course after the second treatment of the patient L.M. (L.M.2, Fig. 3) was $Ra_{av} = 1730$ mg/day which is 18% higher than $Ra(0)$. However, the second LDL apheresis treatment analyzed in

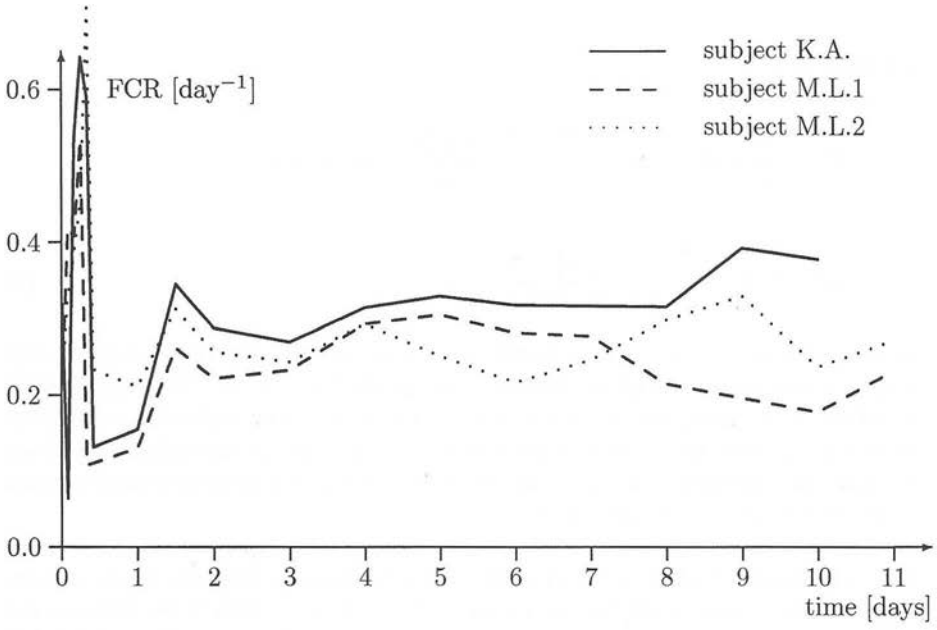

Figure 4. Evolution of the LDL apoB fractional catabolic rate FCR in the post LDL apheresis period. A.K. – control subject. L.M.1 – FH patient, first treatment. L.M.2 – FH patient, second treatment.

the current investigation was separated from the steady state turnover study by about one year.

4. Discussion

Various mathematical models were applied to describe the posttreatment LDL rebound following the LDL apheresis (Franceschini et al., 1991; Weryński et al., 1990; Malchesky et al., 1988, 1990). However, in contrast to the previous studies the results presented in this study attempt to evaluate the dynamic behaviour of LDL apoB synthesis and catabolism rates in response to the disturbance created by LDL apheresis. A programmed or controlled infusion of the tracer, keeping the specific activity constant, could be used for the direct calculation of LDL apoB appearance (synthesis) rate (Ra) in the nonsteady state condition. Controlled tracer infusion, in principle, would allow for the calculation of the Ra time course, which would not depend on the values - constant or variable - of the rate coefficients between the central and peripheral compartments. This approach, which was originally proposed and discussed for a noncompartmental model by Norwich (1973), is based on choosing the time course of the tracer infusion F in such way that a_1 becomes constant. From (12) it is seen that for

a constant a_1

$$R_a = \frac{F}{a_1} + K_{12}(t)e^{-\int_0^t K_{12}(\tau)d\tau}\left(\frac{m_2^*(0)}{a_1} - m_2(0)\right) + \ldots$$

$$+K_{1n}(t)e^{-\int_0^t K_{1n}(\tau)d\tau}\left(\frac{m_n^*(0)}{a_1} - m_n(0)\right) \tag{1}$$

Because $K_{12}(t), \ldots, K_{1n}(t)$ are positive functions of time, the integrals in (1) will decrease with time, and after sufficiently long time $Ra = F/a1$. The experiments in which F is controlled to keep a_1 constant seem to be difficult to perform. However, at least for a short period of time, this type of experiment has been designed and performed to measure the time varying endogenous hepatic output of glucose in the dog (Norwich, 1973).

