0022-3565/00/2923-0846$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2000 by The American Society for Pharmacology and Experimental Therapeutics
JPET 292:846–852, 2000

Vol. 292, No. 3
Printed in U.S.A.

Disposition of Dodecanedioic Acid in Humans

ALESSANDRO BERTUZZI, GELTRUDE MINGRONE, ALBERTO GANDOLFI, ALDO VIRGILIO GRECO, and SERENELLA SALINARI

Istituto di Medicina Interna e Geriatria, Università Cattolica del Sacro Cuore (G.M., A.V.G.); Istituto di Analisi dei Sistemi ed Informatica del CNR (A.B., A.G.); and Dipartimento di Informatica e Sistemistica, Università di Roma "La Sapienza" (S.S.), Rome, Italy

Accepted for publication November 16, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The disposition of dodecanedioic acid (C12) was investigated in six overnight-fasting healthy male volunteers, who received a 165-min i.v. infusion of 42.45 mmol of C12 added to 150 μCi of [1–12-^{14}C]C12. Blood samples were collected up to 360 min after the start of infusion, and concentration of serum labeled C12 was determined. Expired radioactivity (μCi/min) was measured up to 600 min and at 24 h. The 24-h C12 urinary excretion was around 5% of the administered amount. The percentage of C12 oxidized was 81.7 ± 9.5% (mean ± S.D.) of administered amount as estimated from the area under the curve of measured $^{14}CO_2$ expiration rate. 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 first order kinetics. Parameters of model B were estimated for each subject, whereas model A was identified by fitting the pooled data of all subjects. On the basis of estimates obtained from model B, an average calorie delivery of 500 kcal/day was predicted in the plateau phase for the infusion rate of our experiments. When estimated from model A, the maximal rate of tissue uptake was 0.38 ± 0.08 mmol/min, with a maximal calorie delivery of 750 kcal/day. These results appear promising for C12 utilization in parenteral nutrition, because C12 elimination with urine is low, whereas tissue uptake and oxidation are rather efficient.

The first evidence of ω-oxidation of monocarboxylic acids was reported by Verkade and Van der Lee (1934). After the oral administration of triundecylin, a compound that contains undecanoic acid, the straight saturated monocarboxylic acid with 11 carbon atoms, the homologous dicarboxylic acid (DA) 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. Since this first experimental observation, many studies have been performed regarding the fate of DAs, both in experimental animals and in humans (Greco and Mingrone, 1995). Medium-chain DAs (chain length of 6–12 carbon atoms) are rapidly β-oxidized in mitochondria and peroxisomes (Pettersen, 1973; Mortensen et al., 1982; Leighton et al., 1989; Vamecq and Draye, 1989). Pettersen and Haas (1973) showed that DAs with 10 to 16 carbon atoms could be activated in rat liver mitochondria in the presence of CoA and ATP and that the DA with 16 carbon atoms was a substrate for the carnitine palmitoyltransferase. In accordance with these observations, Mortensen and Gregersen (1982) found that the in vitro β-oxidation of dodecanedioic acid (C12) was dependent on ATP, CoA, carnitine, and NAD^+. However, the relative role of mitochondrial and peroxisomal β-oxidation has not been completely elucidated.

Whereas odd-chain DAs give acetyl-CoA and, as a terminal product, malonic acid that cannot be further oxidized, even-chain DAs appear to be completely oxidized (Mingrone et al., 1988, 1991). Succinyl-CoA, produced as an intermediate metabolite of even-chain DAs, is a gluconeogenic substrate that can play an important role in clinical conditions in which glucose metabolism is impaired, such as starvation, sepsis, and diabetes mellitus (Kou and Tserng Shiow-Jen, 1991). The transport of DAs of short-chain length through the cell membrane seems to be mediated by a carrier that has been characterized in rat hepatocytes (Boelsterli et al., 1995) and renal tubules (Sheridan et al., 1983; Ullrich et al., 1984). An active dicarboxylate transport system has also been evidenced in rat mitochondria (Saint-Macary and Foucher, 1985).

