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ANALYSIS BY A MATHEMATICAL MODEL OF DODECANEDIOIC ACID DISPOSAL IN HUMANS

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Abstract

The disposal of dodecanedioic acid (C12) was investigated in 6 overnight-fasting healthy male volunteers, who received a 165 min i.v. infusion of 42.45 mmol C12 added with 150 μCi (1-12)^{14}C dodecanedioic acid. Blood samples were collected up to 360 min after the start of infusion and concentration of labeled C12 in serum was determined. Expired radioactivity (μCi/min) was measured up to 600 min and at 24 h. The 24 h urinary excretion of C12 was found to be around 5% of administered amount. C12 kinetics was described by assuming a single compartment. A saturable rate of C12 tissue uptake (model A) and a linear rate of tissue uptake (model B) were considered. The kinetics of CO_2 produced by C12 oxidation was described by a fast pathway acting in parallel to a slow pathway modeled by a first order kinetics. The parameters of model B were identified for each subject, whereas the more complex model A was reliably identified by fitting the pooled data of all subjects. When estimated as mean population parameters by model A, the apparent distribution volume was 6.91±0.30 L, the rate constant of urinary excretion was 4.72±0.52 mL/min, and the maximal rate of tissue uptake was 0.38±0.08 mmol/min. The percentage of C12 oxidized was 81.7 ± 9.5% (mean±SD over the 6 subjects) of the administered amount as estimated from the AUC of measured $^{14}CO_2$ expiration rate, and 78.4% as estimated by the model. These results appear to be promising for C12 utilization in parenteral nutrition, because C12 elimination with urine is low whereas tissue uptake and oxidation are rather efficient.

Key words: Dodecanedioic acid, oxidation, kinetic parameters, mathematical model.

1. Introduction

The first evidence of the ω-oxidation of monocarboxylic acids was reported by Verkade and Van der Lee in 1933 (27). After the oral administration of triundecylin, a compound which contains undecanoic acid, the straight saturated monocarboxylic acid with 11 carbon atoms, the homologous dicarboxylic acid was recovered in the urine. The term ω-oxidation was used by these Authors to indicate the oxidation of the methyl group of a monocarboxylic acid to a carboxyl group. After this first observation, many studies have been performed on the fate of dicarboxylic acids, both in experimental animals and in humans (7). Medium-chain dicarboxylic acids (DA, chain length from 6 to 12 carbon atoms) are rapidly β-oxidized in mitochondria as well as in peroxisomes (12,17,20,26). While the odd-chain DAs give acetyl-CoA and, as a terminal product, malonic acid which cannot be further oxidized, the even-chain DAs appear to be completely oxidized (13,16). Succinyl-CoA, that is produced as an intermediate metabolite of even-chain DAs, is a gluconeogenic substrate which can play an important role in those clinical conditions in which glucose metabolism is impaired, such as starvation, sepsis and diabetes mellitus (10).

Among the medium-chain DAs, dodecanedioic acid (C12) seems to be the most suitable for nutritional purposes. In fact, the urinary excretion of C12 is low (3–5% of administered dose) (4,14) compared to azelaic acid (DA with 9 carbon atoms) (1) and sebacic acid (DA with 10 carbon atoms) (2,13), and the energy density is high (7.18 kcal per gram of C12 oxidized) (14). The respiratory quotient of dodecanedioic acid (RQ=0.77) is rather low, this reduced RQ representing an advantage in the patients with respiratory distress, where the CO_2 pulmonary exchange is low with subsequent hypercapnia and acidosis. Furthermore, the free fraction of C12 in plasma is higher than the fraction of long-chain (LCT) and medium-chain (MCT) monocarboxylic acids, due to its relatively high water solubility and its low affinity for albumin binding sites (4). Finally, contrary to both LCT and MCT, dodecanedioic acid does not require hydrolysis prior to cellular utilization.

The kinetic analysis of C12 disposal following an intravenous bolus injection has been recently reported in humans, showing a rather efficient tissue uptake, coupled with low elimination in the urine (4). The kinetics of dodecanedioic acid and the effect of its administration on glucose kinetics in rats (3) showed that dodecanedioic acid can supply glucose precursors and undergoes a rapid tissue uptake to an extent comparable, from the point of view of energy supply, to that of glucose.