The modified Matthews method, utilizing a bolus injection of a tracer for the estimation of LDL apoB synthesis and fractional catabolic rates in non-steady state is valid with the assumption that the rate coefficients between the central and peripheral compartments are constant. To verify this assumption we have calculated the rate coefficients using the standard turnover studies in the steady state in patients before and after estrogen treatment (Eriksson et al., 1989). Comparing the changes of the rate coefficients after estrogen treatment to pretreatment values, the following observations can be stated. In all cases FCR showed a substantial increase after chronic estrogen treatment. K_{12} increased in two cases, decreased in two cases and in one case no change was noted. K_{21} increased in three, decreased in one, and showed no substantial change in one case. Similar results have been obtained in a study of the effects of total parenteral nutrition on the LDL rate coefficients (Chait et al., 1981). The latter study involved a different method for estimation of the rate coefficients, in which the coefficient K_{21} was kept constant. However, in these experiments the FCR for LDL apoB increased during total parenteral nutrition in all cases, whereas K_{12} decreased slightly in two cases and increased in three cases (Chait et al., 1981).

These experiments did not show decisively that the coefficients K_{12} and K_{21} are constant. However, in contrast to the consistent changes in one direction of FCR for LDL apoB in the above described investigations, the changes of K_{12} and K_{21} seem to be random. Besides, whereas the changes of FCR may be explained by physiological variations, no physiological explanation for changes in K_{12} and K_{21} can be provided (Chait et al., 1981). The rate constants K_{12} and K_{21} can also be interpreted as stable coefficients of mass transport across microvascular walls, with transport governed by diffusion and convection (Rippe and Haraldsson, 1994). The size of the peripheral compartment can be roughly estimated assuming that this compartment comprises the LDL interstitial fluid pool. Because the ratio of plasma to the interstitial fluid LDL concentration

is about 0.1 and the lipoproteins are excluded from at least 50% of the interstitial space (Sloop et al., 1987), the size of the interstitial LDL pool can be estimated to be about 0.2 - 0.3 of the plasma (central) compartment. If K_{12} and K_{21} represent physiologically interpretable mass transport coefficients, the mass balance equation in steady state, $K_{12}m_2 = K_{21}m_1$ should be fulfilled. In fact the ratio $K_{21}/K_{12} = 0.32$ found in our study is reasonably close to the ratio m_1/m_2 estimated above to be 0.2 - 0.3.

To evaluate the sensitivity of calculations of Ra and FCR to disturbances in the rate coefficients as well as measured data, the estimation of Ra and FCR changes was performed as presented in Appendix III. In this estimation K_{12} and K_{21} were changed by 10%, and the appropriate changes of Ra and FCR were calculated. For all three analysed cases results were very similar, therefore only one case, the second treatment of the patient L.M. (L.M.2) is shown in Fig. 5. The variation in the rate coefficients values influences Ra more than FCR, however, the general pattern of both the Ra and FCR time courses was preserved.

The impact of possible errors in measured data was estimated using Monte Carlo method by adding to the data random deviations of 10% or less (Appendix III). The results of Ra and FCR calculations shown in Fig. 6 suggest that data noise should have rather small impact on Ra and FCR time courses.

FCR displayed an oscillatory pattern in the first two posttreatment days. Interestingly, despite of the fact that LDL receptor function was obviously severely defective in the FH patient, his FCR exhibited a quantitatively similar behaviour to that observed in the normal subject. It should be noted that because samples were taken frequently during the first two days and daily later, the oscillations can be seen only in these days. Very similar pattern was observed in the analysis of the LDL turnover studies which may be explained by the existence of circadian rhythm in the LDL metabolism (Weryński et al., 1999).

The finding of a distinctly elevated Ra and less reduced FCR in the FH patient might be explained by the specific defect in the LDL receptor, which does not allow for the uptake of VLDL and IDL particles. In this case, much smaller amounts of receptor would be sufficient for the effective uptake of LDL particles. However, inefficient removal of VLDL and IDL particles would also result in high LDL appearance as observed in this study. Increased levels of LDL production have indeed been observed in FH (Eriksson et al., 1989; Bilheimer et al., 1979).