Among medium-chain DAs, C12 seems to be the most suitable for nutritional purposes. In fact, the urinary excretion of C12 is low (3–5% of administered dose) (Mingrone et al., 1994; Bertuzzi et al., 1995) compared with azelaic acid (DA with nine carbon atoms) (Bertuzzi et al., 1991) and sebacic acid (DA with 10 carbon atoms) (Mingrone et al., 1991; Bertuzzi et al., 1994), and the energy density is high (7.18 kcal/g of C12 oxidized) (Mingrone et al., 1994). The C12

Received for publication July 7, 1999.

ABBREVIATIONS: DA, dicarboxylic acid; C12, dodecanedioic acid; BMI, body mass index; MH, methylbenzethonium hydroxide; S.A., specific activity.

respiratory quotient (0.77) is rather low, representing an advantage in patients with respiratory distress, in whom 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 both long-chain (LCT) and medium-chain (MCT) monocarboxylic acids, because of its relatively high water solubility and its low affinity for albumin binding sites (Bertuzzi et al., 1995). Finally, contrary to both LCT and MCT, C12 administered in free form as a salt does not require hydrolysis before cellular utilization.

Kinetic analysis of C12 disposition after i.v. bolus injection has been recently reported in humans, showing efficient tissue uptake of C12 coupled with low elimination in urine (Bertuzzi et al., 1995). The kinetics of C12 and the effect of its administration on glucose kinetics in rats showed that C12 can supply glucose precursors and undergoes a rapid tissue uptake to an extent comparable with that of glucose, from the point of view of energy supply (Bertuzzi et al., 1997).

To verify whether the amount of C12 that can be taken up by tissues and oxidized to CO_2 is energetically adequate to consider C12 a suitable fuel substrate in humans, experiments of continuous i.v. infusion of labeled and unlabeled C12 were performed in healthy volunteers. For each subject, measurements of C12 plasma concentration, C12 excretion in the 24-h urine sample, 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 predicts that the rate of C12 tissue uptake and the percentage of oxidation of the C12 amount taken up by tissues are adequate for use of C12 in parenteral nutrition.

Materials and Methods

Chemicals

C12 and azelaic acid (used as internal standard) were obtained from Sigma Chemical Co. (St. Louis, MO). C12 was purified by Real S.r.l. (Como, Italy) and was free from pyrogens and contaminants with a degree of purification of 99.8%, ascertained by gas-liquid chromatography and mass spectrometry. All the other chemicals were of the highest purity available. A 0.4 M solution of C12 salified with NaOH was used for the i.v. infusion. The solutions were sterilized by 0.25-μm ∅ Millipore filters (Molsheim, France) before administration.

Experimental Procedures

An amount of 42.45 mmol of C12, added to 150 μCi of [1–12-^{14}C] C12 [specific activity (S.A.) 117 mCi/mmol], was administered as a 165-min continuous i.v. infusion in six overnight-fasting healthy male volunteers aged 51.2 ± 9.5 years (mean ± S.D.) 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 to 360 min at intervals varying from 5 to 20 min and immediately centrifuged. Plasma samples were frozen at −20°C until analysis. Each subject voided before starting C12 administration, and the 24-h urine sample was collected in a container with 0.1% sodium azide to prevent bacterial growth.

The protocol conformed to the directives given by the Ethical Committee of the Institutional Health Review Board of the Catholic University, School of Medicine, in Rome. 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 rate was automatically computed every minute. Indirect calorimetry was started 0.5 h before and was continued until 600 min after the beginning of C12 infusion.

Expired air was collected over 2-min periods at regular intervals of 20 to 30 min for a total time of 600 min after the beginning of labeled C12 infusion, and another CO_2 sample was collected at 24 h after starting C12 infusion. A 20-liter Douglas bag was used. A 1-M solution of methylbenzethonium hydroxide (MH) in methanol was prepared by adding 20 ml of MH to 36 ml of ethanol; 4 ml of 0.1% phenophthaleine was added as pH indicator. Three-milliliter aliquots of this solution were placed in graduate tubes and titrated with 0.15 N HCl. The next 9 ml of the solution was transferred into a bubbling apparatus to trap CO_2 from the Douglas bag. Following the above procedure, solutions containing 3 mEq of MH were obtained: these solutions trap exactly 3 mmol of CO_2 (Wolfe, 1984). Finally, the MH solution trapping $^{14}CO_2$ was added with 10 ml of 0.4% 2,5-diphenyloxazole (in diphenyl oxazole) toluene in scintillation fluid and counted. Radioactivity was detected by a β-scintillation counter (Packard Tri-Carb 460C, Downers Grove, IL). Quenching was checked by the internal standard method. The $^{14}CO_2$ fluxes were calculated by use of the values of CO_2 production rate measured by indirect calorimetry.