In order to verify whether the amount of dodecanedioic acid that can be taken up by the tissues and oxidized to CO_2 is adequate to consider C12 as a suitable fuel substrate in man, experiments of continuous i.v. infusion of labeled and unlabeled dodecanedioic acid were performed in healthy volunteers. For each subject, measurements of C12 plasma concentration, C12 excretion in the 24 h urine, and labeled CO_2 expiration rate were analyzed by means of a mathematical model with a linear rate of C12 tissue uptake. A more complex model with a saturable rate of tissue uptake was identified by fitting the pooled data of all subjects. The analysis gave a maximal rate of C12 tissue uptake and a percentage of oxidation of the amount of dodecanedioic acid taken up by tissues that appear to be adequate for the use of dodecanedioic acid in parenteral nutrition. Moreover, in order to illustrate the essential features of the model used for the analysis of experimental data, some simulations of the model response are presented.

2. Materials and Methods

Chemicals

Dodecanedioic acid and azelaic acid (used as internal standard) were from Sigma Chemical Co. (St. Louis, MO, USA). Dodecanedioic acid was purified by Real S.r.l. (Como, Italy) and was free from pyrogens and contaminants with a degree of purification, ascertained by gas-liquid chromatography and mass spectrometry, of 99.8%. All the other chemicals were purity available quality or of the highest purity available.

A 0.4 M solution of dodecanedioic acid salified with NaOH was used for the intravenous (i.v.) infusion. The solutions were sterilized by 0.25 μm $\oslash$ Millipore filters (Molsheim, France) before administration.

Experimental procedure

An amount of 42.45 mmoles of C12, added with 150 μCi of (1-12)^{14}C dodecanedioic acid (specific activity 117 mCi/mmol), was administered as a 165 min continuous i.v. infusion in 6 overnight-fasting healthy male volunteers aging 51.2±9.5 years (mean±SD) and with an average body mass index (BMI) of 25.5±2.6 Kg/m^2. Heparinized blood samples (3 ml) were taken from 10 up to 360 min at intervals varying from 5 to 20 min and immediately centrifuged. Plasma samples were frozen at -20^{o}C until analysis. Each subject voided before starting the administration of dodecanedioic acid and the 24 h urine was collected in a container with 0.1% sodium azide to prevent bacterial growth.

The protocol used was in conformity to the directives given by the Ethical Committee of the Institutional Health Review Board of the Catholic University, School of Medicine, in Rome (Italy). Informed consent was obtained in all cases.

CO_2 collection

Indirect calorimetry was performed by an open hood system (Delta-track, Datex Instrumentarium, Helsinki, Finland) and the CO_2 production $\dot{V}CO_2$, was automatically computed every 1 min. Indirect calorimetry was started half-hour before and was continued until 600 min after the beginning of the infusion of dodecanedioic acid.

The expired air was collected over 2 min periods at regular intervals of 20-30 min for a total time length of 600 min after the beginning of the infusion of labeled dodecanedioic acid, and a further sample of CO_2 was collected at 24 hours after starting the infusion of dodecanedioic acid. A 20 L Douglas-bag was used.

A 1 M solution of methylbenzethonium hydroxide (MH) in methanol was prepared by adding 20 mL MH to 36 mL ethanol; 4 mL 0.1% phenophthaleine were added as pH indicator. Aliquots of 3 mL each of this solution were placed in graduate tubes and titrated with 0.15 N HCl. The next 9 mL of the solution were transferred into a bubbling apparatus to trap the CO_2 from the Douglas-bag. Following the above procedure, solutions containing 3 mEq of MH were obtained: these solutions trap exactly 3 mmol CO_2 (30). Finally, the MH solution trapping $^{14}CO_2$ was added with 10 mL of 0.4% 2,5-diphenyloxazole (in PPO) toluene in scintillation fluid and counted.

The radioactivity was detected by a β-scintillation counter (Packard Tri-Carb 460C, Downers Grove, IL, USA). Quenching was checked by the internal standard method. The fluxes of $^{14}CO_2$ were calculated by using the values of $\dot{V}CO_2$ measured by indirect calorimetry.

Dicarboxylic acid analysis

Serum samples. Azelaic acid (100 μg) was added to 1 ml of each serum sample as an internal standard. Proteins were precipited with 0.1 ml of 4 N HCl and dicarboxylic acids extracted twice with 8 volumes of ethylacetate maintaining the solution at 60°C for 15 minutes. The combined extracts were dried in a GyroVap apparatus (Howe, mod. GV1, Gio. De Vita, Rome, Italy) operating at 60°C, coupled with a vacuum pump and a gas trap FTS system (Stone Ridge, New York, USA).

Urine samples. Samples (0.5 ml) from 24-hour urine were added with 50 μg azelaic acid as internal standard, and then treated with cation-exchange resin (Dowex 50 W-X4, 100-200 μm mesh, H^+) to remove salts, concentrated under reduced pressure and filtered through a Millipore HV (0.45 μm) filter. The samples were acidified to pH 1–2 with 4 N HCl, extracted twice with ethylacetate and evaporated in the GyroVap as previously described.