Another interesting result of this study is the finding that the average value of Ra after the first LDL apheresis treatment of the FH patient was virtually equal to the value of Ra calculated from the steady state turnover study performed one month before the first LDL apheresis, suggesting that LDL apheresis has no impact on Ra. The second treatment of the FH patient produced an average Ra which was 18% higher than the average value of Ra after the first treatment. However, these two treatments were separated by about a year.

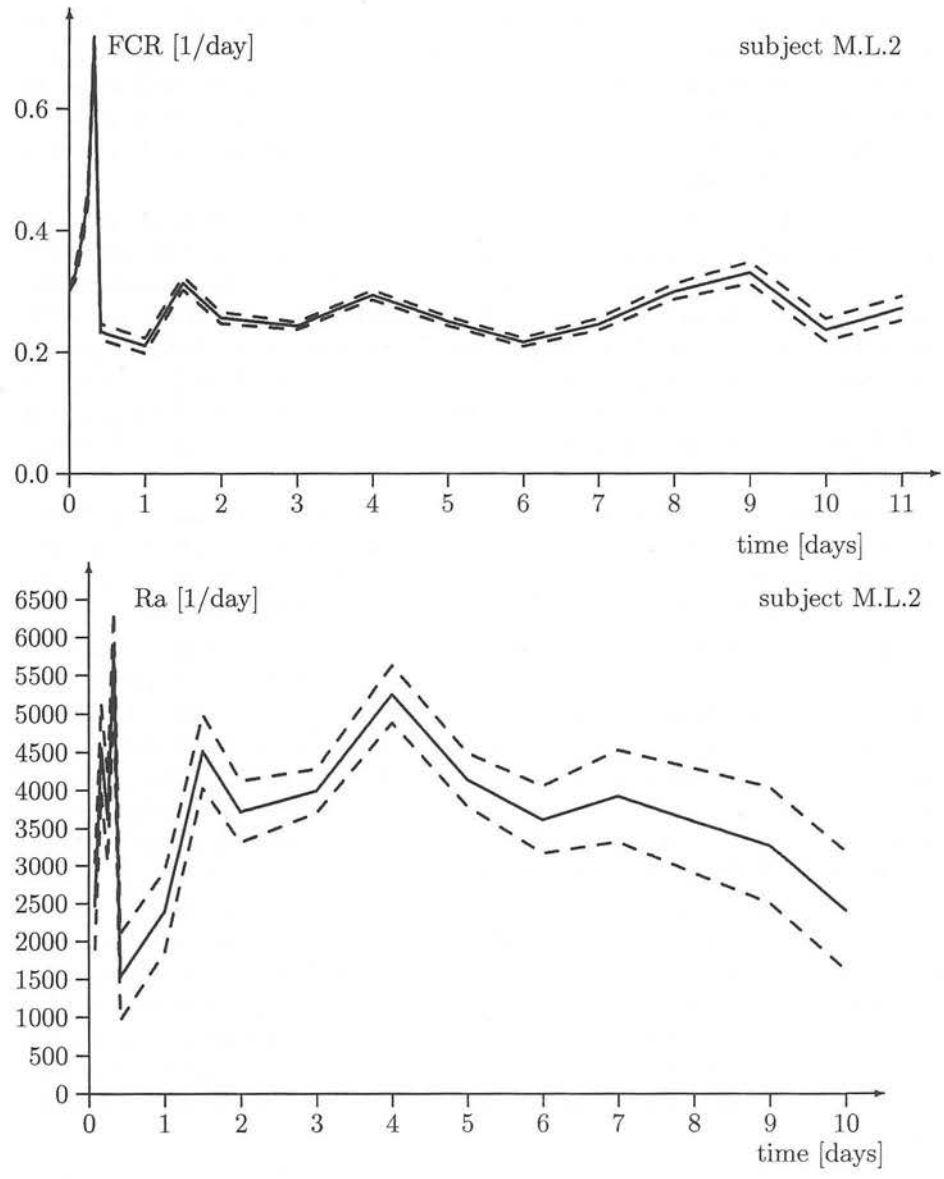

Figure 5. The range of change of LDL apoB fractional catabolic rate FCR and rate of appearance (synthesis) Ra. K_{12} and K_{21} were disturbed by 10%.