DA Analysis

Serum Samples. Azelaic acid (100 μg) was added to 1 ml of each serum sample as an internal standard. Proteins were precipitated with 0.1 ml of 4 N HCl and DAs extracted twice with 8 volumes of ethyl acetate maintaining the solution at 60°C for 15 min. The combined extracts were dried in a GyroVap apparatus (Howe GV1; Gio. De Vita, Rome, Italy) operating at 60°C, coupled with a vacuum pump and a gas trap FTS-System (Stone Ridge, NY).

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

HPLC Analysis. The HPLC of DAs was performed according to a previously described method (Mingrone et al., 1992). The eluate of the peak corresponding to the retention time of C12 standard was collected into a vial, added to scintillation fluid, and counted as specified above.

Mathematical Model of C12 Kinetics and Disposition

The kinetics of C12 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 indicate that C12 binding to human serum albumin can be described by assuming one class of equivalent and independent binding sites (Bertuzzi et al., 1995), so the total C12 concentration was written as

$$c_t = c_f + a\frac{Kc_f}{1 + Kc_f}, \quad (1)$$

where a is the concentration of protein binding sites in the compartment and K is the association constant.

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 (Bertuzzi et al., 1991, 1994, 1997)

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

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

$$g(c_f) = \varrho_t c_f \quad (3)$$

where ϱ_t (l/min) is the rate constant of tissue uptake. Note that the term $g(c_f)$ represents the rate of elimination of C12 in routes different from renal excretion and leading to C12 utilization. The saturable behavior of eq. 2 can be attributed in principle to various processes, from the transport through the cell membrane to the enzymatic reaction in the mitochondria.

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

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

where V is the distribution volume (liters) 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:

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

$$c_f(0) = 0\ . \quad (5)$$

Because the function g may have the form given by eq. 2 or by eq. 3, in the following, we 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 (S.A. of administered C12 = 3.53 mCi/mol), and the radioactivity of C12 in plasma was measured. Thus the quantity

$$y_1(t) = SA \cdot c_t(t) \quad (6)$$

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 C12 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 $\varrho \int_0^{24\ h} c_f(t)dt$.

We assumed that the production of CO_2 resulting from 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

$$y_2(t) = \alpha' u^*(t) + \frac{\alpha}{\tau}\int_0^t \exp^{-\frac{1}{\tau}(t-s)} u^*(s)ds\ , \quad (7)$$

where $u^* = S.A. \cdot g[c_f(t)]$ (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 slow pathways of CO_2 production and elimination, respectively. 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 that is oxidized and the value of $\alpha + \alpha'$ is 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.

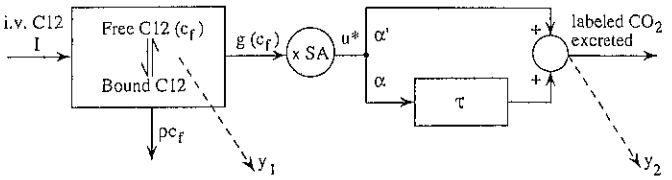

Fig. 1. Block diagram of the model used for the analysis of kinetics and oxidation of C12. The dashed arrows represent measured quantities. For the symbols, see *Materials and Methods.*

A diagram of the kinetics of C12 and the production and excretion of CO_2 is shown in Fig. 1.

Estimation of Unknown Parameters. The identifiability of the unknown parameters of model A (V, α, ϱ, T_m, K_M, α, α', and τ) and model B (V, α, ϱ, ϱ_t, α, α', and τ) was verified by the similarity transformation method (Wajda et al., 1989). For all subjects, the value of the association constant K was set equal to $6.4 \times 10^3\ M^{-1}$, the mean value previously estimated in a group of healthy subjects (Bertuzzi et al., 1995). The parameters of both models were estimated for each subject by simultaneously 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 c.v., a weighted least-squares fit was performed, with weights given by the inverse of the c.v. times the experimental value (Landaw and DiStefano, 1984).