HPLC analysis. The HPLC of dicarboxylic acids was performed according to a previously described method (15). Briefly, the extracted solutes were dissolved in 0.5 ml acetonitrile and added to 10 mg of p–bromophenacylbromide and 30 μl of N,N-diisopropylethylamine as catalyst. The mixture was heated to 60°C for 15 minutes. The derivatives were dissolved in a final volume of 1 ml acetonitrile, and an aliquot of 10 μl was automatically injected into a liquid chromatograph (Hewlett-Packard 1050) with a HP 3396A integrator and a scanning spectrophotometer operating in the 190−600 nm wavelength range (light source: deuterium lamp), noise$< 2.5\times10^{-5}$ AU peak-to-peak at 254 nm with 4 nm bandwidth, flowing water at 1 ml/min.

Dicarboxylic acid derivatives were separated on a LC-18, 4.6 mm i.d., 25 cm length, 5 μm particle size, reversed phase column (Supelco Inc., Bellefonte, PA, USA). The HPLC conditions were as follows: solvent A bidistilled water/methanol (1:1, v/v), solvent B acetonitrile; after 15 min isocratic elution with 15% acetonitrile, a gradient elution was performed from 15% to 100% of B in 80 minutes. The flow rate was 1 ml/min; UV detector operating at 255 nm; chart speed 0.2 cm/min; range of absorbance from −0.300 to 1.000 absorbance units (AU).

The eluate of the peak corresponding to the retention time of C12 standard was collected into a vial, added with scintillation fluid and counted as specified above.

Mathematical model of C12 disposal

The kinetics of dodecanedioic acid was described by a one-compartment model with two routes of elimination, renal excretion and tissue uptake. Because C12 binds to albumin, the total C12 concentration in the compartment, c_t, was represented as the sum of a free concentration, c_f, plus a bound concentration. Previous results (4) indicate that C12 binding to human serum albumin follows an apparent one-step kinetics, so the total C12 concentration was written as

$$c_t = c_f + a\frac{Kc_f}{1+Kc_f}\,, \tag{1}$$

where a is the concentration of albumin binding sites and K is the association constant. In *Eq.* 1, K was set equal to 6.4×10^3 M^{-1} according to (4).

The renal excretion rate of C12 was assumed to be linearly related to the concentration of free C12, with an apparent renal clearance ϱ (L/min). For the rate of tissue uptake, assumed to be a function of free C12 concentration, $g(c_f)$, two different forms were considered: a saturable function of the Michaelis-Menten type as in previous papers (1,2,3)

$$g(c_f) = \frac{T_m c_f}{K_M + c_f} \tag{2}$$

where T_m is the maximal rate of tissue uptake (mol/min) and K_M the uptake constant (M), and a linear function

$$g(c_f) = \varrho_t c_f \tag{3}$$

where ϱ_t (L/min) is the rate constant of tissue uptake.

For the kinetics of total C12 concentration we can write the equation

$$V\dot{c}_t(t) = -\varrho c_f(t) - g(c_f(t)) + I(t)\,, \quad c_t(0) = 0\,, \tag{4}$$

where V is the distribution volume (L) and $I(t)$ the infusion rate (mol/min). Considering the equilibrium between free and bound C12 as instantaneous, and using *Eq.* (1), the kinetics of free C12 was thus described by the following equation:

$$V\dot{c}_f(t) = \frac{1}{1+aK/(1+Kc_f(t))^2}\left[-\varrho c_f(t) - g(c_f(t)) + I(t)\right], \quad c_f(0) = 0\,. \tag{5}$$

Since the function g may have the form given by *Eq.* 2 or by *Eq.* 3, in the following we will denote the model with saturable tissue uptake as model A and the model with linear tissue uptake as model B.

Unlabeled C12 was administered together with a labeled fraction (specific activity of administered C12: $\mathrm{SA} = 3.53\,\mathrm{mCi/mol}$) and the radioactivity of C12 in plasma was measured. Thus the quantity

$$y_1(t) = \mathrm{SA} \cdot c_t(t) \tag{6}$$

was taken as model output. We notice that any possible recycling of radioactivity through C12 metabolites was not taken into account in the model because the concentration of labeled dodecanedioic acid in plasma was directly measured. The amount of C12 excreted in the 24 h urine was also measured, and the corresponding quantity given by the model was computed as $\varrho\int_0^{24\,\mathrm{h}} c_f(t)dt$.