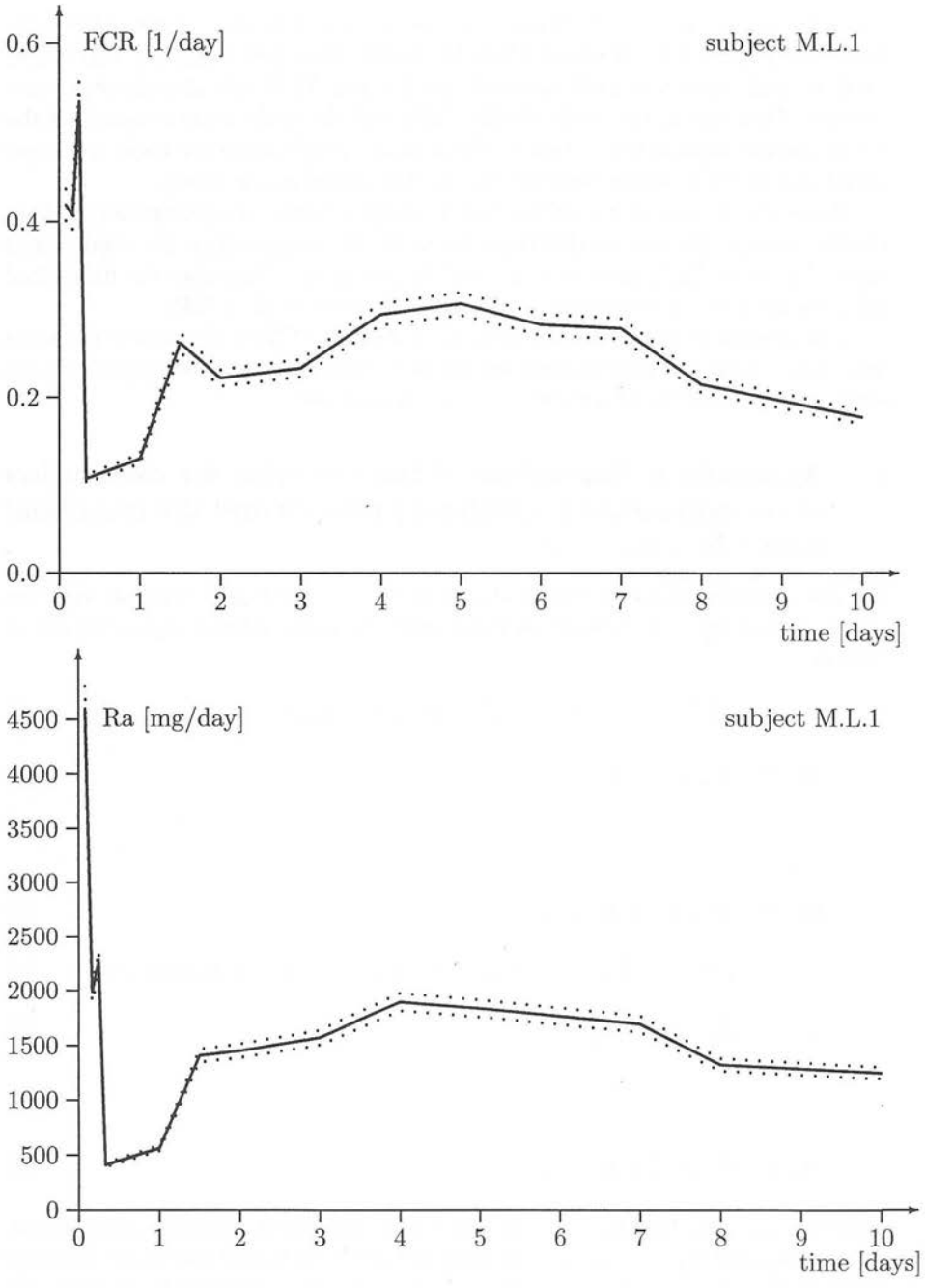

Figure 6. The range of change of LDL apoB fractional catabolic rate FCR and rate of appearance (synthesis) Ra. Measured data were disturbed by 10%.

Also the average FCR values observed in this patient did not differ substantially from the FCR obtained in the steady state (see Results). The rather small overall impact of LDL apheresis on Ra and FCR was also reported previously. Thus, using the radiolabelled LDL and the daily measurements of the urine/plasma radioactivity ratios, Thompson (1980) reported daily averaged variations of FCR which were similar to that found in our study.