The parameters were also estimated as means over the subject population by the naive pooled-data approach (Jacquez, 1996), 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, and the S.E. values of the estimates were determined from the inverse Hessian matrix of the index computed at the optimum (Seber and Wild, 1989). For the individual estimates, S.E. was computed assuming a c.v. of the measurements equal to 0.1.

Results

The 24-h urinary excretion of C12 was 5.1 ± 0.8% (mean ± S.D. over the six subjects) of the administered amount, corresponding to 2.18 ± 0.35 mmol. To evaluate the amount of C12 oxidized in each subject, following Nördenstrom et al. (1983), the ratio between the total radioactivity excreted with the expired air and the amount of labeled C12 infused (percent oxidation) 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 by the trapezoidal approximation. The amount of C12 oxidized, expressed as percent oxidation, was 81.7 ± 9.5% of the administered amount of C12 and, therefore, 86.1 ± 10.1% of the C12 amount that is not lost in urine.

The fitting of the individual data by model A gave unacceptably high S.E. values 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 S.E. values and a limited loss of the goodness of fit. The fitting for two subjects is shown in Fig. 2. The values of the parameters for each subject, together with the mean values, are reported in Table 1, where the BMI of each subject is also given. It can be observed that the values of some parameters (in particular ϱ_t, α, and α +

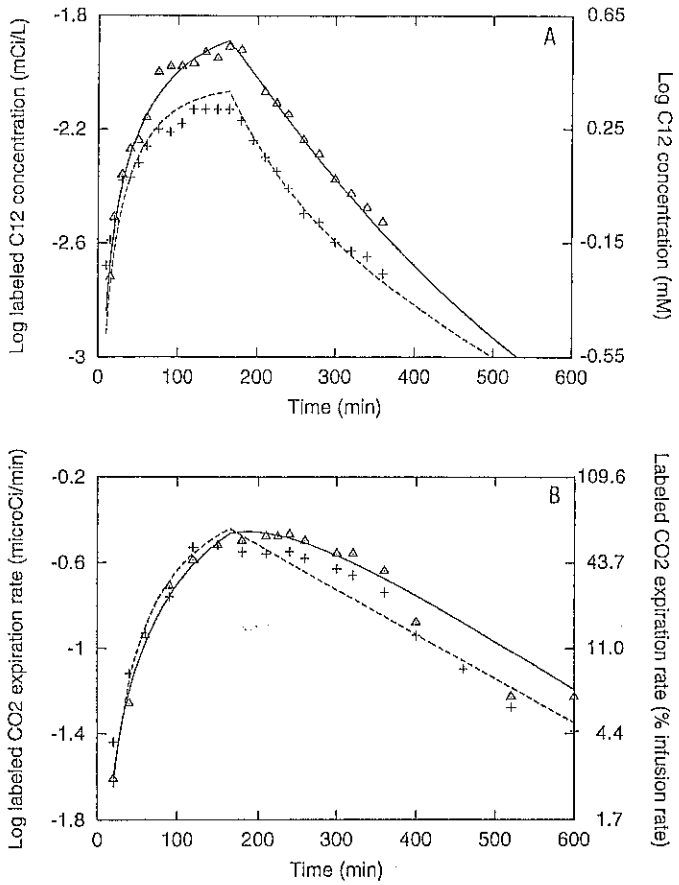

Fig. 2. Experimental data of total concentration of labeled C12 in serum (A) and of $^{14}CO_2$ expiration rate (B) versus time during and after the labeled C12 infusion in two subjects (△, subject 1 of Table 1; +, subject 6 of Table 1). The continuous and dashed lines represent the optimal fitting curves predicted by model B with the parameter values reported in Table 1. The right y-axis of A gives the unlabeled C12 concentrations computed from the S.A. of infused C12. The right y-axis of B gives the labeled CO_2 expiration rate as a percentage of the labeled carbon infusion rate.

α') 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 S.E. values by fitting the pooled data of all subjects. The estimated parameters should be considered in this case as direct estimates of the mean parameter values over the subject population. The parameters of model B were 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 versus 323), so that model A was found to be preferable on the basis of both Akaike and Schwarz criteria (Landaw and DiStefano, 1984). The population means of model parameters, estimated by model A, are reported in Table 2. 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 3A 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. 3B, the measured values of the $^{14}CO_2$ expiration rate and the best-fitting curve are depicted. A rapid increase in 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 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 $\int_0^{24h} y_2(t)dt$, was found to be equal to 117.6 mCi, that is, 78.4% of the infused amount of label.