We assumed that the production of CO_2 due to C12 oxidation, as well as the transport and excretion of this CO_2 fraction in the expired air, can be represented by a fast pathway, in which the C12 taken up by tissues is instantaneously transformed into CO_2 and excreted in expired air, acting in parallel to a slow pathway simply modeled by a first-order kinetics with time constant τ. Thus, denoting by y_2 the $^{14}CO_2$ expiration rate (mCi/min), which is the measured quantity, we have

$$y_2(t) = \alpha'\, u^\star(t) + \frac{\alpha}{\tau}\int_0^t \mathrm{e}^{-\frac{1}{\tau}(t-s)} u^\star(s)\, ds\,, \tag{7}$$

where $u^\star(t) = \mathrm{SA}\, g(c_f(t))$ (mCi/min) is the amount of ^{14}C taken up by tissues as C12 in the unit time, and α' and α are the fractions of labeled carbon atoms enrouted in the fast and, respectively, in the slow pathway of CO_2 production and elimination. The possibly incomplete C12 oxidation may cause a certain fraction of label to be retained within the body as C12 or other compounds, oxidizable with time constants larger than the time horizon of the experiment. Moreover, labeled carbon atoms can be lost in the urine as compounds other than C12. Therefore, we assumed that the coefficients α and α' in *Eq.* (7) are such that $\alpha+\alpha' \leq 1$. Although the relationship between the fraction of C12 taken up by tissues which is oxidized and the value of $\alpha+\alpha'$ is rather complex, depending on the fate of the different C12 metabolites, the sum $\alpha+\alpha'$ can be taken, in a first approximation, as representative of this fraction.

Estimation of unknown parameters

The identifiability of the unknown parameters of model A (V, a, ϱ, T_m, K_M, α, α', and τ) and model B (V, a, ϱ, ϱ_t, α, α', and τ) was verified by the similarity transformation method (28). The parameters of both models were estimated for each subject by simultaneouly fitting the individual data of labeled C12 concentration and $^{14}CO_2$ expiration rate at the available time points, plus the measurement of the C12 amount excreted in the 24 h urine. Under the assumption that all the measurements had a constant coefficient of variation (CV), a weighted least-squares fit was performed, with weights given by the inverse of the coefficient of variation times the experimental value (11).

The parameters were also estimated as means over the subject population by the naive pooled data approach (9), that is by fitting the pooled data of all subjects. A weighted least-squares index was used, with weights given by the inverses of the sample estimates of the variance of the data at each time.

The least-squares index was minimized by means of a quasi-Newton algorithm with the BFGS updating formula (29), and the standard errors (SE) of the estimates were determined from the inverse Hessian matrix of the index computed at the optimum (22). For the individual estimates, the standard errors were computed assuming a CV of the measurements equal to 0.1.

3. Results

The 24 h urinary excretion of C12 was 5.1±0.8% (mean±SD over the 6 subjects) of the administered amount, corresponding to 2.18±0.35 mmoles. To evaluate the amount of C12 oxidized in each subject, following (18), the ratio between the total radioactivity excreted with the expired air and the amount of labeled C12 infused (percent oxidation, PO) was computed. The total radioactivity excreted was determined by reporting the individual data of $^{14}CO_2$ expiration rate versus time and computing the enclosed area from 0 to 24 h (area under the curve, AUC) by the trapezoidal approximation. The amount of C12 oxidized, expressed as PO, was 81.7±9.5% of the administered amount of C12 and, therefore, 86.1±10.1% of the C12 amount which is not lost in urine.

The fitting of the individual data by model A gave unacceptably high values of the standard errors of parameters T_m and K_M, leading to the conclusion that a Michaelis-Menten tissue uptake could not be reliably identified on the basis of the available individual data. On the contrary, the parameters of model B were estimated with acceptable standard errors and a limited loss of the goodness of fit. The fitting for two subjects is shown in Fig. 1. The values of the parameters for each subject, together with the mean values, are reported in Table 1. The BMI of each subject is also given. It can be observed that the values of some parameters (in particular ϱ_t, α, and the sum $\alpha+\alpha'$) tend to discriminate subjects 1 and 2 (smaller BMI) from subjects 5 and 6 (larger BMI).