Recently, Arends et al. (1993) found, using a stable isotope technique, that on the average there is no difference between Ra measured on the second and eight day after LDL apheresis in healthy subjects. However, for individual subjects substantial differences were noted (Arends et al., 1993).

The presented method of calculation of Ra and FCR in the nonsteady state may have several practical applications and could obviously be applied to the study of the turnover of a variety of plasma proteins.

5. Appendix I. Derivation of the formulae for calculation of the appearance (synthesis) rate Ra and the fractional catabolic rate FCR

For the compartmental structure shown in (1) and the tracer infusion with the known rate F into the central compartment, the mass balance equations are as follows.

$$\dot{m}_1 = -(FCR + K_{21} + ... + K_{n1})m_1 + K_{12}m_2 + ... + K_{1n}m_n + Ra \tag{2}$$

$$\dot{m}_2 = -K_{12}m_2 + K_{21}m_1 \tag{3}$$

$$\vdots$$

$$\dot{m}_n = -K_{1n}m_n + K_{n1}m_1 \tag{4}$$

$$\dot{m}_1^* = -(FCR + K_{21} + ... + K_{n1})m_1^* + K_{12}m_2^* + ... + K_{1n}m_n^* + F \tag{5}$$

$$\dot{m}_2^* = -K_{12}m_2^* + K_{21}m_1^* \tag{6}$$

$$\vdots$$

$$\dot{m}_n^* = -K_{1n}m_n^* + K_{n1}m_1^* \tag{7}$$

where $m_1, \ldots, m_n$ and $m_1^*, \ldots, m_n^*$ are tracee and tracer amounts respectively. $\dot{m}_1, \ldots, \dot{m}_n$ and $\dot{m}_1^*, \ldots, \dot{m}_n^*$ are the time derivatives of tracee and tracer amounts respectively. $K_{12}, \ldots, K_{1n}$ and $K_{21}, \ldots, K_{n1}$ are rate coefficients between the central and peripheral compartments. Ra is the appearance (synthesis) rate (mass/time) of tracee, and FCR is the fractional catabolic rate. Because Ra

and FCR are, in general, functions of time, the system is, in general, in a nonsteady state. From (5):

$$FCR + K_{21} + \cdots + K_{n1} = \frac{K_{12}m_2^* + \cdots + K_{1n}m_n^* + F - \dot{m}_1^*}{m_1^*}. \tag{8}$$

Inserting (8) into (2) one gets

$$Ra = (K_{12}m_2^* + \cdots + K_{1n}m_n^* + F - \dot{m}_1^*)\frac{m_1}{m_1^*} +$$

$$+\dot{m}_1 - K_{12}m_2 - \cdots - K_{1n}m_n.$$

Because for the specific activity $a_1 = \frac{m_1^*}{m_1}$ the following identity holds:

$$\dot{m}_1 - \frac{\dot{m}_1^*}{a_1} = -\frac{\dot{a}_1}{a_1}m_1$$

therefore Ra can be expressed as follows

$$Ra = \frac{F}{a_1} - \frac{\dot{a}_1}{a_1}m_1 + K_{12}(\frac{m_2^*}{a_1} - m_2) + \cdots + K_{1n}(\frac{m_n^*}{a_1} - m_n). \tag{9}$$

Solutions of equations (3) to (4) are as follows.

$$m_2 = m_2(0)e^{-\int_0^t K_{12}(\tau)d\tau} + \int_0^t m_1(\tau)e^{-\int_\tau^t K_{12}(\delta)d\delta} K_{21}(\tau)d\tau \tag{10}$$

$$\vdots$$

$$m_n = m_n(0)e^{-\int_0^t K_{1n}(\tau)d\tau} + \int_0^t m_1(\tau)e^{-\int_\tau^t K_{1n}(\delta)d\delta} K_{n1}(\tau)d\tau \tag{11}$$

where $m_2(0), \ldots, m_n(0)$ are the initial (for $t = 0$) masses of tracee in the peripheral compartments.