Discussion

Because C12 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 used as a convenient method for measuring the concentration of the compound in plasma (Jacquez, 1996) and to quantitate its oxidation. The administration of unlabeled C12 at a relatively high infusion rate was intended to give evidence to the possible presence of a saturable mechanism in the C12 tissue uptake.

In the mathematical model proposed, the kinetics of labeled C12 plasma concentration was described by a simple one-compartment model, to reduce the number of unknown parameters, while maintaining an adequate overall fitting of the experimental data. To describe the rate of C12 tissue uptake, as already done in the analysis of kinetic data of azelaic and sebacic acids (Bertuzzi et al., 1991, 1994), a saturable function was used in model A. This assumption is suggested by the observations on the active transport of mono acids and DAs across cellular membranes (Sheridan et al., 1983; Ullrich et al., 1984; Boelsterli et al., 1995; Saint-Macary and Foucher, 1985; Stremmel, 1988) and by the saturable nature of the enzymatic process of oxidation. We note, however, that this process is further complicated by the backinhibition due to β-oxidation products of DAs (Poosch and Yamazaki, 1989). 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 estimated value of the C12 distribution volume, obtained as a mean of the individual estimates (Table 1) or as a population estimate (Table 2), is not far from the sum of plasma volume plus the volume of the rapidly equilibrating interstitial water (10.7 liters for a 70-kg human; Bischoff, 1975). This estimate of the distribution volume does not strictly represent a physiological space, because the distribution kinetics was modeled by a single compartment, whose volume will generally be lower than the sum of plasma volume plus the volumes of peripheral compartments. Note that the low value obtained for the distribution volume suggests that intracellular spaces are not involved, thus favoring the view that the saturable behavior of tissue uptake may be mainly caused by the saturation of the transport system at the cellular membrane level.

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). The individual estimates of a are dependent on the assumption that K is constant among individuals; thus, our estimates should be considered apparent concentrations of sites with a fixed K value. However, it

TABLE 1

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

BMI values (kg/m^2) are indicated in parentheses.

Parameter	Subjects						Mean
	1 (22.7)	2 (23.3)	3 (25.2)	4 (24.5)	5 (28.6)	6 (28.7)	
				Liter			
V	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
				mM			
a	0.28 ± 0.22	0.74 ± 0.12	[a]	0.50 ± 0.09	0.74 ± 0.19	0.84 ± 0.12	0.53
				ml/min			
ϱ	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	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
				min			
τ	149 ± 22	175 ± 12	208 ± 18	195 ± 14	214 ± 11	163 ± 13	184

[a] Estimated a virtually equal to zero.

TABLE 2

Estimates ± S.E. 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
	liter
V	6.91 ± 0.30
	mM
a	0.87 ± 0.12
	ml/min
ρ	4.72 ± 0.52
	mmol/min
T_m	0.38 ± 0.08
	mM
K_M	3.06 ± 1.08
α	0.59 ± 0.02
α'	0.24 ± 0.02
	min
τ	151 ± 11