The parameters of the model with saturable tissue uptake of C12 (model A) were estimated with acceptable standard errors by fitting the pooled data of all subjects. The estimated parameters are to be intended in this case as direct estimates of the mean parameter values over the subject population. The parameters of model B were also estimated in the same way, obtaining values that substantially agree with the means of the individual estimates reported in Table 1. However, the least-squares index at the optimal values of parameters was larger than for model A (339 *vs.* 323), so that model A was found to be preferable on the basis of both Akaike and Schwarz criteria (11). The population means of model parameters, estimated by model A,

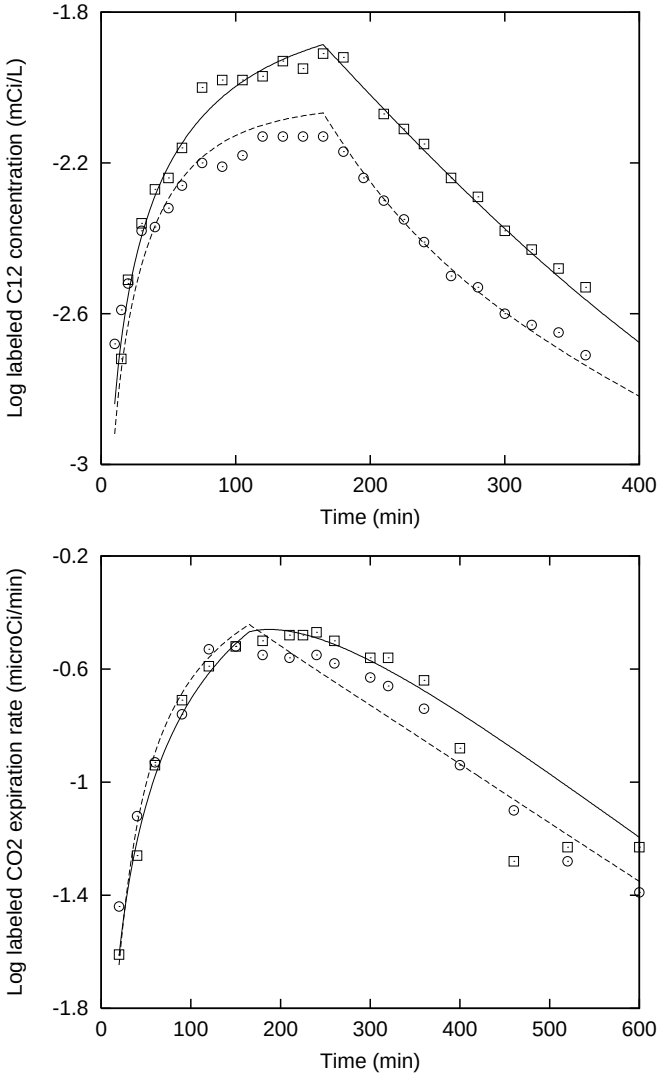

Fig. 1. Experimental data of total concentration of labeled C12 in serum (upper panel) and of $^{14}CO_2$ expiration rate (lower panel) vs. time during and after the labeled C12 infusion in two subjects (squares, subject 1 of Table 1; circles, subject 5 of Table 1). The continuous and dashed lines represents the optimal fitting curves predicted by model B. The parameter values are reported in Table 1.

are reported in Table 2. We can note that the values of parameters V, ϱ, α, α', and τ are in substantial agreement with the means of individual estimates given in Table 1.

Figure 2, panel A, shows the experimental data of the total concentration of labeled C12 in serum, together with the optimal fitting curve given by model A with the parameters reported in Table 2. The amount of C12 excreted in the 24 h urine, as predicted by the model (2.18 mmol), was coincident with the average measured value. In Fig. 2, panel B, the measured values of the $^{14}CO_2$ expiration rate and the best fitting curve are depicted. A rapid increase of the radioactivity in the expired CO_2 was observed, with a prolonged plateau reached before the end of C12 infusion and maintained up to about 300 min. Then the expired radioactivity

Table 1. Estimates (±SE) of the individual kinetic parameters for C12 disposal and CO_2 expiration, obtained by the model with linear tissue uptake (model B).

Parameter	Subjects (BMI)						Mean
	1 (22.7)	2 (23.3)	3 (25.2)	4 (24.5)	5 (28.6)	6 (28.7)	
V, L	6.13±0.20	4.38±0.19	6.74±0.24	6.98±0.28	6.70±0.34	7.41±0.33	6.39
a, mM	0.09±0.07	0.74±0.12	— (⋆)	0.50±0.09	0.74±0.19	0.84±0.12	0.48
ϱ, mL/min	3.29±0.37	3.15±0.36	2.16±0.22	3.51±0.42	7.83±1.31	6.79±1.01	4.45
ϱ_t, mL/min	55.6±5.59	52.1±0.30	46.4±1.16	81.2±5.47	117±16	137±15	81.6
α	0.72±0.04	0.70±0.03	0.67±0.05	0.53±0.03	0.59±0.03	0.53±0.03	0.62
α'	0.18±0.03	0.29±0.03	0.33±0.03	0.24±0.02	0.22±0.02	0.21±0.02	0.24
τ, min	149±22	175±12	208±18	195±14	214±11	163±13	184

(⋆) Estimated a virtually equal to zero

Table 2. Estimates (±SE) of the population means of kinetic parameters for C12 disposal and CO_2 expiration, obtained by the model with nonlinear tissue uptake (model A).