For nonzero initial conditions the solutions of the equations describing transport of tracer, (5) to (7), are exactly the same as (10) to (11) except that the masses of the tracer should be denoted with the asterisk. Using these results in (9), the formula for Ra is now as follows:

$$Ra = \frac{F}{a_1} - \frac{\dot{a}_1}{a_1}m_1 + K_{12}(t)e^{-\int_0^t K_{12}(\tau)d\tau}(\frac{m_2^*(0)}{a_1} - m_2(0)) + \ldots$$

$$K_{1n}(t)e^{-\int_0^t K_{1n}(\tau)d\tau}(\frac{m_n^*(0)}{a_1} - m_n(0)) +$$

$$+K_{12}(t)\int_0^t e^{-\int_\tau^t K_{12}(\delta)d\delta} K_{21}(\tau)m_1(\tau)(\frac{a_1(\tau)}{a_1(t)}-1)d\tau+\ldots$$

$$+K_{1n}(t)\int_0^t e^{-\int_\tau^t K_{1n}(\delta)d\delta} K_{n1}(\tau)m_1(\tau)(\frac{a_1(\tau)}{a_1(t)}-1)d\tau. \tag{12}$$

In principle, knowing time courses of the rate coefficients, Ra could be calculated from (12). In real experimental situation, these time courses are very unlikely to be known. In this paper it is assumed that the rate coefficients for LDL transport and metabolism between compartments are constant, which substantially simplifies the formula (12) as is shown below.

Specification of the initial conditions $m_2(0),\ldots,m_n(0)$ and $m_2^*(0),\ldots,m_n^*(0)$ can be done by a proper design of the experiments. In the following, it is assumed that the disturbance (apheresis treatment) produced a rapid change of the mass of the tracee in the central compartment. This change should be so fast that, at the termination of the disturbance, the steady state values of the masses of the tracee in the peripheral compartments did not change substantially. For the two compartment model, suitable for representation of the LDL transport and metabolism, the appropriate considerations are presented in Appendix II. Thus, at the termination of the disturbance ($t=0$) the steady state conditions are still valid:

$$m_2(0)=\frac{m_{1s}K_{21}}{K_{12}},\cdots,m_n(0)=\frac{m_{1s}K_{n1}}{K_{1n}},$$

where m_{1s} is the pretreatment steady state value of m_1.

Also the case should be considered where the infusion of the tracer starts at $t=0$ and the tracer from the previous infusions is absent in the system. In this condition, $m_2^*(0)=m_n^*(0)=0$.

For the experimental conditions as described above, and for constant (time invariant) mass transport coefficients between the peripheral and central compartments, Ra can be expressed as follows:

$$Ra=\frac{F}{a_1}-\frac{\dot{a}_1}{a_1}m_1-m_{1s}(K_{21}e^{-K_{12}t}+\ldots+K_{n1}e^{-K_{1n}t})+$$

$$+\int_0^t m_1(\tau)(\frac{a_1(\tau)}{a_1(t)}-1)\times$$

$$\times(K_{12}K_{21}e^{-K_{12}(t-\tau)}+\ldots K_{1n}K_{n1}e^{-K_{1n}(t-\tau)})d\tau. \tag{13}$$

Applying the same assumptions and the experimental conditions as used to derive (13), solution of (6) to (7) yields:

$$m_2^* = K_{21} \int_0^t m_1^* e^{-K_{12}(t-\tau)} d\tau \tag{14}$$

$$\vdots$$

$$m_n^* = K_{n1} \int_0^t m_1^* e^{-K_{1n}(t-\tau)} d\tau. \tag{15}$$

Inserting equations (14) until (15) into (8) yields:

$$FCR = \frac{1}{m_1^*}(K_{12}K_{21} \int_0^t m_1^* e^{-K_{12}(t-\tau)} d\tau + \ldots$$

$$+K_{1n}K_{n1} \int_0^t m_1^* e^{-K_{1n}(t-\tau)} d\tau) - (K_{21} + \ldots + K_{n1}) - \frac{\dot{m}_1^*}{m_1^*} + \frac{F}{m_1^*}. \tag{16}$$