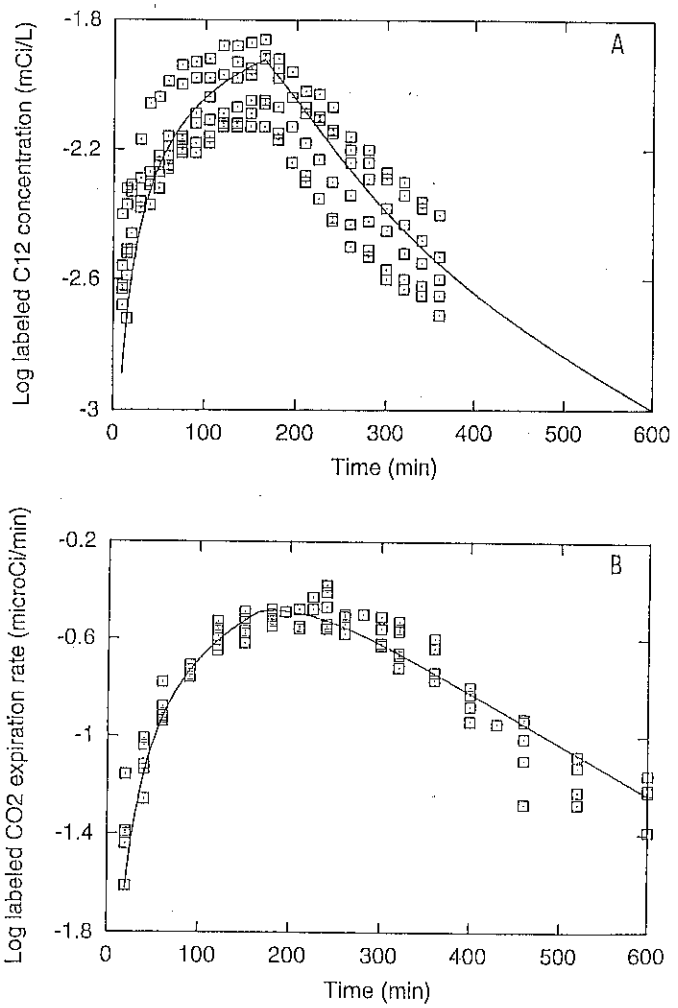

Fig. 3. Experimental data (⊡) of total concentration of labeled C12 in serum (A) and of $^{14}CO_2$ expiration rate (B) versus time during and after the labeled C12 infusion in six subjects. The continuous lines represent the optimal fitting curves predicted by model A following the naive pool approach. The parameter values are reported in Table 2.

was verified that the estimate of a obtained by assuming different values for K varied less than would be expected if the product aK were constant. The total amount of binding sites, $aV = 6.01$ mmol, estimated by model A from the pooled data, is comparable with the amount of albumin binding sites in plasma [5.95 mmol, assuming the albumin concentration in plasma equals 0.6 mM, 3.1 binding sites per molecule (Bertuzzi et al., 1995), and plasma volume equals 3.2 liters]. This result suggests a limited extent of C12 binding to interstitial proteins. When a linear tissue uptake was assumed, the estimated number of binding sites (3.39 mmol with the mean values) was, on the contrary, markedly smaller than the number of albumin sites in plasma. This result appears to be indirect evidence of the presence of a saturable tissue uptake of C12. In fact, the protein binding and the saturable tissue uptake influence the descending branch of C12 plasma concentration (after the end of infusion) in opposite ways: the protein binding produces an upward concavity, whereas the saturable tissue uptake produces a downward concavity. If both these nonlinearities are actually present, the data will show a reduced (or almost absent) upward concavity. This point was verified by simulations of the model with reasonable parameter values. So, if data are fitted with a model containing only protein binding, the binding site concentration is likely to be underestimated, leading to possibly fewer binding sites than the albumin binding sites in plasma.

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 DAs change with the chain length (Sheridan et al., 1983; Ullrich et al., 1984). We found that azelaic acid is likely to be actively secreted (Bertuzzi et al., 1991), whereas sebacic acid appears to be reabsorbed (Bertuzzi et al., 1994).

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 first order kinetics. The fast pathway accounts for the observed rapid increase in radioactivity in the expired air after the beginning of 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 gluconeogenic 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 complex (the recovery of labeled CO_2 after administration of labeled bicarbonate has been indeed represented in the literature by two- or three-compartment models; see Issekutz et al., 1968; Pallikarakis et al., 1991), we chose a simple model that nonetheless 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 the value determined from the area under the curve of the experimental data of expired radioactivity (81.7%). The sum $\alpha + \alpha'$ was estimated to be less 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, that was 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; Selkurt, 1971).

As can be seen from the individual estimates in Table 1, the values of the parameters ϱ_t, α, and $\alpha + \alpha'$ appear to be different in subjects 1 and 2 (having smaller BMI) from subjects 5 and 6 (with larger BMI). In particular, for the subjects with higher BMI, a 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 significant 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 (Damsbo et al., 1991). Hence, in our overweight subjects, C12 is likely to be more effectively stored to compensate this deficiency.