Parameter	Estimate±SE
V, L	6.91 ± 0.30
a, mM	0.87 ± 0.12
ρ, mL/min	4.72 ± 0.52
T_m, mmol/min	0.38 ± 0.08
K_M, mM	3.06 ± 1.08
α	0.59 ± 0.02
α'	0.24 ± 0.02
τ, min	151 ± 11

gradually declined but was still detectable at 24 h (2.35±0.36 nCi/min, data not shown). The total radioactivity excreted with the expired air, computed by the model as $\int_0^{24\,\mathrm{h}} y_2(t)dt$, was found to be equal to 117.6 mCi, that is 78.4% of the infused amount of label.

4. Discussion

Because dodecanedioic acid is an exogenous substrate, the kinetics of labeled C12 has been assumed as representative of the kinetics of unlabeled C12. Therefore, the isotopic label was utilized as a convenient method for measuring the concentration of the compound in plasma (9), and to quantitate its oxidation. The administration of unlabeled dodecanedioic acid at a relatively high infusion rate was intended to give evidence to the possible presence of a saturable mechanism in the C12 tissue uptake.

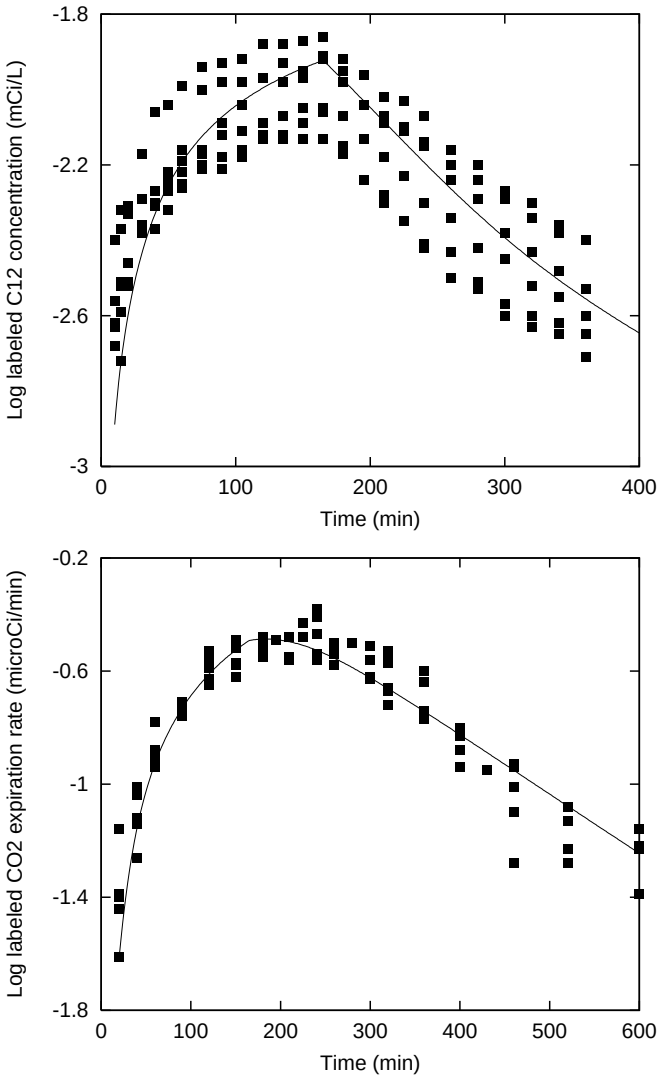

Fig. 2. Experimental data (squares) of total concentration of labeled C12 in serum (upper panel) and of $^{14}CO_2$ expiration rate (lower panel) vs. time during and after the labeled C12 infusion in six subjects. The continuous lines represent the optimal fitting curves predicted by model A following the naive pool approach. The parameter values are reported in Table 2.