If a two compartment model is used ($n = 2$) and a bolus injection of the tracer is performed just after the termination of the disturbance ($t = 0$), then $F = 0$ and Ra and FCR can be expressed as follows:

$$Ra = -\frac{\dot{a}_1}{a_1} m_1 - K_{21} m_{1s} e^{-K_{12}t}$$

$$+K_{12}K_{21} \int_0^t m_1(\tau)(\frac{a_1(\tau)}{a_1(t)} - 1) e^{-K_{12}(t-\tau)} d\tau \tag{17}$$

$$FCR = K_{12}K_{21} \int_0^t \frac{m_1^*(\tau)}{m_1^*(t)} e^{-K_{12}(t-\tau)} d\tau - \frac{\dot{m}_1^*}{m_1^*} - K_{21}. \tag{18}$$

6. Appendix II. Evaluation of the change of LDL apoB mass in the peripheral compartment after LDL apheresis treatment

During LDL apheresis the change of the LDL apoB mass in the central compartment, m_1, can be described by the exponential function of the form (Malchesky et al., 1988)

$$m_1 = m_1(0) e^{-\frac{K_E t}{V_P}} \tag{19}$$

where $m_1(0)$ is the LDL apoB mass in the central compartment before LDL apheresis, K_F is the value of the clearance of LDL apheresis, and V_P is the plasma volume.

The change of the LDL apoB mass during LDL apheresis in the peripheral compartment can be calculated using (10) and (19)

$$m_2(T) = m_2(0)e^{-K_{12}T} + m_1(0)K_{21}\int_0^T e^{-\frac{K_F t}{V_P}} e^{-K_{12}(T-t)}dt \tag{20}$$

where T is the LDL apheresis duration time.

Taking into account that in the steady state $m_2(0) = \frac{m_1(0)K_{21}}{K_{12}}$, solution of (20) can be presented in the form

$$\frac{m_2(T)}{m_2(0)} = e^{-K_{12}T}[1 + \frac{K_{12}}{\alpha - K_{12}}(1 - e^{-(\alpha - K_{12})T})] \tag{21}$$

where $\alpha = \frac{K_F}{V_P}$.

Thus, knowing K_{12} from the steady state turnover study as well as LDL apheresis intensity and duration time T the postreatment $m_2(T)$ can be calculated.

In the studied cases K_{12} was found to be lower than 1.32 day^{-1}, T about 2h and α about 12 day^{-1}. Taking in (21) $K_{12} = 1.2$ day^{-1}, $T = 0.83$ day^{-1}, $\alpha = 12$ day^{-1} one obtains $m_2(T) = 0.96m_2(0)$. This means that the assumption that $m_2(T) = m_2(0)$ involves an error of less than 4%.

7. Appendix III. Estimation of Ra and FCR changes in response to disturbances in the rate coefficients and measured data.

A. Disturbances in rate coefficients. The sensitivity of $Ra(t)$ and $FCR(t)$ calculations to changes in K_{12} and K_{21} was evaluated using the sensitivity coefficients as follows:

$$\Delta Ra(t) = |\frac{\partial Ra(t)}{\partial K_{12}}|\Delta K_{12} + |\frac{\partial Ra(t)}{\partial K_{21}}|\Delta K_{21} \tag{22}$$

$$\Delta FCR(t) = |\frac{\partial FCR(t)}{\partial K_{12}}|\Delta K_{12} + |\frac{\partial FCR(t)}{\partial K_{21}}|\Delta K_{21} \tag{23}$$

These changes were calculated for 10% disturbances in K_{12} and K_{21}. In Fig. 5 the range of change of Ra, $Ra(t) - \Delta Ra(t), Ra(t) + \Delta Ra(t)$ and the range of change of FCR, $FCR(t) - \Delta FCR(t), FCR(t) + \Delta FCR(t)$ are shown using dashed lines.

B. Evaluation of disturbances in measured data using Monte Carlo method. For every data point of m_1, m_1^* and a_1 there was a random variation added using a random number generator in the ranges

$$\{m_1 - 0.1m_1, m_1 + 0.1m_1\},$$

$$\{m_1^* - 0.1m_1^*, m_1^* + 0.1m_1^*\},$$

$$\{a_1 - 0.1a_1, a_1 + 0.1a_1\}.$$

After 5000 calculations the mean values of Ra and FCR, denoted $\overline{Ra}$ and $\overline{FCR}$, as well as standard deviations (SD) were determined.

The results of calculations are depicted in Fig. 6 in which the ranges shown using the dotted lines are $\overline{FCR} + 3SD$ and $\overline{Ra} + 3SD$.

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