The estimates of parameters obtained by the linear model provide an estimate of the calorie delivery in the plateau phase for the infusion rate used (0.257 mmol/min). Taking into account the incomplete oxidation of C12, the calorie delivery of C12 can be computed as $(\alpha + \alpha')\varrho_t c_{ss} \times 230.3 \times 7.18$ (where c_{ss} is the steady-state concentration of free C12, found by setting the time derivative in eq. 5 equal to zero; 230.3 is the C12 molecular weight; and 7.18 cal/mg is the C12 energy density), obtaining 500 kcal/day when the mean value of parameters in Table 1 is used. An estimate of the maximal rate of C12 tissue uptake was obtained with model A and the pooled data. The estimated maximal rate of tissue uptake (T_m = 0.38 mmol/min) is relatively high and larger than the value obtained for sebacic acid (0.24 mmol/min), the DA with 10 carbon atoms (Bertuzzi et al., 1994). The estimated maximal calorie delivery of C12 can be computed as $(\alpha + \alpha')T_m \times 230.3 \times 7.18$, corresponding to 750 kcal/day. Therefore, this fuel substrate appears capable of supplying an energy amount comparable with that usually given by other alternative lipid substrates such as MCT emulsion (450 kcal/day; Pakula et al., 1999).

References

Bertuzzi A, Gandolfi A, Salinari S, Mingrone G, Arcieri-Mastromattei E, Finotti E and Greco AV (1991) Pharmacokinetic analysis of azelaic acid disodium salt. *Clin Pharmacokinet* **20**:411–419.

Bertuzzi A, Gandolfi A, Salinari S, Mingrone G and Greco AV (1994) Pharmacokinetics of dicarboxylic acids in man. *IEEE Eng Med Biol* **13**:472–478.

Bertuzzi A, Mingrone G, De Gaetano A, Gandolfi A, Greco AV and Salinari S (1997) Kinetics of dodecanedioic acid and effect of its administration on glucose kinetics in rats. *Br J Nutr* **78**:143–153.

Bertuzzi A, Mingrone G, Gandolfi A, Greco AV and Salinari S (1995) Pharmacokinetic analysis of dodecanedioic acid in humans from bolus data. *J Parenter Enteral Nutr* **19**:498–501.

Bischoff KG (1975) Some fundamental considerations of the applications of pharmacokinetics to cancer chemotherapy. *Cancer Chemother Rep* **59**:777–793.

Boelsterli UA, Zimmerli B and Meier PJ (1995) Identification and characterization of a basolateral dicarboxylate/cholate antiport system in rat hepatocytes. *Am J Physiol* **268**:G797–G805.

Damsbo P, Vaag A, Hother-Nielsen O and Beck-Nielsen R (1991) Reduced glycogen synthase activity in skeletal muscle from obese patients with and without type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **34**:239–245.

Greco AV and Mingrone G (1995) Dicarboxylic acids, an alternate fuel substrate in parenteral nutrition: An update. *Clin Nutr* **14**:143–148.

Issekutz B, Paul P, Miller HI and Bortz WM (1968) Oxidation of plasma FFA in lean and obese humans. *Metabolism* **17**:62–73.

Jacquez JA (1996) *Compartmental Analysis in Biology and Medicine*, BioMedware, Ann Arbor, MI.

Kou Y and Tserng Shiow-Jen J (1991) Metabolic conversion of dicarboxylic acids to succinate in rat liver homogenates. *J Biol Chem* **266**:2924–2929.

Landaw EM and DiStefano IIIJJ (1984) Multiexponential, multicompartmental, and noncompartmental modeling. II. Data analysis. *Am J Physiol* **246**:R665–R677.

Leighton F, Bergseth S, Rortveit T, Christiansen EN and Bremer J (1989) Free acetate production by rat hepatocytes during peroxisomal fatty acid and dicarboxylic acid oxidation. *J Biol Chem* **264**:10346–10350.

Mingrone G, Greco AV, Bertuzzi A, Arcieri-Mastromattei E, Tacchino RM, Marino F, Finotti E and Castagneto M (1991) Tissue uptake and oxidation of disodium sebacate in man. *J Parenter Enteral Nutr* **15**:454–459.

Mingrone G, Greco AV, De Gaetano A, Tataranni A, Raguso C and Castagneto M (1994) Pharmacokinetic profile of dodecanedioic acid, a proposed alternative lipid substrate for parenteral nutrition. *J Parenter Enteral Nutr* **18**:225–230.