In the mathematical model proposed, the kinetics of labeled C12 plasma concentration was described by a simple one-compartment model, in order to reduce the number of unknown parameters, while maintaining an adequate overall fitting of the experimental data. As suggested by literature data on the active transport of mono and dicarboxylic acids across cellular membranes (21,24), we used a saturable function to describe the rate of C12 tissue uptake in model A, as already done in the analysis of kinetic data of azelaic and sebacic acids (1,2). Alternatively, a simpler model with a linear rate of tissue uptake (model B) was considered. In both models A and B, the C12 binding to protein binding sites was taken into account by assuming that these sites are characterized by the binding constant of C12 to serum albumin. The saturable rate of tissue uptake and the protein binding of C12 introduce nonlinearities in model equations.

The effects of these nonlinearities are illustrated by simulations, reported in Figs. 3A and 3B, in which two different infusion rates were used. The main qualitative effects can be observed in the descending branch of the log of total C12 concentration versus time. In the case of linear rate of tissue uptake with no protein binding, the descending branch would be a straight line, but the presence of C12 binding to albumin produces an upward concavity (panel A, dashed curves). On the contrary, when there is no protein binding and the rate of tissue uptake is saturable, the descending branch shows a downward concavity, as seen in the upper dotted curve of panel A. This effect is no longer detectable in the lower dotted curve obtained with the smaller infusion rate, as expected because at low concentrations the Michaelis-Menten term behaves as a linear term. When the model contains the two nonlinearities, the two above effects partially compensate, as shown by the continuous curves. Moreover, the presence of a saturable tissue uptake produces a rounding in the top of the curve of CO_2 expiration rate versus time (see the dotted and continuous curves in panel B). This effect is obviously more clearly detectable when the C12 concentration is higher.

The estimated value of the C12 distribution volume, obtained as mean of the individual estimates (Table 1) or as population estimate (Table 2), is not far from the sum of plasma volume plus the volume of the rapidly equilibrating interstitial water (10.7 L for a 70-kg man, according to (5)). However, our estimate of the distribution volume does not represent strictly a physiological space, because the distribution kinetics was modeled by a single compartment, whose volume will be in general lower than the sum of plasma volume plus the volumes of peripheral compartments. The concentration a of albumin-equivalent binding sites is smaller than the concentration of the albumin binding sites in plasma (see Tables 1 and 2). However, the total amount of binding sites, $aV = 6.01$ mmol, estimated by model A using the pooled data, is comparable to the amount of albumin binding sites in plasma (5.95 mmol, assuming the albumin concentration in plasma equal to 0.6 mM, 3.1 binding sites per molecule (4), and plasma volume equal to 3.2 L). This result suggests a limited extent of C12 binding to interstitial proteins. When a linear tissue uptake was assumed, the estimated amount of binding sites was, on the contrary, smaller than the amount of albumin sites in plasma: this result appears to be an indirect evidence of the presence of a saturable tissue uptake of C12. The low value of the rate constant ρ of C12 excretion in the urine suggests a tubular reabsorption of C12. This finding confirms that the modalities of urinary excretion of dicarboxylic acids change with the chain length. In fact, while azelaic acid is actively secreted, both sebacic acid and C12 are reabsorbed (2,25).

The complex processes of C12 metabolism up to CO_2 production and excretion in expired air have been modeled assuming the coexistence of a fast pathway together with a slow pathway represented as a first-order kinetics. The fast pathway accounts for the observed rapid increase of radioactivity in the expired air after the beginning of the labeled C12 infusion. Two major determinants could account for the slow CO_2 elimination pathway. The large fraction of CO_2 carried by the formation of bicarbonate can represent one determinant of the slow pathway. Moreover, succinic acid, which is a gluconeogenetic precursor, can be derived from C12 β-oxidation and then the labeled glucose released from the liver can be taken up by tissues and oxidized to $^{14}CO_2$. Although the phenomena involved from C12 oxidation to expiration of CO_2 appear to be very complex (the recovery of labeled CO_2 following administration of labeled bicarbonate has been indeed represented in the literature by two- or three-compartment models (8,19)), we chose a simple model which however guaranteed a reasonable fitting of the data.

An incomplete recovery of ^{14}C in the expired CO_2 was also allowed in the model, and the fraction of radiocarbon expired in 24 h predicted by model A (78.4%) is in good agreement with

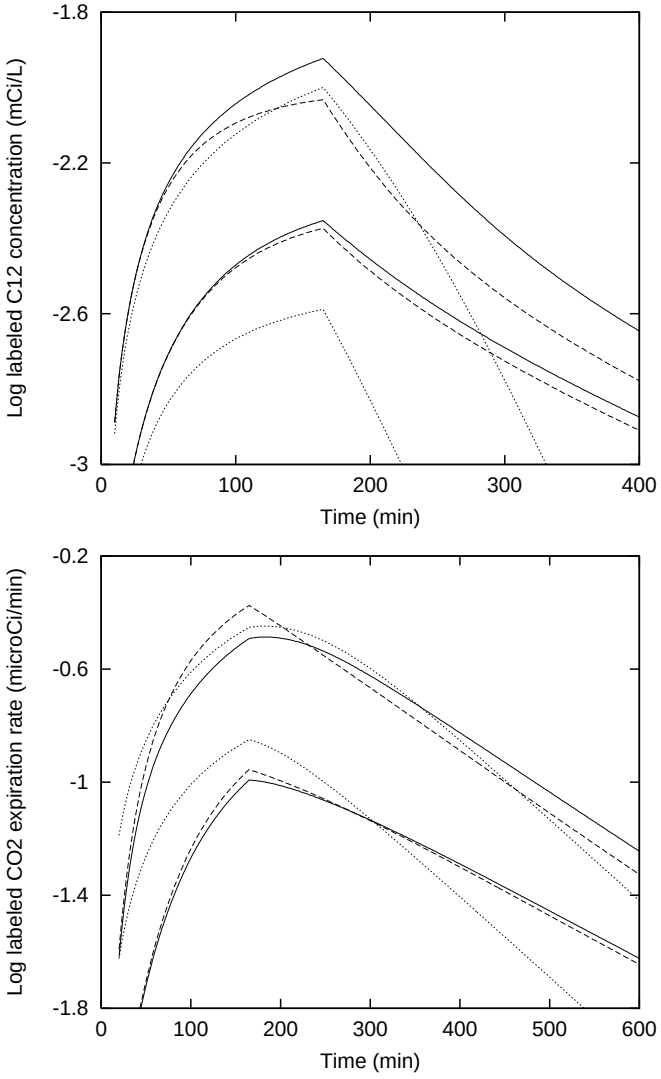

Fig. 3. Simulations of total C12 concentration versus time (upper panel) and of CO_2 expiration rate (lower panel). Upper curves: model A with the parameters of Table 2 and the infusion rate of 0.257 mmol/min (continuous lines); model A with $a = 0$ and the other parameters unchanged (dotted lines); model B with $\varrho_t = T_m/K_M$ (values of Table 2) and the other parameters unchanged (dashed lines). Lower curves: simulations performed as for upper curves except that the infusion rate was 0.086 mmol/min.

the value determined from the AUC of the experimental data of expired radioactivity (81.7%). The sum $\alpha+\alpha'$ was estimated to be smaller than 1 (0.83 by model A and 0.86 by model B) and was in close agreement with the percentage of label, not lost as C12 in the urine, which is expired in 24 h (86.1%). These results suggest that the C12 taken up by tissues was not completely oxidized to CO_2, with the possible storage of labeled carbons into compounds not rapidly oxidizable, or that a portion of labeled carbon atoms was lost in the urine as bicarbonate. The percentage of $^{14}CO_2$ following the fast pathway, as estimated by model A ($100\alpha'/(\alpha+\alpha') = 28.9\%$), seems to approximate the physiological value of CO_2 dissolved in plasma plus the CO_2 carried by the

formation of carbamino compounds into erythrocytes (8% and 27%, respectively, according to (23)).

As can be seen from the individual estimates of Table 1, the values of the parameters ϱ_t, α, and the sum $\alpha+\alpha'$ appear to be different in subjects 1 and 2 (having smaller BMI) with respect to subject 5 and 6 (with larger BMI). In particular, for the subjects with higher BMI, larger ϱ_t and smaller $\alpha+\alpha'$ were found. This result indicates that individuals with higher BMI might present a faster uptake, but a less complete utilization of C12, with a significative apparent storage within the body. It is ascertained that in obese subjects glucose storage under glycogen form in both muscles and liver is impaired and restored only after weight loss (6). Hence, in our overweight subjects, C12 is likely to be more efficiently stored to compensate this deficiency.

Concerning the tissue uptake, only the estimation of the population mean parameters using the pooled data allowed us to find a maximal rate of C12 tissue uptake. This maximum rate ($T_m = 0.38$ mmol/min) is relatively high, and larger than the value (0.24 mmol/min) which has been obtained for sebacic acid, the dicarboxylic acid with 10 carbon atoms (2). Taking into account the incomplete oxidatin of C12 as estimated by model A, the maximal caloric delivery of C12 would be computed by $(\alpha+\alpha')T_m \times 230 \times 7.18$ (where 230 is the C12 MW and 7.18 cal/mg is the C12 energy density) corresponding to 31.25 kcal/h. Therefore, this fuel substrate might supply an energy amount of about 750 kcal/day, which is a good result for parenteral nutrition.

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