Mingrone G, Tacchino RM, Castagneto M, Finotti E and Greco AV (1992) Use of even numbered carbon atom dicarboxylic salts in parenteral nutrition as fuel substrate. *J Parenter Enteral Nutr* **16**:32–38.

Mingrone G, Tacchino RM, Greco AV, Arcieri-Mastromattei E, Marino F, Finotti E and Castagneto M (1988) Preliminary studies of a dicarboxylic acid as an energy substrate in man. *J Parenter Enteral Nutr* **13**:299–305.

Mortensen PB and Gregersen N (1982) The biological origin of ketotic dicarboxylic aciduria II: In vivo and in vitro investigations of the β-oxidation of C8–C16 dicarboxylic acids in unstarved, starved and diabetic rats. *Biochim Biophys Acta* **710**:477–484.

Mortensen PB, Kølvraa S and Gregersen N (1982) Cyanide insensitive and clofibrate enhanced β-oxidation of dodecandioic acid: Evidence of peroxisomal β-oxidation of dicarboxylic acids. *Biochim Biophys Acta* **713**:393–397.

Nördenstrom J, Carpentier YA, Askanazi J, Robin AP, Elwyn DH, Hensle TW and Kinney JM (1983) Free fatty acid mobilization and oxidation during total parenteral nutrition in trauma and infection. *Ann Surg* **198**:725–735.

Pakula R, Konikoff FM, Moser AM, Greif F, Tietz A, Gilat T and Rubin M (1999) The effects of short term lipid infusion on plasma and hepatic bile lipids in humans. *Gut* **45**:453–458.

Pallikarakis N, Sphiris N and Lefevre P (1991) Influence of the bicarbonate pool on the occurrence of $^{13}CO_2$ in exhaled air. *Eur J Appl Physiol* **63**:179–183.

Pettersen JE (1973) Metabolism of hexadecanedioic acid and its mono-L-carnitine ester. *Biochim Biophys Acta* **306**:1–19.

Pettersen JE and Haas M (1973) ATP-dependent activation of dicarboxylic acids in rat liver. *Biochim Biophys Acta* **326**:305–313.

Poosch MS and Yamazaki RK (1989) The oxidation of dicarboxylic acid CoA esters via peroxisomal fatty acyl-CoA oxidase. *Biochim Biophys Acta* **1006**:291–298.

Saint-Macary M and Foucher B (1985) Comparative partial purification of the active dicarboxylate transport system of rat liver, kidney and heart mitochondria. *Biochem Biophys Res Commun* **133**:498–504.
Seber GAF and Wild CJ (1989) *Nonlinear Regression*, John Wiley & Sons, New York.
Selkurt EE (ed) (1971) *Physiology*, Little, Brown and Company, Boston, MA.
Sheridan E, Rumrich G and Ullrich KJ (1983) Reabsorption of dicarboxylic acids from the proximal convolution of rat kidney. *Pfluegers Arch* **399**:18–28.
Stremmel W (1988) Fatty acid uptake by isolated rat heart myocytes represents a carrier-mediated transport process. *J Clin Invest* **81**:844–852.
Ullrich KJ, Fasold H, Rumrich G and Klöss S (1984) Secretion and contraluminal uptake of dicarboxylic acids in the proximal convolution of rat kidney. *Pfluegers Arch* **400**:241–249.
Vamecq J and Draye JP (1989) Peroxisomal and mitochondrial β-oxidation of monocarboxylyl-CoA, ω-hydroxymonocarboxylyl-CoA and dicarboxylyl-CoA esters in tissues from untreated and clofibrate-treated rats. *J Biochem* **106**:216–222.
Verkade PE and Van der Lee J (1934) Researches on fat metabolism II. *Biochem J* **28**:31–40.
Wajda S, Godfrey KR and Rabitz H (1989) Similarity transformation approach to identifiability analysis of nonlinear compartmental models. *Math Biosci* **93**:217–248.
Wolfe RR (1984) *Tracers in Metabolic Research*, Alan R. Liss, New York.

Send reprint requests to: Alessandro Bertuzzi, Istituto di Analisi dei Sistemi ed Informatica del CNR, Viale Manzoni 30, 00185 Rome, Italy. E-mail: bertuzzi@iasi.rm.cnr.it